PROCEEDINGS OF VII. INTERNATIONAL AGRICULTURAL, BIOLOGICAL, LIFE SCIENCE CONFERENCE AGBIOL 2025

07-10 SEPTEMBER 2025

ISTANBUL, TÜRKİYE

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WELCOME NOTES

You are welcome to our VII. AGBIOL Conference that is organized by Trakya University, Beykent University and International Researchers Association. The aim of our conference is to present scientific subjects of a broad interest to the scientific community, by providing an opportunity to present their work as oral or poster presentations that can be of great value for global science arena. Our goal was to bring three communities, namely science, research and private investment together in a friendly environment of Edirne, Turkey in order to share their interests and ideas and to get benefit from the interaction with each other.

In September 2018, we organized the first AGBIOL Conference with more than 700 scientists and researchers from all over the world with over 800 scientific papers. Due to COVID-19 situation, II. AGBIOL 2020 has organized fully on-line event which was one of the biggest online conferences in recent years in the world with 499 papers and 1133 authors with 333 oral and 166 e-poster presentations from 55 countries. Due to COVID-19 situation, AGBIOL 2021 was organized online again. AGBIOL 2022 conference was organized with a worldwide participation from 44 countries over 522 papers contributed by over 1300 authors. AGBIOL 2023 was organized with a record and worldwide participation from 33 countries 833 papers contributed by over 2000 authors with 522 oral and 311 poster presentations. AGBIOL 2024 consisted of 835 papers contributed by about 2000 authors with worldwide recodr participation again from 55 countries with 522 oral and 311 poster presentations.

There is a worldwide record participation from 60 countries 988 papers contributed over 2300 authors with 400 oral and 588 poster presentations in AGBIOL 2025.

The AGBIOL 2025 is normal participation as well as with online participation in Beykent University in Istanbul, Turkey on 07-10 September, 2025. The program included oral talks by invited prominent scientists and oral and e poster presentations by participants in selected topics from the submitted abstracts focusing on Agriculture, Biology and Life Sciences topics.

With care for our nature and environment, we aim the green congress, meaning that as little as possible papers will be used. Abstract book is published in electronic book and is distributed to the participants by e mail for online participants. All the e-posters are prepared in electronic form and then submit to via the conference e mail and exhibited in electronical poster boards as well as in online e poster hall in our web page during the conference.

The participants with paid conference fee accessed all the normal and virtual presentation talks in each session, as well as to visit the virtual poster hall via preliminary provided. The abstracts were published in the Conference Abstract and Proceedings Book. Participants might send us their full papers, which based on their preferences are published either in our Conference Abstract and Proceedings Book or in selected International Indexed Scientific Journals.

Conference Topics:

Agriculture, Forestry, Life Sciences, Agricultural Engineering, Aquaculture and Biosystems, Animal Science, Biomedical science, Biochemistry and Molecular Biology, Biology, Bioengineering, Biomaterials, Biomechanics, Biophysics, Bioscience, Biotechnology, Botany, Chemistry, Chemical Engineering, Earth Sciences, Environmental Science, Food Science, Genetics and Human Genetics, Medical Science, Machinery, Pharmaceutical Sciences, Physics, Soil Science.

We would like to thank all of you for joining this conference and we would like to give also special thanks to TUBITAK and collaborators for giving us a big support to organize this event.

Prof Dr Yalcin KAYA
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CONTENTS

WELCOME NOTES 3

ORGANIZING COMMITTEE 4

INVITED SPEAKERS 5

SCIENTIFIC COMMITTEE 6

EFFECT OF IRON FOUNDRY DUST ON THE ENERGY EFFICIENCY OF HYDROPHILIC NANOCOATED PHOTOVOLTAIC PANELS **16**

TECHNICAL AND ECONOMIC ANALYSIS OF THE SOLAR ENERGY SYSTEM DESIGNED FOR EDIRNE PROVINCE AGRICULTURAL RESEARCH INSTITUTE BUILDING IN THE CASE OF ROOF AND FACADE APPLICATION **25**

IMPACT OF REGULAR WATER-BASED CLEANING ON THE ENERGY EFFICIENCY OF PHOTOVOLTAIC PANELS **31**

QUANTUM-CHEMICAL STUDY OF NOVEL MULTIFUNCTIONAL POLYMER BY SUBSTITUTION REACTION OF GRAFTING ACETYLSALICYLIC ACID **38**

ASSESSMENT OF THE TRANSITION FROM CONVENTIONAL SOIL TILLAGE TO MINIMUM TILLAGE TECHNOLOGY IN A MAIZE CROP WITH THREE HYBRIDS – A THREE-YEAR CASE STUDY IN LANURILE, CONSTANȚA COUNTY **45**

INNOVATIVE AGRICULTURE THROUGH RECYCLING: SUSTAINABLE BIOLOGICAL PRODUCTION SYSTEMS FROM DISCARDED MATERIALS **58**

SOME TAXONOMIC, ECOLOGICAL AND ETHNO-ENTOMOLOGICAL DATA ON THE LOCUSTS (ORTHOPTERA) OF THE HABITATS OF VLORA **72**

DAIRY SECTOR VALUE CHAINS IN BOSNIA AND HERZEGOVINA **81**

ANTISTAPHYLOCOCCAL STUDY OF THE ESSENTİAL OİL OF MENTHA PULEGİUM L. FROM ALGERİA **92**

THE HOME GARDENS AS BIOCULTURAL RESERVE OF LOCAL PLANT DIVERSITY AND TRADITIONAL KNOWLEDGE **98**

STATIC ANALYSIS OF FUNCTIONALLY GRADED POROUS SANDWICH BEAMS **107**

INVESTIGATION OF THE EQUIVALENT MECHANICAL PROPERTIES OF TPMS LATTICE STRUCTURES **121**

PRODUCTION AND CHARACTERIZATION OF MESOPOROUS ACTIVATED CARBON FROM CAROB TREE BIOMASS FOR ENVIRONMENTAL APPLICATIONS **138**

BOLTING IN SPINACH; GENETIC AND ENVIRONMENTAL FACTORS **146**

POSSIBLE MORPHOLOGICAL EVIDENCES FOR A NEW SPECIES OF *EUPALOPSELLUS* (ACARIFORMES: EUPALOPSELLIDAE): INSIGHTS FROM A FEMALE SPECIMEN COLLECTED FROM TÜRKİYE **161**

GIS MAPPING OF SOIL MACRONUTRIENTS FOR NUTRIENT MANAGEMENT IN HAZELNUT **166**

CLASSIFICATION OF CURRENT ARTIFICIAL INTELLIGENCE APPLICATIONS ACCORDING TO THEIR FUNCTIONALITY **172**

EFFECTS OF CHEMICAL AND ORGANOMINERAL FERTILIZERS ON MORPHOLOGICAL CHARACTERISTICS AND YIELD **186**

BALANCE, BACTERIA, AND THE BLACK BELT: IMPACT OF PROBIOTICS ON GASTROINTESTINAL SYMPTOMS DURING JUDO TRAINING AND JUDO COMPETITIONS 192

BIOSURFACTANT POTENTIAL OF PROBIOTIC MICROORGANISMS ISOLATED FROM FERMENTED CABBAGE: A SUSTAINABLE APPROACH TO FUNCTIONAL BIOPRODUCTS 201

HEAT AND COLD STRESS IN WATER BUFFALOES (BUBALUS BUBALIS): A REVIEW 212

AN OBSERVATION ON AN ANOMALY IN THE SCE SETA DISRUPTING BILATERAL SYMMETRY IN *TYCHEROBIUS IZMIRENSIS* AKYOL & KOÇ (ACARIFORMES: CAMEROBIIDAE) 218

SPATIOTEMPORAL PATTERNS OF TRACE ELEMENTS (LI, MN, NI, BA) IN RIVERS FEEDING THE İSTANBUL STRAIT, TÜRKIYE 227

PHYSIOLOGICAL EFFECTS OF LOW AND HIGH TEMPERATURE STRESS ON CORN WITH MYCORRHIZAL SYMBIOSIS 232

PROBABILISTIC ASSESSMENT OF FLUORIDE-RELATED HEALTH RISKS IN GROUNDWATER OF THE FELENT STREAM WATERSHED, KÜTAHYA 243

SPATIAL–TEMPORAL VARIATIONS OF FLUORIDE IN SURFACE WATERS OF FELENT STREAM (TÜRKIYE) AND ASSOCIATED HEALTH RISKS 250

ENVIRONMENTAL IMPACTS OF GREENHOUSE GAS EMISSIONS AND PROPOSED SOLUTIONS FOR THEIR REDUCTION 257

FROM SOIL TO SURVIVAL: PGPB-TRIGGERED DEFENSE AND ADAPTATION IN PLANTS 266

TOXIC DIALOGUES: HERBICIDES, MICROBIAL VOICES, AND THE ECOSYSTEM'S RESPONSE 281

THE IMPORTANCE OF MARKER ASSISTED SELECTION IN BLACKBERRY 292

SOFT CHEMISTRY NANOCOMPOSITE OF MGO DOPED PVC FABRICATION FOR PHOTOCATALYTIC APPLICATIONS 300

VALORIZATION OF FOOD INDUSTRY WASTES FROM CEREALS AND PULSES INTO FUNCTIONAL FOOD ADDITIVES 314

THE ROLE OF ORGANIC FERTILIZER ON CROP PRODUCTION IN AFGOI-SOMALIA 323

INTERVAL VALUED PYTHAGOREAN FUZZY AHP-INTERVAL VALUED PYTHAGOREAN FUZZY TOPSIS METHOD FOR SOLAR POWER PLANT SITE DECISION 332

LEGAL ISSUES FACED BY FARMERS AND AWARENESS OF AGRICULTURAL LAW IN THE TRAKYA REGION 340

MOLECULAR MARKER-BASED APPROACHES IN DETERMINING ALMOND GENETIC DIVERSITY: A REVIEW OF RECENT STUDIES 350

THE EFFECTS OF GLOBAL WARMING ON THE WATER QUALITY OF LAKES AND RIVERS 358

CHLORINE CONTAMINATION IN DILUTE NITRIC ACID PRODUCTION: SOURCES, EFFECTS, AND PREVENTION METHODS 365

SHORT-TERM EFFECTS OF DIFFERENT ORGANIC FERTILIZERS ON SOIL CHEMICAL PROPERTIES AND PLANT GROWTH IN MELON AND WATERMELON CULTIVATION 371

IDENTIFICATION OF SNP MARKERS ASSOCIATED WITH GENES CONTROLLING POTASSIUM AND SODIUM CONCENTRATION IN WALNUT FRUIT BY GWAS METHOD 382

PROPOLIS AGAINST *BOTRYTIS CINEREA*: A REVIEW OF STUDIES COMBINING IN VITRO AND IN VIVO TESTS 394

BIOCONTROL ACTIVITY OF Trichoderma harzianum AGAINST *Fusarium culmorum* CAUSING ROOT AND CROWN ROT IN WHEAT UNDER SALT STRESS CONDITIONS402

POLY(4,4"-BIS(2'-FLUORENYLIDENEIMINO)-3,3"-DIMETHYLBIPHENYL): ELECTROSYNTHESIS, CHARACTERIZATION AND APPLICATION IN ORGANIC PHOTOVOLTAIC CELL 410

ASSESSMENT OF RIPARIAN VASCULAR FLORA OF THE RIVER SHKUMBIN NEAR ELBASAN AREA, ALBANIA 416

HORTICULTURAL PRACTICES IN POT CULTIVATION OF RHODODENDRON SPECIES: A CASE FROM TURKEY 423

EVALUATION OF THE ANTIOXIDANT ACTIVITY OF DAUCUS CAROTA L. ESSENTIAL OIL FROM EL-TAREF -ALGERIA 432

RECENT ADVANCES IN ALMOND MICROPROPAGATION AND FUTURE PERSPECTIVES 438

ASSESSING THE IMPACT OF GLOBAL WARMING ON SOIL HEALTH AND AGRICULTURAL PRODUCTIVITY 447

PRE-TREATMENT WITH GILABURU EXTRACT ALLEVIATES H2O2-INDUCED CYTOTOXICITY IN ENDOTHELIAL CELLS 466

A PRELIMINARY ANALYSIS OF FUNCTIONAL TRAIT COMPOSITION IN ADRIATIC SEA FISHERIES 471

SHORT-TERM EFFECTS OF DIFFERENT ORGANIC FERTILIZERS ON SOIL CHEMICAL PROPERTIES AND PLANT GROWTH IN MELON AND WATERMELON CULTIVATION 478

SMART BIOPOLYMER-BASED PACKAGING SYSTEMS AND CURRENT APPLICATIONS IN FOOD QUALITY TRACEABILITY 488

ENHANCING ENERGY EFFICIENCY IN AGRICULTURAL PRACTICES 508

APPLICATION OF LIPOSOMAL ENCAPSULATED ANTIMICROBIAL BIOACTIVE COMPONENTS IN FOOD PRODUCTS AS NATURAL PRESERVATION 512

REASONS FOR THE UNDERDEVELOPMENT OF CANOLA IN TÜRKİYE 519

ANTI-NUTRITIONAL SUBSTANCES CONTAINED IN RAPESEED 525

DETERMINATION OF THE STABILITY OF POLYPHENOL OXIDASE FROM BLACK MULBERRY TOWARDS DIFFERENT ORGANIC SOLVENTS AND SURFACTANTS 535

BIOCHEMICAL AND AROMATIC CHANGES IN PERSIMMON (DIOSPYROS KAKI L.) FRUITS HARVESTED IN DIFFERENT PERIODS 541

EFFECTS OF AQUAFABA AS ALTERNATIVE PLANT ADDITIVE ON PHYSICAL, TEXTURAL AND SENSORY CHARACTERISTICS OF EGGLESS TURKISH PASTA (ERIŞTE) 556

PHYTOCHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITIES OF GINGER (Zingiber officinale) **561**

SOUS-VIDE: AN INNOVATIVE APPROACH TO COOKING AQUATIC PRODUCTS **570**

FISH SPECIES SETTLING IN AN ARTIFICIAL REEF MODEL CREATED FROM FALLEN CONCRETE PIERS OF PIPES INTAKE FROM THE SEA IN KONYAALTI, ANTALYA .. **577**

EXPLORING THE AVAILABILITY OF BULGARIAN PLANT GENETIC RESOURCES IN THE INTERNATIONAL CROP NETWORKS **583**

SOME RADICAL SCAVENGING ACTIVITIES OF OLIGOMERIC PROANTHOCYANIDINS OBTAINED FROM Pinus brutia Ten. BARKS **590**

APPLICATION OF ADAMS-BASHFORTH-MOULTON METHOD IN PREDICTING VEGETABLE PRODUCTION IN ALBANIA **595**

CYTOTOXIC EFFECTS OF OLIGOMERIC PROANTHOCYANIDINS OBTAINED FROM Pinus brutia Ten. BARKS **607**

EXPLORATORY FACTOR ANALYSIS OF FRUIT FARMERS' ECO-FRIENDLY AGRICULTURAL TECHNIQUES APPLICATIONS IN TERMS OF SUSTAINABLE AGRICULTURE-ENVIRONMENT RELATIONS: EVIDENCE FROM EĞIRDIR LAKE IN TURKEY **611**

SOME TECHNOLOGICAL PROPERTIES AND BIOACTIVE COMPONENTS OF LEAVENED AND UNLEAVENED FLATBREADS SUBSTITUTED WITH GERMINATED MILLET FLOUR **628**

OPTIMISATION OF THE CHEMICAL COMPOSTING OF CATTLE MANURE BY MEANS OF NITRIC ACID **635**

NEW DATA ON DISTRIBUTION OF WULFENIA BALDACCII DEGEN **640**

SOCIO-ECONOMIC IMPACTS OF REDUCING AIR PARTICULATE MATTER (PM10) POLLUTION IN KOCAELI USING BENMAP-CE MODEL **644**

INFLUENCE OF SYNTHESIS METHODS ON STRUCTURAL AND MORPHOLOGICAL PROPERTIES OF MAGNETITE NANOPARTICLES: A COMPARATIVE STUDY OF PRECIPITATION AND SOLVOTHERMAL TECHNIQUES **657**

PHENOTYPIC ANALYSIS OF LEAF SENESCENCE IN ANTIRRHINUM MAJUS L. **666**

REFLECTION OF NATURE ON LIFE: BIOMIMICRY AND SUSTAINABILITY **674**

EXPERIMENTS ON REVEALING EFFECTS OF DIFFERENT MEDIA STRENGTH, AND SUCROSE-DEPENDENT IN ADVENTITIOUS ROOT CULTURES OF RADISH (*Raphanus sativus* L.) **683**

WHAT HAPPENS WHEN PLASTICS ARE DISPOSED INTO THE ENVIRONMENT? MICROPLASTIC IN POLLUTION THE OCEANS **691**

EXAMINING THE EFFECT OF SUSTAINABILITY-FOCUSED CERTIFICATION IN PALM OIL PRODUCTION ON PRODUCER AND CONSUMER AWARENESS **700**

EFFECT OF POROSITY RATIO ON SOUND ABSORPTION IN STRUCTURES PRODUCED WITH 3D PRINTING **707**

PLANT BY-PRODUCTS AS BIOACTIVE GOLDMINES: INSIGHTS FROM SUNFLOWER SEED HUSKS AND POMEGRANATE PEELS **754**

APPLICATION OF SOUS-VIDE PROCESSING ON SELECTED FRUITS AND EVALUATION OF ITS EFFECTS ON QUALITY CHARACTERISTICS **766**

EFFECT OF NANOPARTICLES ON MORPHOLOGICAL TRAITS AND SECONDARY METABOLITES OF SNAPDRAGON 801

EVALUATION OF THE ADHESION STRENGTH OF BIOCERAMIC COATINGS ON LIGHTWEIGHT METALS 809

DEVELOPMENTAL NEUROTOXICITY OF CHLORPYRIFOS IN VIVO: STUDY OF CHOLINESTERASE ACTIVITY AS A BIOLOGICAL INDICATOR 819

BIOACTIVE SYNERGY OF CINNAMON –TURMERIC: IN VITRO EXPLORATION OF ANTIOXIDANT, ANTI-HEMOLYTIC AND ANTI-INFLAMMATORY EFFECTS AND THEIR IMPLICATIONS FOR HUMAN HEALTH 828

NEW APPROACHES FOR ARTIFICIAL INTELLIGENCE-ASSISTED RETINOSCOPES 839

OPTIMIZING GERMINATION OF NIGELLA SATIVA L. WITH GIBBERELLIC ACID AND SEED PRIMING TECHNIQUES 844

SPATIOTEMPORAL PATTERNS OF TRACE ELEMENTS (LI, MN, NI, BA) IN RIVERS FEEDING THE İSTANBUL STRAIT, TÜRKIYE 850

THE EFFECTS OF CHITOSAN AND ESSENTIAL OIL APPLICATIONS ON FRUIT CRACKING PREVENTION AND QUALITY CRITERIA IN 0900 ZIRAAT CHERRY VARIETY (PRUNUS AVIUM. L) 855

INVESTIGATION OF PECTIN AND XANTHAN GUM DEGRADATION BY INTESTINAL BACTERIA 865

ASSESSMENT OF ETHYLENE EFFECTS ON POST-HARVEST CHARACTERISTICS OF CUT SNAPDRAGON FLOWERS 872

ASSESSING THE IMPACT OF GLOBAL WARMING ON SOIL HEALTH AND AGRICULTURAL PRODUCTIVITY 878

SHORT-TERM EFFECTS OF DIFFERENT ORGANIC FERTILIZERS ON SOIL CHEMICAL PROPERTIES AND PLANT GROWTH IN MELON AND WATERMELON CULTIVATION 896

CYTOTOXIC ACTIVITIES OF DIFFERENT EXTRACTS OF *INONOTUS TAMARICIS* (Pat.) *MAIRE* FROM SOUTHWEST ANATOLIA, TÜRKİYE 906

CURRENT STATUS AND POTENTIAL APPLICATION OF MICROALGAE PRODUCTION 911

THE ROLE OF THE WOMEN ON ACHIEVING THE LDN 920

ENDOPHYTIC BACTERIA FROM ECHIUM AMOENUM FISCH & MEY. AS A NEW ELICITOR OF PHENOLIC BIOSYNTHESIS 927

THE IMPACT OF DILL LEAVES ON THE SENSORY QUALITY OF TUNA PATE 933

FROM INVASION TO CONSUMER TABLE: AS A MODEL: LIONFISH 938

USE OF ORIGANUM SPECIES ESSENTIAL OILS IN THE CONTROL OF PLANT DISEASES 941

FROM SOIL TO SURVIVAL: PGPB-TRIGGERED DEFENSE AND ADAPTATION IN PLANTS 950

ASSESSMENT OF CATAGLYPHIS NODUS (BRULLÉ, 1833) IN THE ECOSYSTEMS AROUND NARTA LAGOON AS ENVIRONMENTAL INDICATOR 964

CURRENT STATUS OF SOME FISHERY COOPERATIVES IN GIRESUN (EASTERN BLACK SEA REGION) AND SOCIO-DEMOGRAPHIC CHARACTERISTICS OF ARTISANAL FISHERMEN 971

PHYTOCHEMICAL ANALYSIS AND IDENTIFICATION OF BIOACTIVE COMPOUNDS IN SPINACH LEAVES (*SPINACIA OLERACEA L.*) 977

EFFECT OF SALINITY STRESS ON BIOCHEMICAL, GROWTH, AND YIELD CHARACTERISTICS OF WHEAT 987

ANTIOXIDANT ACTIVITIES OF DIFFERENT EXTRACTS OF INONOTUS CUTICULARIS (BULL. EX FR.) KARST. FROM SOUTHWEST ANATOLIA, TÜRKİYE 998

MACRO ELEMENTAL RISK ANALYSIS OF WATER IN TWO MAJOR DAM LAKES OF THE MARMARA REGION, TÜRKIYE 1004

BLACK CARBON CONTENT IN AIRBORNE PM2.5 DURING THE WINTER BURGAS, BULGARIA 1010

THE CORRELATION BETWEEN BLACK CARBON CONTENT IN AIRBORNE PM2.5, CONCENTRATION OF OTHER AIR POLLUTANTS AND METEOROLOGICAL CONDITIONS DURING THE WINTER – BURGAS, BULGARIA 1021

SUSTAINABILITY ASSESSMENT OF OPEN AND CLOSED SYSTEMS IN MICROALGAE CULTIVATION: A REVIEW 1032

SYNTHESIS AND EVALUATION OF THE ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF AN ESTER DERIVATIVE OF MALONIC ACID 1036

ASSESSMENT OF THE IMPACT OF SURFACE WATER TABLE IRRIGATION ON THE PHYSICOCHEMICAL PROPERTIES OF SOILS IN OUED M'ZAB (ALGERIA) 1044

RESEARCH ON CONVENTIONAL WHEAT CULTIVATION TECHNOLOGY, INCLUDING SOIL TILLAGE AND PHASED FERTILIZATION, APPLIED TO THREE WHEAT VARIETIES UNDER NON-IRRIGATED CONDITIONS, IN THE PEDOCLIMATIC CONDITIONS OF GĂLBINAȘI, BUZĂU COUNTY, DURING THE 2023–2024 AGRICULTURAL YEAR 1053

NUTRACEUTICAL POTENTIAL OF ACHILLEA MILLEFOLIUM. HEALTH BENEFITS, DERMATOLOGICAL USE AND SAFETY 1066

RESEARCH ON CONVENTIONAL WHEAT CULTIVATION TECHNOLOGY, INCLUDING SOIL TILLAGE AND PHASED FERTILIZATION, APPLIED TO THREE WHEAT VARIETIES UNDER NON-IRRIGATED CONDITIONS, IN THE PEDOCLIMATIC CONDITIONS OF GĂLBINAȘI, BUZĂU COUNTY, DURING THE 2023–2024 AGRICULTURAL YEAR 1079

BIOCHEMICAL AND AROMATIC CHANGES IN PERSIMMON (DIOSPYROS KAKI L.) FRUITS HARVESTED IN DIFFERENT PERIODS 1093

PRESENCE OF NOSEMOSIS IN HONEY BEES IN ISPARTA PROVINCE, TÜRKİYE ... 1109

EFFECTS OF CHITOSAN COATED IRON OXIDE AND SILVER NANOPARTICLES ON GROWTH AND BIOCHEMICAL CHARACTERISTICS OF SNAPDRAGON (ANTIRRHINUM MAJUS) 1114

PRODUCTION AND MODES OF ACTION OF BACTERIOCINS PRODUCED BY ENTEROCOCCAL STRAINS 1121

IN VITRO ANTIOXIDANT AND ANTIDIABETIC OF MAESA KAMERUNENSIS MEZ. PLANT EXTRACT 1129

ANTIDIABETIC AND ANTIOXIDANT ACTIVITY OF TWO SAPONINS ISOLATED FROM M. KAMERUNENSIS MEZ. 1135

CORIANDRUM SATIVUM L.: A REVIEW ON BOTANICAL CHARACTERISTICS AND AGRICULTURAL SIGNIFICANCE 1142

MULTI-SITE EVALUATION OF NITROGEN DYNAMICS IN INHIBITOR-ENRICHED UREA APPLICATIONS UNDER FIELD AND CONTROLLED CONDITIONS 1148

PROPOLIS AGAINST BOTRYTIS CINEREA: A REVIEW OF STUDIES COMBINING IN VITRO AND IN VIVO TESTS 1155

IMPACT OF MASTITIS PATHOGENS ON BLOOD ANTIOXIDANT ENZYMES IN TRANSITION DAIRY COWS 1162

A WEARABLE 3D-GLOVE SYSTEM FOR HYPERSPECTRAL AND CONTACT IMAGING OF ROOT AND RHIZOSPHERE MICROBIOMES : A CONCEPTUAL DESIGN 1169

ENVIRONMENTAL IMPACTS OF GREENHOUSE GAS EMISSIONS AND PROPOSED SOLUTIONS FOR THEIR REDUCTION 1178

THE EFFECTS OF GLOBAL WARMING ON THE WATER QUALITY OF LAKES AND RIVERS 1188

EFFECT OF SOME ELICITORS ON TAXOL PRODUCTION IN HAZELNUT CELL SUSPENSION CULTURE 1195

PRESENCE OF *merA* GENE AND MERCURY RESISTANCE AMONG PATHOGENIC BACTERIA 1200

PHENOTYPIC AND GENOTYPIC DETECTION OF BIOFILM PRODUCTION OF *STAPHYLOCOCCUS AUREUS* FROM CHICKEN MEAT 1209

APPROACHES TO OBTAINING OPTIMUM BENEFIT FROM MICROBIAL PRODUCTS USED IN PLANT PRODUCTION 1218

ASSESSMENT OF HABITAT HEALTH IN ÇARDAK LAGOON *USING CARCINUS AESTUARII* NARDO, 1847 AS A BIOINDICATOR 1229

LIGHT-EMITTING DIODE LIGHT QUALITY INFLUENCES GERMINATION, GROWTH AND BIOCHEMICAL INDICES OF *ARNEBIA EUCHROMA* L. 1237

IMPACT OF NANOPARTICLES ON MORPHOLOGICAL TRAITS AND SECONDARY METABOLITES OF SNAPDRAGON 1246

IMPROVING THE EFFICIENCY OF BIO-COAGULATION AND FLOCULATION FOR TURBIDITY CONTROL IN WASTEWATER TREATMENT 1254

EVALUATION OF SUGAR, ORGANIC ACID, AND AROMA PROFILE IN BITTER MELON (*MOMORDICA CHARANTIA* L.) 1261

STUDY OF THE INTERACTIONS OF POLYMERIC BINARY MIXTURES: PHYSICO-CHEMICAL AND RHEOLOGICAL CHARACTERISATION 1275

THE ROLE OF SOIL CONDITIONERS IN CEREAL PRODUCTION 1284

A THERMODYNAMIC STUDY OF A SOLAR ADSORPTION AIR-CONDITIONING SYSTEM 1295

DETERMINATION OF ELEMENTS IN HONEY, HONEYBEE, SUNFLOWER AND BEE DRINKING WATER SAMPLES COLLECTED FROM THE ENEZ DISTRICT, EDIRNE (TURKEY) 1303

GENETIC AND SPECIES DIVERSITY OF FISH IN ALBANIA'S MAJOR LAKES 1323

DETERMINATION OF CHLOROPHYLL CONTENT IN MAIZE AND BEANS (2M+1B) SOWN AT DIFFERENT RATES IN THE SAME ROW 1331

RAPID ASSESSMENT OF THREAT STATUSES OF FISH SPECIES IN DRIN RIVER 1348

INFLUENCE OF TEMPERATURE ON FERMENTATION RATE IN PRODUCTION OF DIFFERENT WINE TYPES 1357

MIGRATION OF FRESHWATER FISHES IN TÜRKİYE AND OBSTACLES ENCOUNTERED BY FISHES DURING MIGRATION 1365

EFFECTS OF BLENDING DIFFERENT RATIOS OF COTTONSEED OIL WITH DIESEL FUEL IN A SINGLE-CYLINDER DIESEL ENGINE 1370

ASSESMENT OF INCEPTISOL AND ENTISOL SOIL CLASSES COMMONLY FOUND IN TURKEY IN TERMS OF FERTILITY 1409

ULTRASONIC EXTRACTION and ANTIOXIDANT ACTIVITY of Elaeagnus umbellata FRUIT 1416

EVALUATION OF THE POTENTIAL FOR RAINWATER HARVESTING IN THE AGRO-INDUSTRY: A CASE STUDY OF THE TABLE OLIVE INDUSTRY 1425

FUNCTIONAL NOODLES ENRICHED WITH BELL PEPPER POWDER: EFFECTS ON COLOR, TEXTURE, AND COOKING QUALITY 1431

EFFECTS OF FUNCTIONAL OILSEED POWDERS ON THE QUALITY ATTRIBUTES OF CRACKERS..... 1440

CARBON FOOTPRINT OF AGRICULTURAL ACTIVITIES AND THE EMISSION REDUCTION POTENTIAL OF WIND ENERGY IN ALIAĞA DISTRICT 1450

THE CARBON BORDER ADJUSTMENT MECHANISM (CBAM) AND TURKISH AGRICULTURE: A SUSTAINABLE TRANSITION 1458

IDENTIFICATION OF GENE REGIONS ASSOCIATED WITH IMPORTANT DISEASES OBSERVED IN TOMATO PLANTS USING MOLECULAR MARKERS..... 1466

MACRO ELEMENTAL RISK ANALYSIS OF WATER IN TWO MAJOR DAM LAKES OF THE MARMARA REGION, TÜRKIYE 1477

PHENOLIC PROFILE OF DIFFERENT PARTS OF INULA GRAVEOLENS DETERMINED BY HPLC-DAD 1483

IN VITRO A-AMYLASE AND A-GLUCOSIDASE INHIBITORY ACTIVITIES *OF INULA GRAVEOLENS* EXTRACTS 1493

KNOWLEDGE ABOUT THE GENETICAL, MORPHOLOGICAL AND PHENOLOGICAL DIVERSITY OF THREE LINDEN SPECIES (*TILIA CORDATA* MILL., *TILIA PLATYPHYLLOS* SCOP. AND *TILIA TOMENTOSA* MOENCH.) IN BOSNIA AND HERZEGOVINA AND POTENTIAL FOR BETTER MANAGEMENT OF LINDEN FORESTS 1500

SOURCES OF INSTITUTIONAL SUPPORT FOR SUSTAINABLE MANAGEMENT OF TREES FROM THE GENUS Tilia sp. IN BOSNIA AND HERZEGOVINA 1517

CORRELATION OF BIOMARKERS OF COMBINED STRESS TOLERANCE IN EGGPLANTS AND EVALUATION WITH POLAR HEATMAP 1531

A RESEARCH ON IN VITRO MICROPROPGATION OF GERANIUM (Pelargonium graveolens) 1539

A RESEARCH ON IN VITRO MICROPROPGATION OF GINGER (Zingiber officinale Roscoe) 1547

AI AGAINST HONEY ADULTERATION: EFFECTIVE AND RELIABLE HONEY BOTANICAL ORIGIN AUTHENTICATION 1554

CONSERVED MOTIFS IDENTIFICATION OF THIONIN PROTEIN OF TWENTY-SIX PLANT SPECIES 1566

BIOINFORMATICS STRUCTURAL ANALYSIS OF THIONIN PROTEIN IN SIX PLANT SPECIES OF BRASSICACEAE FAMILY 1572

SUSTAINABLE TOURISM IN TÜRKIYE AND THE BALKAN COUNTRIES: POLICY EVOLUTION AND KEY CHALLENGES 1578

SMART MATURITY MONITORING IN OLIVE ORCHARDS: UTILIZING VIS/NIR TOOLS FOR OPTIMAL HARVEST DECISION-MAKING IN ALBANIA 1587

COMPLIANCE OF ALBANIAN FISHERIES LEGISLATION WITH EU REGULATIONS: A CRITICAL ASSESSMENT AND HARMONIZATION PERSPECTIVE 1598

IMPLEMENTATION OF A SMART IRRIGATION SYSTEM FOR WATER AND ENERGY WASTE REDUCTION USING ARDUINO 1605

FIRST RECORD OF A PROTIST PATHOGEN OF *Labidostomis rufa* (COLEOPTERA: CHRYSOMELIDAE) (WALTL, 1838) IN ISPARTA, TÜRKİYE 1613

VIABILITY AND VIGOUR OF CARROT (DAUCUS CAROTA L.) SEEDS IN DIFFERENT FERTILIZATION REGIMES 1618

MORPHOLOGICAL AND DECORATIVE EVALUATION OF NEW, PROMISING GLADIOLUS VARIETIES 1625

MONITORING OF THE GREENHOUSE WHITEFLY (TRIALEURODES VAPORARIORUM) IN THE LUSHNJA DISTRICT 1632

INTEGRATED CONTROL OF THE CODLING MOTH (*CARPOCAPSA POMONELLA*) IN THE KORCA REGION 1636

RADIOGRAPHIC EVALUATION OF THE ROLE OF TITANIUM SCAFFOLD POROSITY ON BONE REGENERATION AND IMPLANT STABILITY IN TTA 1641

OXIDANT STATUS OF SPIRULINA EXTRACTS 1649

RESEARCHING COGNITIVE TECHNOLOGIES IN BROADCAST OPERATIONS 1654

THE DEVELOPING NEW BREAD WHEAT VARIETIES WITH EVALUATING YIELD AND QUALITY TRAITS IN EDIRNE 1666

AGBIOL 2025 CONFERENCE STUDENT ORGANIZING TEAM 1674

OUR SPONSORS 1675

EFFECT OF IRON FOUNDRY DUST ON THE ENERGY EFFICIENCY OF HYDROPHILIC NANOCOATED PHOTOVOLTAIC PANELS

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ABSTRACT

In this study, the impact of dust accumulation an important factor affecting the efficiency of photovoltaic (PV) panels on energy production was experimentally investigated, with a specific focus on hydrophilic nanocoating and iron foundry dust. Two equivalent 100W PV panels were installed on the campus of the Faculty of Engineering at Trakya University. One of the panels (PV Panel A) was treated with a hydrophilic nanocoating, while the other panel (PV Panel B) was left untreated in its standard state. The experimental study was conducted over a five-week period from April 22 to May 27, 2025. During this time, the energy output of both PV panels was continuously recorded. According to the analysis of the experimental results, the total energy production of the hydrophilic nanocoated PV Panel A was measured as 13,485.6 Wh, whereas the standard PV Panel B produced a total of 10,197.0 Wh. It was observed that PV Panel A, with the hydrophilic nanocoating, generated 3,288.6 Wh (32.3%) more energy than PV Panel B during the test period. At the conclusion of the study, energy production graphs for both PV panels were plotted, and relevant analyses were presented.

Keywords: Energy, Photovoltaic Panel, Hydrophilic Nanocoating, Iron Foundry Dust

INTRODUCTION

In recent years, due to climate change, energy crises, economic concerns, and environmental issues, there has been a significant shift toward renewable energy sources for electricity generation. With technological advancements and the continuously growing global population, the demand for energy is increasing rapidly. Among alternative energy solutions to conventional sources such as oil, coal, and nuclear power, solar energy stands out as a promising option for the future. However, not all of the solar radiation reaching the Earth can be utilized for electricity generation through photovoltaic (PV) panels. Incoming solar radiation, which spans a wide spectrum, is divided into direct, diffuse, and reflected components due to atmospheric interactions. Approximately 30% of this radiation is reflected back by the atmosphere. Even under completely clear skies, only about 80% of the incoming radiation reaches the Earth's surface. On average, only about 50% of solar radiation is usable under normal daily conditions [1]. Several factors affect the efficiency of converting solar radiation into electrical energy through the photovoltaic effect. These include the inability of solar cells to fully absorb the radiation, the partial usability of photon energy, increased cell temperature, shading effects on the modules, and surface contamination of the module cells [1]. These

efficiency-reducing factors have long been the subject of extensive research, and alternative solutions continue to be sought. One of the key issues is dust accumulation on the PV panel surface and the resulting degradation of cell performance [2]. The reduction in energy efficiency of PV panels varies depending on the amount and type of dust accumulated on their surfaces. While many studies have focused on the general performance impact of dust on PV panels, relatively few have examined how different types of dust affect performance [2]. The extent of dust pollution varies significantly depending on the geographic location where solar energy systems are installed [3]. A variety of pollutants from different sources are encountered in urban and rural environments. For instance, particles such as limestone, ash, soil, sand, and general dust are transported differently depending on the region [4–5]. Additionally, dust accumulation becomes a significant issue in urban areas due to air pollution and increased industrial activity [6]. Dust and particulates released from fossil fuel combustion and construction activities lead to a reduction in solar irradiance. Consequently, this decline in irradiance negatively affects voltage and current values, resulting in a decrease in PV panel performance [7]. Moreover, atmospheric deposits such as dust, air pollution, sea salt, and organic residues, in combination with meteorological conditions like wind speed, temperature fluctuations, humidity, snowfall, and hailstorms, can further reduce system efficiency [8–11]. Studies in the literature investigating the effects of dust and surface contamination on PV panel performance have particularly focused on environmental factors such as wind, precipitation, temperature, humidity, and solar radiation. It has been found that the smaller the dust particle size, the greater the shading effect on PV panels. Higher solar radiation, wind speeds, and atmospheric humidity also lead to thicker dust accumulation on PV surfaces, thereby reducing efficiency. On the other hand, some surface cleaning occurs naturally following rainfall, and steeper panel tilt angles facilitate the removal of dust from surfaces [12].

In another study, the accumulation of dust on PV panels under the influence of wind barriers was analyzed using computational fluid dynamics (CFD) simulations. Variables such as dust particle size, wind barrier height, and porosity were considered. Shear stress transport models were used to simulate airflow behavior around PV systems with wind barriers. The results showed that as the wind barrier height increased, the rate of dust accumulation on PV panels decreased to 8.62%, 7.98%, 7.54%, 7.27%, and 6.47%, respectively. The study identified the optimal wind barrier height as 2.5 meters [13]. A recent study conducted in Iraq investigated the impact of dust particles on PV panel performance. The experimental study was carried out at the University of Technology in Baghdad from March 15 to May 15, 2024. When dust density reached 10 g/m², the power output of the PV panel decreased by 34%. Furthermore, a nonlinear relationship was observed between the fill factor (FF), dust density, and conversion efficiency. Under a constant irradiance of 750 W/m², the PV panel efficiency dropped from 19.8% to 6.3%. Based on the study's findings, dust accumulation was found to reduce PV efficiency by 7.8% to 19.2%. The research emphasized the importance of developing effective cleaning strategies, especially in regions like Iraq where high dust levels and extreme temperatures are common [14].

Unlike the aforementioned studies, the present research involved the installation of a solar energy conversion system consisting of two PV panels with equivalent output power on the campus of the Faculty of Engineering at Trakya University. A hydrophilic nanocoating was applied to the surface of one panel to prevent dust accumulation, while the other panel was left in its standard state. For the experimental procedure, industrial iron foundry dust was uniformly distributed over the surface of all cells on both PV panels. Over a continuous five-week period, the voltage and current values of both panels exposed to iron foundry dust were recorded, and energy output was calculated and analyzed accordingly.

MATERIALS AND METHODS

A solar energy conversion system was installed on the south-facing side of the Engineering Faculty campus at Trakya University, as shown in Figure 1. The experimental setup consists of two polycrystalline photovoltaic panels, each with an equivalent output power of 100 W, along with a data recording system to monitor and analyze energy production values. The technical specifications of the photovoltaic panels used in the solar conversion system are presented in Table 1. Before the start of the experiment, both PV panels in the test setup were washed with water and allowed to dry completely. The initial conditions of the PV panels and the experimental setup are illustrated in Figure 1.

Figure 1. Experimental Setup

The technical specifications of the hydrophilic nanocoating applied to the surface of PV Panel A are presented in Table 1.

Table 1. Technical specifications of the nano coating material

Feature	Value
Density	0.81 g/ml
Viscosity	1.2 cP
Boiling point	78 ºC
Ignition point	≤16.6 ºC
Active components	<5% wt
Volatile Organic Content (VOC)	>85% wt
Color	Whitish transparent/translucent
Solvent	Ethanol

After the PV panel surfaces dried, a hydrophilic nanocoating in liquid form was manually applied to the surface of PV Panel A using a sponge. The properties of this liquid coating material are presented in Table 2. No treatment was applied to the surface of PV Panel B, which

was left in its standard condition. Subsequently, industrial iron dust obtained from a manufacturing site in an industrial region was uniformly sprinkled over all the cells on the surfaces of both PV panels. During this process, the tilt angle of the PV panels was fixed at 10° to ensure that the iron foundry dust would remain on the panel surfaces for a longer period without falling off.

Table 2 Technical specifications of solar PV module

Feature	Value
Brand and Module Cell type	Tommatech Pollycrystalline
Maximum Power (Pmax)	100Wp
Voltage at maxime Power (Vmp)	19.73V
Current at Maximum Power (Imp)	5.58A
Open Circuit Voltage (Voc)	23.22V
ShortCircuit Current (Isc)	5.81A
Maximum System Voltage	1000V
Maximum Series Fuse Rating	6.%A

Figure 2 illustrates the application of iron foundry dust onto the photovoltaic cells of PV Panel A and PV Panel B.

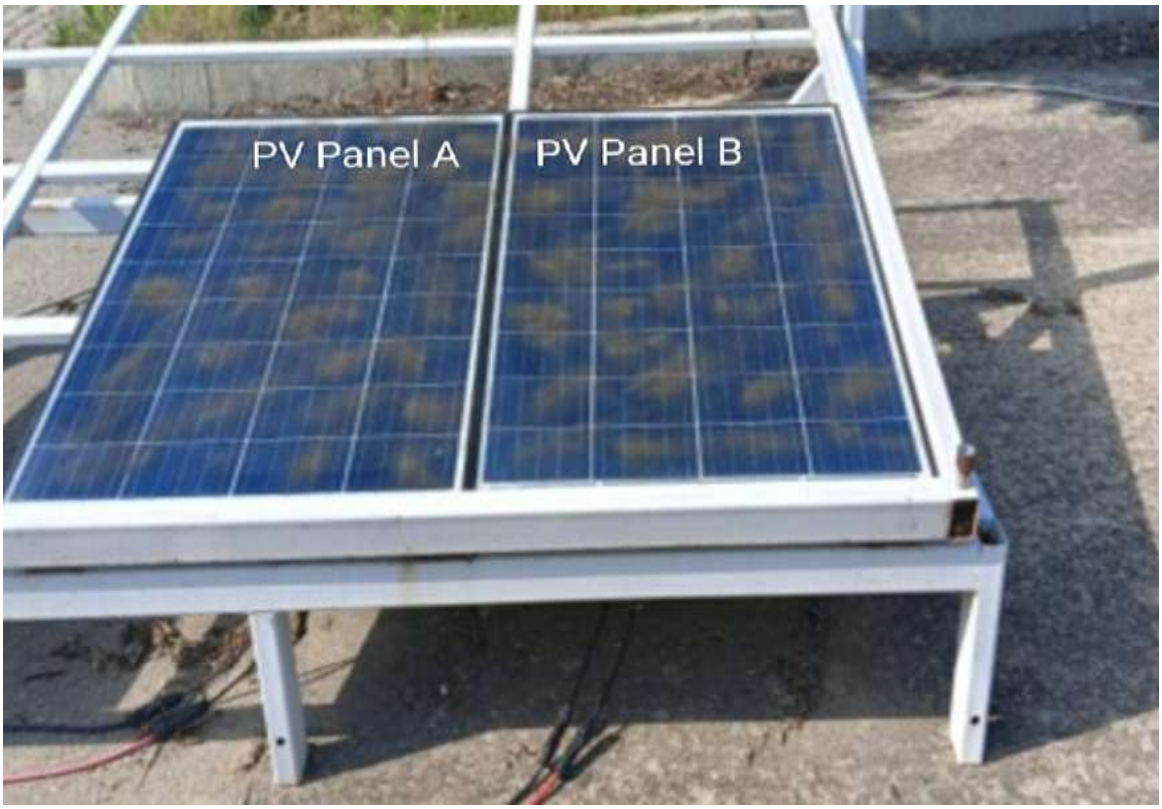

Figure 2. Iron Foundry Dust Deposited on the Surfaces of PV Panels

Following the application of iron foundry dust shown in Figure 2, voltage and current values produced by the PV panels were recorded daily from 07:00 AM to 07:00 PM over a period of five weeks. The control panel of the data acquisition device used for recording the data is shown in Figure 3.

Figure 3. Datalogger Used for Data Acquisition in the Experimental Setup

At the conclusion of the experimental study, the recorded voltage and current data were retrieved from the memory card of the system, and the energy output of the PV panels was calculated. Subsequently, graphs illustrating the power output and energy production of the PV panels were plotted.

RESULTS AND DISCUSSION

During the five-week experimental study, a random day May 11, 2025 was selected to capture instantaneous images of the photovoltaic surfaces of PV Panel A and PV Panel B, which are presented in Figure 4.

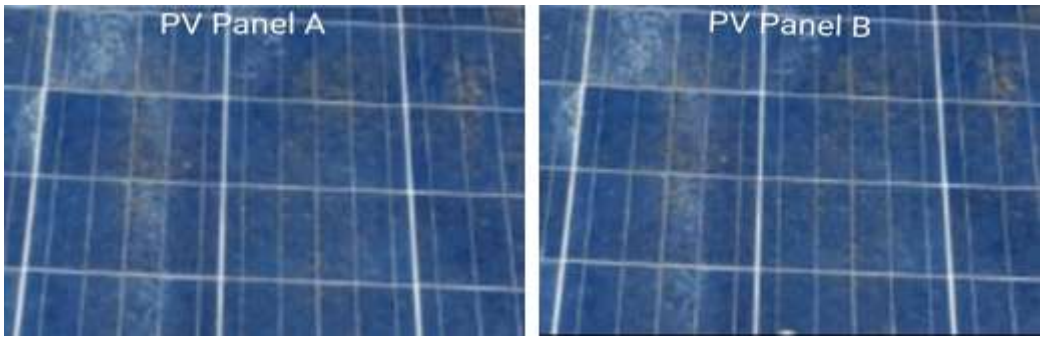

Figure 4. Surface Images of the PV Panels

During the experiments, May 11, 2025 was selected as a random day to capture images of the PV panel surfaces. As shown in Figure 4, a higher concentration of iron foundry dust was observed on the surface of PV Panel B, which was not treated with hydrophilic nanocoating. Under these conditions, the power output of PV Panel B was found to be lower, as illustrated in Figure 5.

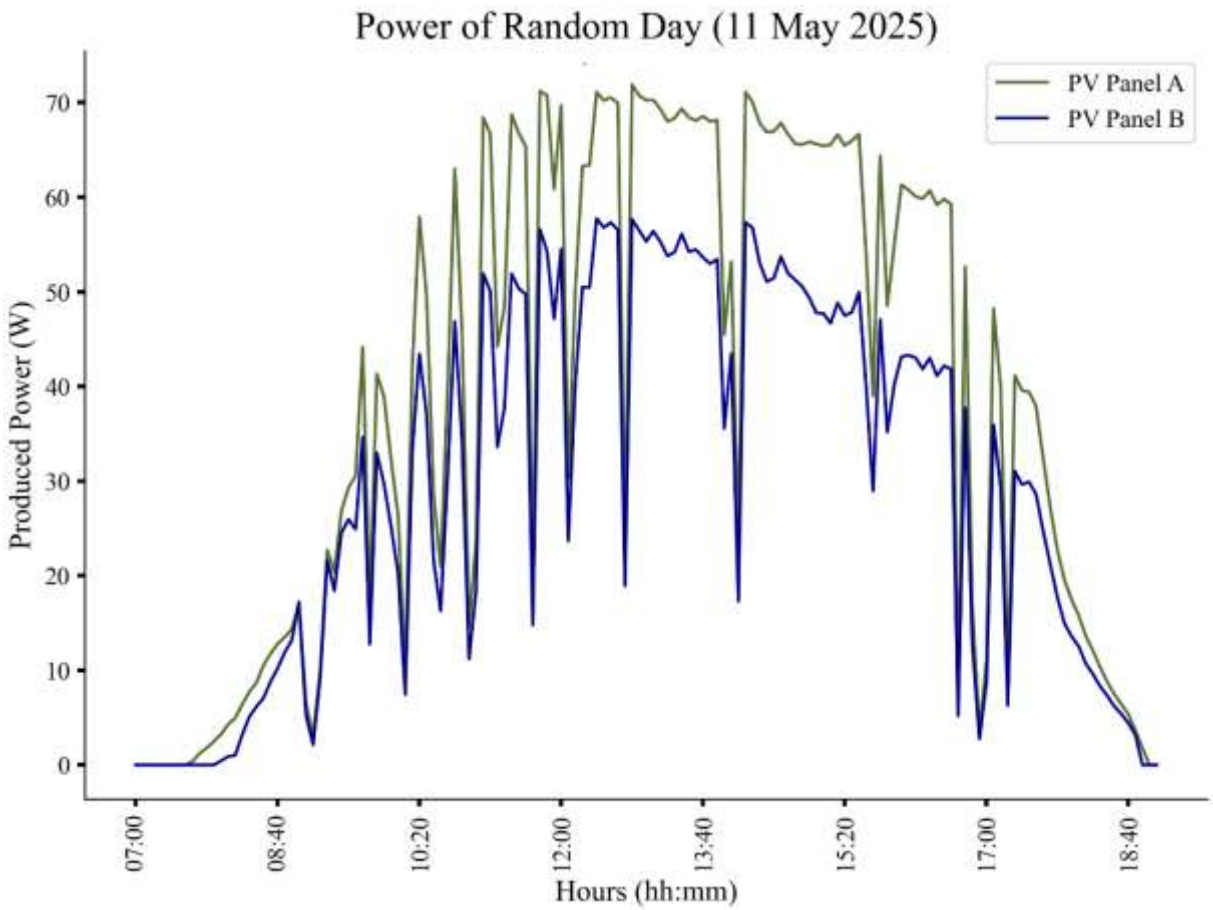

Figure 5. Comparison of PV Panels' Power Output on a Random Day

As observed in Figure 5, PV Panel A, which was coated with the hydrophilic nanocoating, generated higher power throughout the day. Notably, between 12:00 and 15:00 when solar irradiation was most intense PV Panel A produced a peak power output of 70 W. During the same period, the power output of the standard PV Panel B did not exceed 55 W.

Similarly, Figure 6 shows that, consistent with the randomly selected day, the power output of PV Panel A remained higher throughout the day. Analysis of the experimental results identified May 26, 2025 as the day with the highest power output for both PV panels.

As shown in Figure 6, on May 26, 2025, during the period of highest solar irradiation between 12:00 and 15:00, PV Panel A produced power close to 80 W. In contrast, the standard PV Panel B achieved a maximum power output of 65 W during the same time interval. These results indicate a significant decrease in power output for PV Panel B, which was not treated with the hydrophilic nanocoating.

Analysis of the experimental data revealed that the lowest power output from the PV panels occurred on May 16, 2025. As observed in Figure 7, even on the day with the lowest output, PV Panel A with the hydrophilic nanocoating maintained a higher power output compared to PV Panel B.

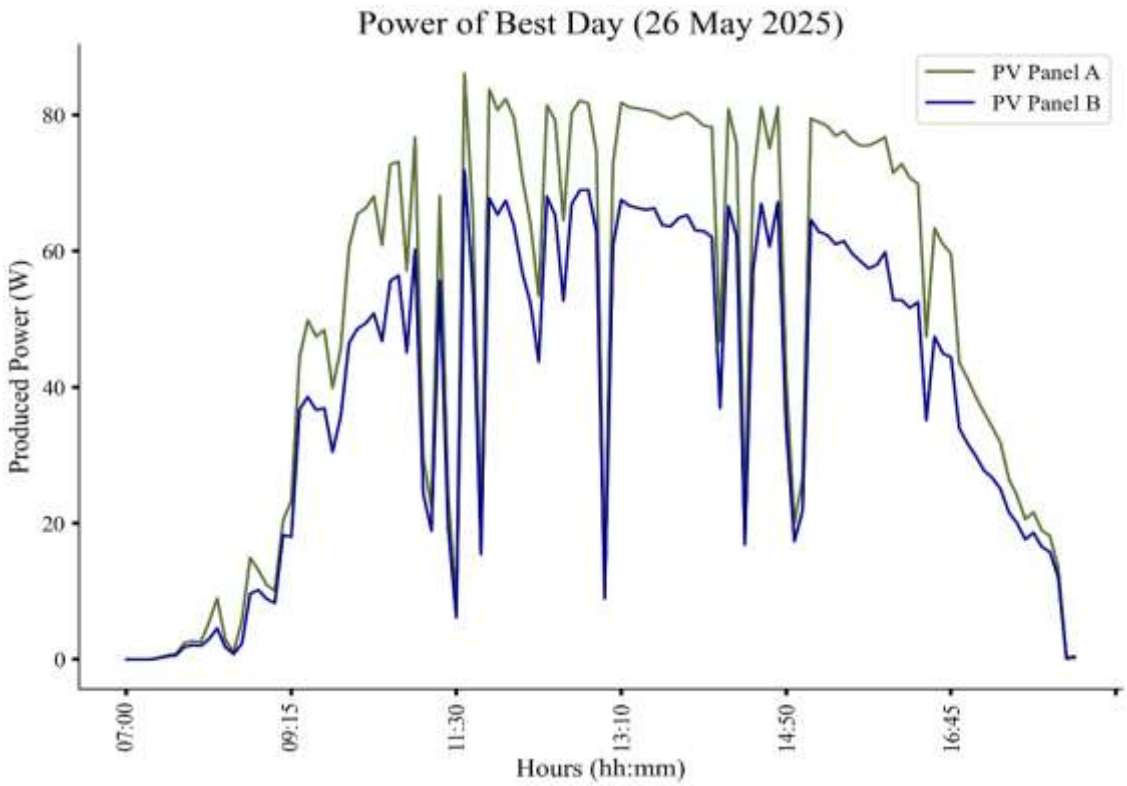

Figure 6. Comparison of PV Panels' Power Output on the Day of Maximum Power Generation

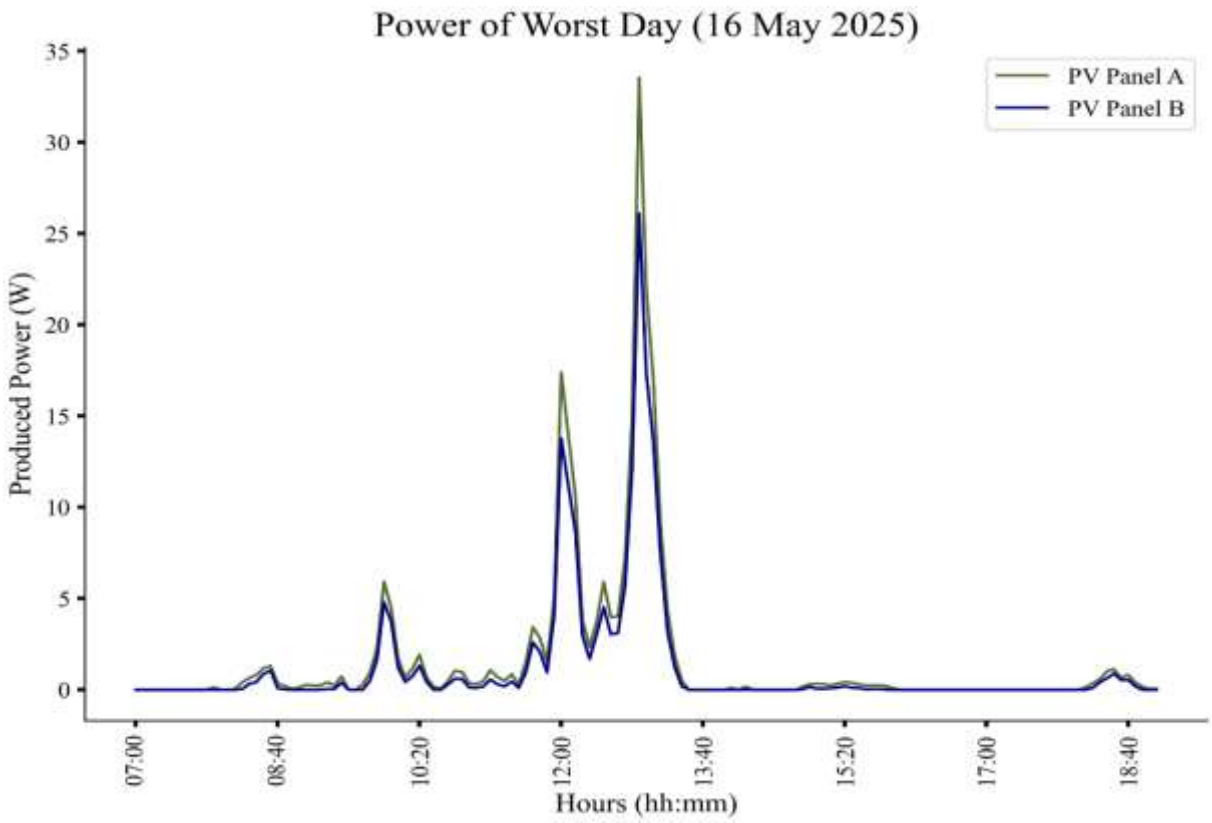

Figure 7. Comparison of PV Panels' Power Output on the Day of Minimum Power Generation

As observed in Figure 7, on May 16, 2025, solar irradiation was low during the day, with insufficient sunlight recorded particularly between 12:00 and 13:40. Around 13:00, PV Panel A produced an instantaneous power output close to 35 W. During periods of low irradiation, even PV Panel A, which was treated with hydrophilic nanocoating, was unable to deliver sufficient power output. As shown in Figure 8, throughout the entire experimental period from April 22, 2025, to May 27, 2025, PV Panel A consistently generated more energy compared to the standard PV Panel B.

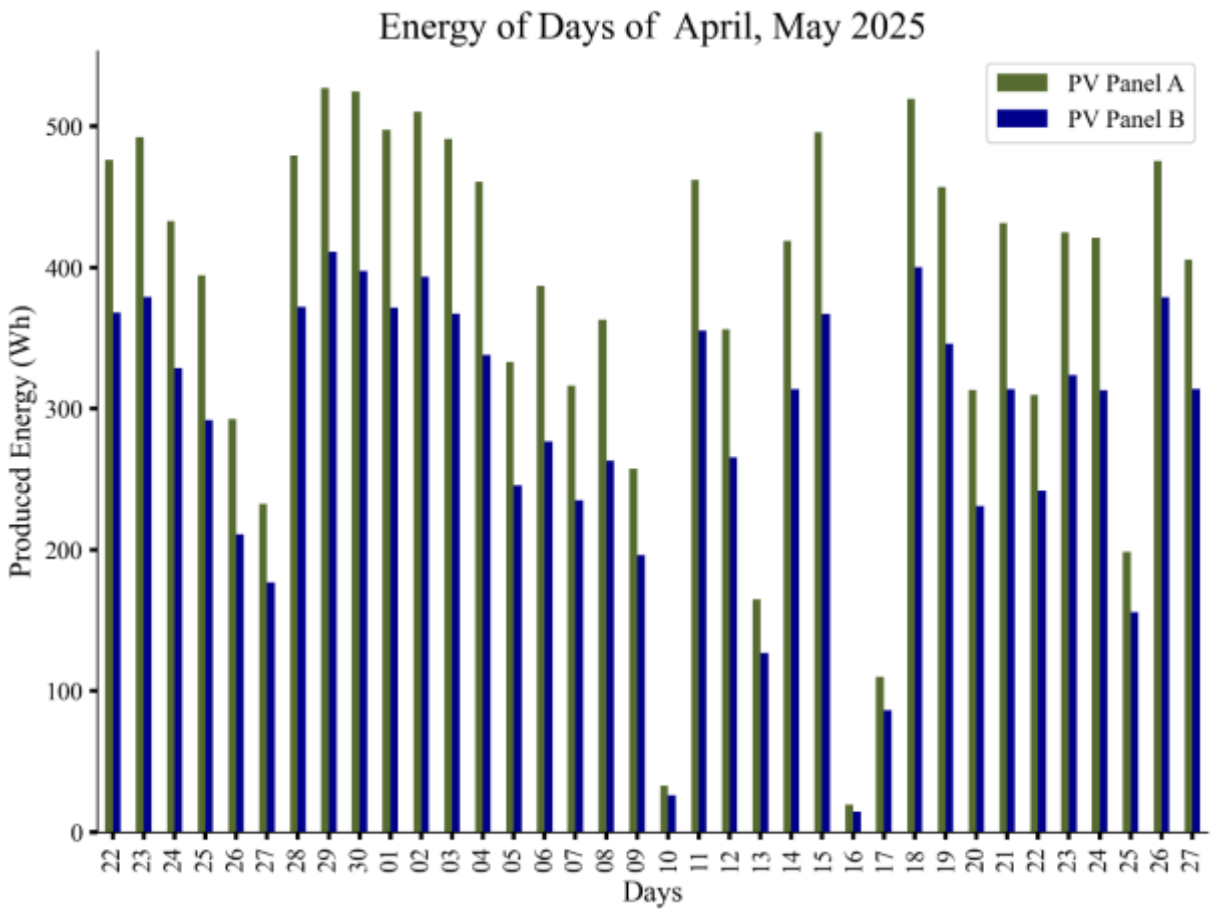

Figure 8. Comparison of Daily Total Energy Output from the PV Panels

As shown in Figure 8, on May 10, 13, 16, and 17, solar irradiation levels were very low, resulting in insufficient energy generation from both PV panels. Based on these results, it was determined that the application of the hydrophilic nanocoating on PV Panel A improved energy efficiency.

CONCLUSION

In this study, the effect of dust accumulation, which significantly reduces the energy output of photovoltaic (PV) panels, was investigated with a focus on hydrophilic nanocoating and iron dust. Throughout all experimental days, the PV Panel A treated with hydrophilic nanocoating generated more energy than the untreated PV Panel B. Analysis of the results showed that, under the same conditions and exposure to iron dust, PV Panel A produced 32.3% more energy compared to the standard PV Panel B.

On the day of highest energy production, May 26, 2025, PV Panel A generated 475.4 Wh of energy, while PV Panel B produced 378.7 Wh. PV Panel A with hydrophilic nanocoating produced 96.7 Wh (25.5%) more energy than the standard PV Panel B on this day. On the day

of lowest production, May 16, 2025, energy outputs of 19.5 Wh and 14.6 Wh were recorded for PV Panel A and PV Panel B, respectively. PV Panel A generated 4.9 Wh (33.6%) more energy than PV Panel B on that day. Over the entire recording period, the total energy production of the hydrophilic nanocoated PV Panel A was 13,485.6 Wh, compared to 10,197.0 Wh for the standard PV Panel B. This corresponds to an increase of 3,288.6 Wh (32.3%) in total energy output for PV Panel A relative to PV Panel B throughout the recording period.

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TECHNICAL AND ECONOMIC ANALYSIS OF THE SOLAR ENERGY SYSTEM DESIGNED FOR EDIRNE PROVINCE AGRICULTURAL RESEARCH INSTITUTE BUILDING IN THE CASE OF ROOF AND FACADE APPLICATION

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ABSTRACT

Solar energy is considered a sustainable resource due to its infinite availability, safety, and the fact that it does not emit greenhouse gases into the atmosphere. Numerous academic studies have been conducted on photovoltaic (PV) panels used in electricity generation from solar energy. While rooftop installations are the most common applications in buildings, interest in alternative installations such as balcony railings, terrace shading systems, and facade cladding is increasing due to various constraints and site-specific conditions. In high rise buildings in particular, limited roof space or an unfavorable roof orientation makes PV facade applications a viable alternative. However, for such applications, accurately modeling the solar energy conversion system and conducting thorough technical and economic analyses are of critical importance. Therefore, before installation, the system should be modeled to analyze investment cost, energy efficiency, and payback period. One of the most commonly used software tools for modeling and simulating solar energy systems is PV*Sol. In this study, a 75-kW self consumption solar energy system was designed and analyzed using PV*Sol software, based on the location data of the Agricultural Research Institute Building in Edirne, Turkey. Two scenarios were evaluated: installation on the South facing roof and, on the South, facing facade of the building. The results show that for both applications, the total investment cost is ₺2,235,600. The payback period is 7.9 years for the rooftop installation and extends to 8.5 years for the facade application. Additionally, it was determined that the rooftop system would prevent approximately 49.244 kg of CO_2 emissions annually.

Keywords: Energy, Solar Energy, PV*Sol Software, PV Panel, Sustainability

INTRODUCTION

In many countries around the world, the continued reliance on fossil based energy sources leads to environmental pollution, economic challenges, and climate change. Therefore, the transition to renewable energy and the diversification of energy sources have become increasingly important. Solar energy is widely preferred due to its inexhaustible nature, cleanliness, and the absence of harmful emissions. However, before installing a solar energy system, it is crucial to select appropriate system components based on the specific site conditions and to conduct thorough technical and economic analyses. Various simulation programs are used for this purpose, offering results that closely approximate real world values. One of the key parameters influencing these analyses is the proper positioning of photovoltaic

(PV) panels. As is well known, PV panels in solar power plants in Türkiye are typically installed facing south at an optimal tilt angle based on the location's geographical characteristics. However, in certain cases such as when the roof orientation is unsuitable, the available roof area is insufficient, or shading is present installing PV panels vertically on the South facing facade of a building becomes a viable alternative. Vertical PV panels on building facades are particularly suitable for high rise structures [1–2]. Moreover, the application of solar energy systems in the construction sector plays a significant role in urban transformation projects [3–4]. The depletion of fossil fuel reserves and the rising cost of energy have further increased interest in renewable energy sources in many parts of the world [5–6–7]. Integrating PV panels into the facades of tall buildings provides substantial surface area for energy generation [8]. However, conventional silicon based PV panels are often heavy, which poses challenges for vertical facade installations and reduces their adoption [8–9].

A review of the literature reveals several studies aimed at improving the energy efficiency of building integrated photovoltaic (BIPV) systems. In one approach, phase change materials and carbon foam were applied to the rear surface of facade mounted PV panels, resulting in an energy efficiency improvement of between 12.64% and 27.35% [10]. In two case studies conducted in Lisbon, Portugal, predictive models were developed to estimate the potential of rooftop and facade PV panels using light detection and ranging (LiDAR) data and local meteorological records. According to the analyses, PV installations on roofs and facades could meet approximately 50% to 75% of the total electricity demand [11]. In a modeling and simulation study where the parameters limiting the integration of photovoltaic (PV) panels were categorized into three groups facade functionality, suitability, and surrounding obstructions it was determined that approximately 31% of the total vertical façade area is suitable for PV system installations. Although there are certain constraints regarding material selection and geometric uncertainties in façade integrated PV systems, the study emphasized that such installations are necessary for urban renewal projects [12]. In another simulation study, the potential for installing solar energy systems on the rooftops and facades of buildings in densely populated areas of Singapore was analyzed. The structural characteristics of the buildings, the distance between buildings, and plot dimensions were examined for their impact on rooftop and façade installations. Simulations were conducted for 12 existing districts in Singapore. The results suggested that standardizing roof shapes, façade orientations, and building heights could increase the feasibility of PV installations and reduce shading effects [13].

In Nigeria, a study focused on reducing energy consumption through PV panel installations on the façades of institutional buildings. The research investigated façade design, the selection of suitable solar panels, and the choice of façade wall materials. It was concluded that thin film and monocrystalline PV panels are suitable for façade applications. Moreover, the integration of PV systems into façades was found to offer not only renewable energy generation but also thermal benefits that contribute to the building's heating requirements [14]. Another study analyzed key parameters such as heat transfer across the façade, cooling demand, and daylight utilization. It was found that more than 30% of the building area had a daylight factor ranging between 1.0% and 3.5%. From an economic perspective, it was observed that the payback period of double laminated monocrystalline PV glass is relatively long [15]. In a recent study, the design and retrofitting of solar energy systems in existing buildings were examined by quantitatively investigating the relationships between PV capacity, tilt angles, and resulting building energy consumption. Using a numerical simulation tool, the fluctuations in indoor temperatures throughout the year, changes in energy consumption, and PV power generation potential were thoroughly evaluated both before and after retrofitting. The findings showed that all improvement scenarios led to reductions in indoor temperatures and energy consumption

during the summer months. Additionally, this configuration was projected to reduce carbon dioxide (CO_2) emissions by 128.90 tons per year through energy savings in existing buildings [16].

In contrast to previous studies in the literature, this study presents a technical and economic analysis of vertically mounted photovoltaic panels (at a 90° angle) on the south-facing external wall of a building, specifically in cases where there is insufficient space on the south facing roof for solar energy system installation.

MATERIALS AND METHODS

This study aims to evaluate the energy production and economic performance of photovoltaic (PV) panels to be integrated into the roof and south-facing façade of the Agricultural Research Institute building located in Edirne, Türkiye. The solar energy system, with a planned output capacity of 75 kW, was designed using the PV*Sol simulation software. The system was configured using 108 CWT690 monocrystalline PV modules, each with a capacity of 690 W, along with one SUN2000-60KTL-M0 inverter and other system components. For the simulations, the tilt angle was set to 32° for the rooftop installation and 90° for the south facing vertical facade, based on the site's geographic location. A visual representation of the installation site is shown in Figure 1. The study focuses on a grid connected self-consumption PV system, in which panels are mounted both on the roof and vertically on the façade. Energy efficiency under both scenarios was examined in detail. Based on the results of the simulation analyses, the total annual electricity generation from the rooftop mounted and vertically façade-integrated PV panels was calculated and illustrated graphically.

Figure 1. Location view of the Agricultural Research Institute Building [17].

For the simulation analysis, the building location shown in Figure 1 was first selected, followed by the configuration of other system components. Additional system specifications are presented in Table 1.

Table 1. PV Generator Module Area [17].

	Module Area
PV Modules	108 x CWT690-132TNB12-V (v2)
Manufacturer	CW Energy Engineering Trade and Industry Limited Company
Tilt Angle (°)	32 / 90
Azimuth Angle (°)	Southwest 225
Mounting Type	Roof-integrated rear ventilation / Wall-integrated rear ventilation
PV Generator Area (m²)	335,5
Shading (%)	0

As shown in Table 1, a surface area of 335.5 m² was utilized for the arrangement of photovoltaic panels.

RESULTS AND DISCUSSION

Based on the components of the solar energy conversion system designed for the Agricultural Research Building, the electricity production estimates obtained from the PV Sol simulation software are presented in Table 2.
In the case of implementing a grid-connected, self-consumption solar energy conversion system on the building's roof, an annual electricity production of 1406 kWh would be achieved, as shown in Table 2.

Table 2. Production Estimate [17].

	Production Estimate (South, Roof)	Production Estimate (South, Wall)
PV generator output (kWp)	74,52	74,52
Annual Specific Energy Yield (kWh/kWp)	1406.00	926.10
System Performance Ratio (PR) (%)	89,06	88,83
Electricity Exported to the Grid (kWh/year)	104,786	69,024
rid Feed-in During the First Year (Including Module Performance Degradation) (kWh/Year)	104,786	69,024
Consumption in Standby Mode (Inverters) (kWh/Year)	11	11
Prevented CO_2 Emissions (kg/year)	49,244	32,436

As shown in Table 2, when photovoltaic panels are integrated into the south-facing wall surface of the building instead of being installed on the roof, the annual electricity production decreases to 926 kWh. Along with the reduction in electricity generation due to the integration of photovoltaic panels into the building wall, the amount of prevented carbon dioxide (CO_2) emissions also decreases.
The financial analysis results of two different design implementations, installed with the same investment cost, are presented in Table 3. When the solar energy conversion system is applied to the roof, the payback period is 7.9 years, whereas integrating the system into the building's south-facing wall increases the payback period to 8.5 years.

Table 3. Financial Analysis [17].

	Gain from Roof Application	Gain from Wall Application
Total Investment Cost (₺)	2,235,600.00	2,235,600.00
Internal Rate of Return (IRR) (%)	59,46	55,53
Payback Period (Years)	7,9	8,5
Electricity Production Cost (₺/kWh)	1.1824	1,7951
Energy Balance / Grid Feed-in Concept	Full Grid Feed-in	Full Grid Feed-in

As shown in Table 3, the unit cost of electricity generated is lower for the roof installation, depending on the system design. However, despite these advantages, certain practical challenges encountered in roof applications such as insufficient space and the absence of a south-facing roof indicate that photovoltaic panels may be integrated into the building's south-facing wall instead.

Based on the simulation results, the total energy yield from the solar energy system is estimated to be 104,786 Wh for the roof installation (with a south orientation of 32°) and 69,024 Wh for the integration on the south-facing wall (90° orientation).

CONCLUSION

In this study, a 75 kW self-consumption solar energy conversion system for the Agricultural Research Institute Building in Edirne Province was designed using PV Sol simulation software, considering both the south-facing roof installation and integration on the south-facing wall surface, followed by technical and economic analyses. For both designs, the investment cost was 2,235,600 TRY. The payback period for the roof installation was found to be 7.9 years, whereas the integration of photovoltaic panels on the building's south facing wall extended this period to 8.5 years. Moreover, the unit cost of energy production was determined to be lower for the roof application. Additionally, it was observed that implementing the solar energy system on the roof would prevent approximately 49,244 kg of CO_2 emissions annually. Despite these advantages, when challenges such as unsuitable south orientation of the roof for mounting, insufficient roof area, and shading are encountered, integrating photovoltaic panels perpendicularly on the building's south facing wall emerges as a viable alternative solution.

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IMPACT OF REGULAR WATER-BASED CLEANING ON THE ENERGY EFFICIENCY OF PHOTOVOLTAIC PANELS

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ABSTRACT

Solar energy is abundant in many countries worldwide and is easier to use compared to other renewable energy sources. However, the overall conversion efficiency of photovoltaic (PV) panels used for electricity generation from solar energy remains relatively low. Maximizing the efficiency of solar energy systems requires maintaining the cleanliness of PV panel surfaces. In this study, the effect of periodic cleaning of PV panel surfaces with only water on energy efficiency was investigated. An experimental solar energy conversion system was installed on the campus of Trakya University Faculty of Engineering. The setup included two polycrystalline PV panels (PV A and PV B), each with a nominal output power of 260 W. Initially, both PV panels were cleaned. Subsequently, PV A was left uncleaned, while PV B was washed with only water at 30 day intervals. Daily energy production data from both panels were recorded over a five month period. At the end of this period, the energy output of PV B, which was cleaned monthly with water, was approximately 3% higher than that of the uncleaned PV A. The experimental results indicate that washing PV panels with water every 30 days can improve energy efficiency. Future studies are expected to build upon these findings by comparing more frequent cleaning intervals to evaluate their effects on energy performance in greater detail.

Keywords: Energy, PV panel, Water cleaning, Efficiency

INTRODUCTION

In recent years, electricity generation from solar energy has become increasingly preferable among renewable energy sources. Compared to other alternative energy systems, solar energy systems are expected to become even more widespread in the future due to their advantages such as ease of installation, the absence of moving parts, economic feasibility, and long operational lifespan. While environmental concerns related to the use of fossil based energy sources persist globally, solar energy is considered a promising solution for electricity generation [1–3]. It is estimated that by the year 2050, photovoltaic (PV) panels will contribute approximately 25% of the world's total electricity production [4]. Despite these developments, the conversion efficiency of photovoltaic panels remains relatively low under current conditions. Furthermore, depending on operational factors, this efficiency may decline even further. The accumulation of dust and dirt on PV panel surfaces significantly affects the system’s energy efficiency. Dust particles on the panel surface not only block light from reaching the solar cells but also lead to an increase in cell temperature. As a result of these adverse conditions, energy losses increase, reducing the output power of the PV panel. Global case studies have shown that coal dust (up to 64%), fine sand (32%), and gypsum dust (30%) can reduce energy output significantly [1]. On the other hand, although the efficiency of PV panels varies depending on production technology, factors such as solar irradiance, ambient temperature, wind speed, and tilt angle also contribute to performance losses [5–6]. After the

effects of solar irradiance and temperature, dust accumulation is the most significant factor causing a decrease in PV output power [7], as dust formation restricts the transmission of solar radiation to the module cells [8–9].

A review of the literature on improving energy efficiency through PV panel cleaning reveals that manual cleaning may damage photovoltaic surfaces due to the pressure exerted by chemical cleaning solutions used to remove accumulated dust and particulates [10]. Furthermore, the use of sprinkler systems for PV panel cleaning can lead to excessive water consumption, which poses a challenge in regions with limited water resources and from a sustainability perspective [11].Waterless cleaning methods, such as self-cleaning superhydrophobic and superhydrophilic coatings, rely on gaseous substances reacting with hot surfaces, but controlling energy efficiency under these conditions is difficult. Therefore, further research is needed regarding factors such as transparency, anti reflective properties, and durability [12]. In one study conducted under the climatic conditions of Oman, a six month performance evaluation of a PV system was carried out to assess the impact of dust accumulation and determine optimal cleaning intervals. Using a manual cleaning method, the study found that efficiency dropped by 28% in the uncleaned PV array [13].

Unlike previous studies, this research experimentally investigates the effect of water only cleaning on energy efficiency in a PV system installed at Trakya University Faculty of Engineering Campus, located in Edirne, a region with relatively low dust levels. The PV panel surfaces were cleaned at 30 day intervals using only water.

MATERIALS AND METHODS

To investigate the effect of surface contamination and dust accumulation on photovoltaic (PV) panels, a solar energy system was installed at the Trakya University Faculty of Engineering Campus, as shown in Figure 1. In the experimental setup, two identical PV panels with a rated power of 260 W were used. The technical specifications of the PV panels employed in the study are presented in Table 1. At the beginning of the experiment, both PV Panel A and PV Panel B were cleaned using only water and a sponge. Subsequently, the energy production data from both panels were recorded over a five month period. Following the initial cleaning, PV Panel A was left unwashed and unmaintained throughout the entire test period. In contrast, PV Panel B was cleaned every 30 days using only water and a sponge. In other words, while PV Panel A remained completely uncleaned, PV Panel B was washed a total of five times. This setup enabled an evaluation of the impact of water based cleaning on the energy efficiency of PV panels. At the end of the five month experimental period, the total energy output of both panels was analyzed and visualized through comparative graphs.

The voltage and current values obtained from the PV panels were recorded throughout the experimental period using a system specific data logger, as shown in Figure 2. Based on these voltage and current values, the output power and the total amount of energy produced by the PV panels were calculated.

Figure 1. Photovoltaic Panel Installation

Figure 2. Data logger system used for recording PV panel voltage and current values

All technical specifications of the PV panels used in the solar energy conversion system are presented in Table 1.

Table 1 Technical specifications of solar PV module

Feature	Value
Maximum Power (Pmax)	260 W
Power Tolerance	0~+3%
Maximum Power Voltage (Vmp)	31.1 V
Maximum Power Current (Imp)	8.37 A
Open Circuit Voltage (Voc)	38.1 V
Short Circuit Current (Isc)	8.98 A
Nominal Operating Cell Temperature (NOCT)	45±2°C
Maximum System Voltage	1000 VDC
Maximum Series Fuse Rating	15 A
Operating Temperature	-40°C~+8°C
Application Class	A
Weight	18.5 kg
Dimension	1650×992×40 (mm)

RESULTS AND DISCUSSION

Following the experimental study, the energy production values obtained from PV Panel A (which was not cleaned) and PV Panel B (which was cleaned once every 30 days using only water) were analyzed. After the initial conditions, PV Panel B, which was cleaned with water once every 30 day interval, consistently produced more energy each month compared to the uncleaned PV Panel A. At the end of the five month period, the total energy outputs of both PV panels were compared. The cumulative energy production values for PV Panel A and PV Panel B are presented in Figure 3.

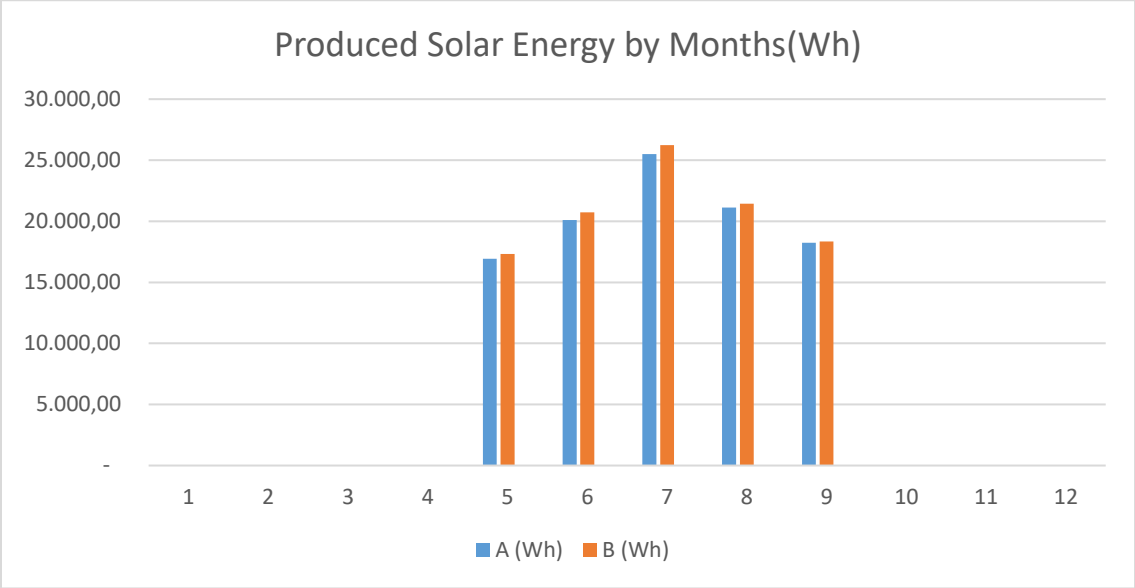

Figure 3. Monthly Total Energy Production of the PV Panels

As shown in Figure 3, PV Panel B, which was cleaned only once every 30 days, produced more energy each month. When the total energy production was examined on a monthly basis, a larger difference in energy output between PV Panel B and PV Panel A was observed during the 6th, 7th, and 8th months. This suggests that dust and dirt accumulation on the PV panel surfaces have a greater negative impact on the output power of the panels during months with higher solar irradiance levels. Based on the results of this study, it was concluded that regular cleaning and washing of PV panels leads to an increase in energy efficiency. At the end of the experimental study, surface images of both PV panels under the current conditions

were captured and are presented in Figure 4.Throughout the study, the surface of PV Panel B, which was cleaned once every 30 days using only water, appeared significantly cleaner compared to the surface of PV Panel A, which was never cleaned, as illustrated in Figure 4.

Figure 4. Surface Contamination Images of PV Panels After the Experiment

As shown in Figure 4, dust and dirt accumulation on the surface of PV Panel A has been found to reduce its energy efficiency. Therefore, regular cleaning or washing of PV panels is essential to prevent the formation of contamination and dust. In Figure 5, the output power profiles of PV Panel A and PV Panel B are compared for a randomly selected day in July, the month with the highest solar irradiance during the five month experimental period.
As observed in Figure 5, the output power of PV Panel B, which was cleaned with water, consistently remained higher than that of PV Panel A throughout the day. Although instantaneous output power of PV panels may fluctuate during the day, the critical parameter is the total amount of energy generated from a PV panel over the course of the day.

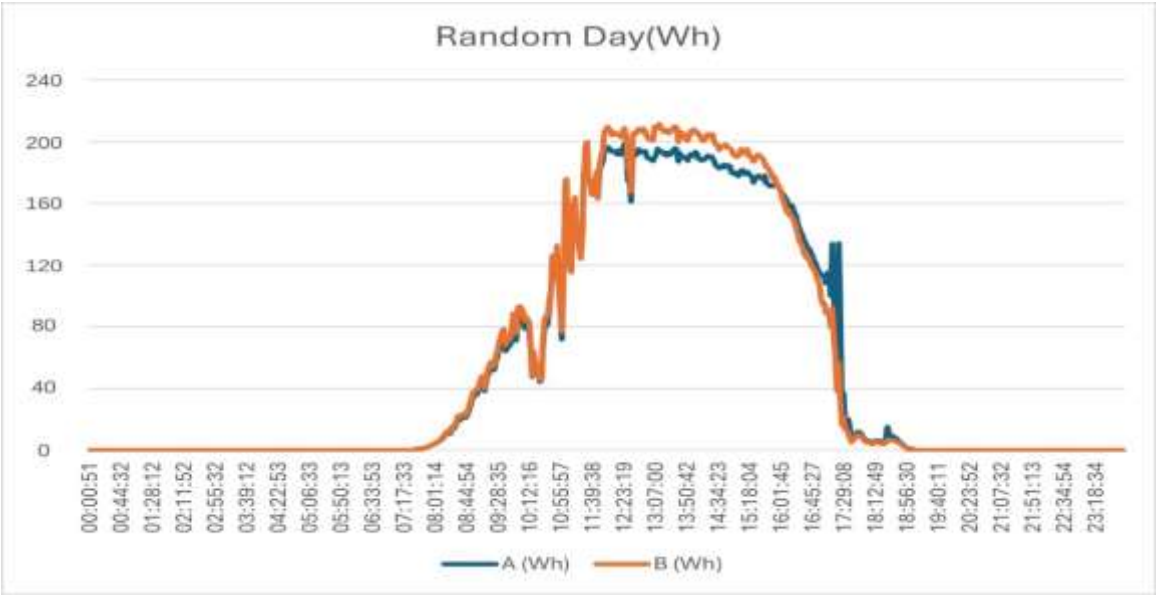

Figure 5. Comparison of the Output Power of PV Panels on a Randomly Selected Day in July

For the randomly selected day, it is observed that the output power of PV Panel B, which was cleaned with water, increases significantly during periods of high solar irradiance, particularly around midday.

CONCLUSION

In this study, the effect of dust and dirt accumulation on the surface of photovoltaic (PV) panels used in solar energy systems on energy efficiency was investigated. PV Panel B, which was manually cleaned with water at 30 day intervals on the installed solar energy system, produced approximately 3% more energy over a five month period. Data analysis from the system revealed that during the five month timeframe, the water cleaned PV panel generated 3122 Wh more energy compared to the uncleaned panel. Considering the large number of photovoltaic panels used in solar power plants, it is evident that regular cleaning with water alone can result in significantly higher energy yields throughout the year. In conclusion, regular cleaning of PV panel surfaces not only increases energy efficiency but also extends the operational lifespan of photovoltaic cells. Future studies using different cleaning agents may provide clearer insights into potential increases in energy efficiency.

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QUANTUM-CHEMICAL STUDY OF NOVEL MULTIFUNCTIONAL POLYMER BY SUBSTITUTION REACTION OF GRAFTING ACETYLSALICYLIC ACID

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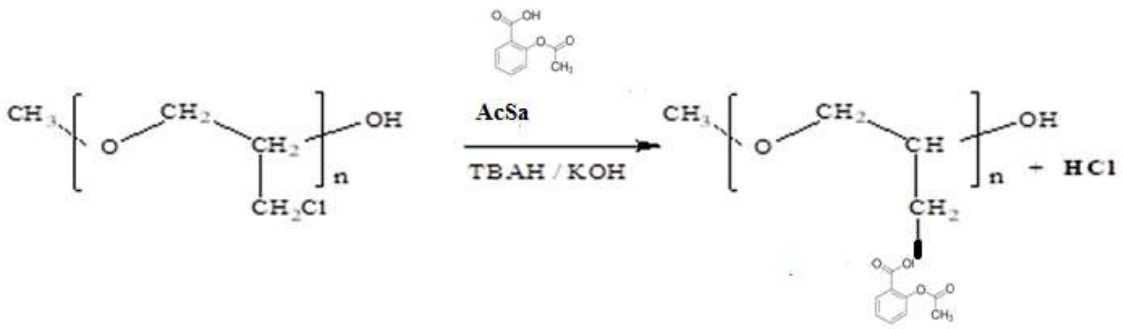
ABSTRACT

During these last years, the important evolution in the computer field and the progress made in the methods of calculation of quantum chemistry make it possible to give a good prediction and a good description of the electronic properties of a given entity. In this work, we have used theoretical study of the substitution reaction by grafting of salicylic acetyl on a unit of epichlorohydrin. We have determined the purely theoretical parameters of the dipole moment (DM), the energy of the frontier orbitals, HOMO, LUMO and the energy gap by two methods HF and DFT, we have also calculated the characters DEN (Normal Electronic Demand) or DEI (request Electronic Inverse) on the substitution reaction of salicylic acetyl (aspirin) + poly (epichlorohydrin); we have calculated the HOMO/LUMO gap energies for the two combinations. The electronic chemical potential μ, and the nucleophilicity index (N).
Local reactivity descriptors of aspirin and epichlorohydrin were calculated using the population analyzes of Mulliken and Hirshfeld by the method DFT/6-31 G(d,p) and also using the analysis of ESP population. A theoretical spectral study of the final product: IR, ^{13}C NMR was carried out.
Keywords: Epichlorohydrin, HOMO, DFT, salicylic acetyl, nucleophilicity, Computational Chemistry.

INTRODUCTION

Computer-aided chemistry (Computational Chemistry), its applications can be used for elucidation and analysis of chemical structures, the processing of chemical information or even theoretical chemistry Justyna Krupa and al.2023. Very precise calculation algorithms are used to minimize the total energy according to the structural parameters and to predict the most stable structure of the studied compounds Mikołaj Sadowski andKarolina Kula, and al.2024.
Computational chemistry methods such as molecular simulations, density-functional theory (DFT), and ab initio approaches are crucial for analyzing the behavior of molecules during the

reaction and the transformation of forms before and after the reaction, which is essential for addressing challenges related to multifunctional polymer utilization and environmental impact.
In this work, the main objective is to use a theoretical study using Gaussian09, of the substitution reaction by grafting of AcSa on units of epichlorohydrin polymer, to be able to confirm the obtaining of the new multifunctional polymer schematic 1. The energy separation of the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) has been used as a simple indicator of kinetic stability Ping Bao,Xiaowei Wang and al.2024. Peng Xi,Fengling Sun.2024 noted that the HOMO-LUMO gap reflects the chemical stability of the molecule. In general, an atom with a higher density HOMO should have a higher ability to remove an electron, while an atom with a higher density occupied by a LUMO should have a higher force to gain an electron R. G. Parr and W. Yang1989 . On the other hand Mulliken atomic charges play an important role in understanding the chemical reactivity of compounds R. S. Mulliken.1932,1939 .
The aim of the present work is to predict the nucleophilic and electrophilic attack sites of some classes of chemical reactions using reactivity indices derived from the density functional theory (DFT). First, we have calculated the global and local nucleophilicity indices. Then, we have conducted a theoretical study of a substitution reaction: epichlorohydrin and acetylsalicylic acid. The local and global reactivity indices derived from the DFT are very reliable for the prediction of electrophilic or nucleophilic attack sites provided that efficient methods (B3LYP) are used for the energy and geometric calculations of the reactants.



Schematic 1 : Chemical modification of PECH by AcSa

MATERIAL AND METHOD

Optimized Geometries and Frontier Molecular Orbitals

Gaussian software was used in order to carry out all the structure optimizations studied with the following DFT. The calculation was launched with the correlation exchange functional B3LYP and the Gaussian basis 6-31G (p,d). The latter has the same denomination, where the set of functions p added to the hydrogens and the set of functions d added to the other heavy atoms is well defined.
First, the theoretical calculation was made with the DFT method using the functional B3LYP (Becke Lee-Yang- Parr) and the base 6-31Gdp, for the latter. Optimization with this method gave us almost similar conformations, the latter having the lowest possible formation energy, hence the most stable conformation.

RESULTS AND DISCUSSION

OPTIMIZATION OF OBTAINED PRODUCT

Calculation details The DFT/B3LYP and 6-311++G(d,p) methods were used to optimize the molecular structures of polymer obtained. The programs Gaussian09W and GaussView5.0 were utilized to carry out all the necessary DFT calculations and visualizations. we obtained E= -1070.545578 au

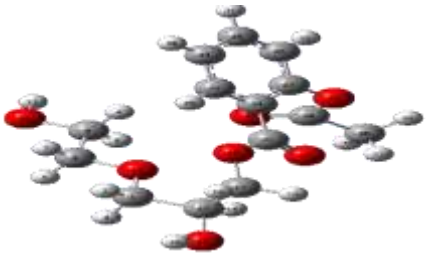


Figure 1 : B3LYP/6-31G(d) computed structure of the graft polymer

HOMO-LUMO

Green and brown colors represent positive and negative phases, respectively. Data from the HOMOLUMO analysis are presented in Figure 2 .

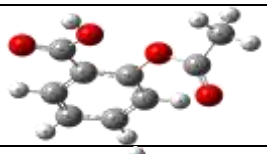
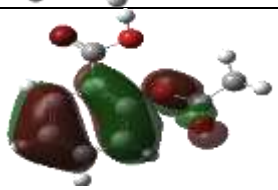
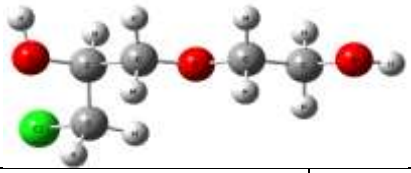
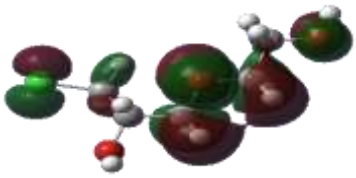
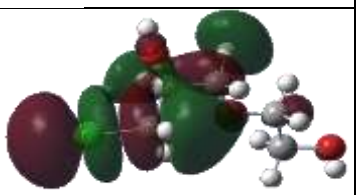
	HOMO	*LUMO*
acetyl salicylic		
		
epichlorohydrin		
		

Figure 2: B3LYP/6-31G(d) HOMO–LUMO of *acetyl salicylic and epichlorohydrin*

To clarify the characters DEN (Normal Electronic Demand) or DEI (Inverse Electronic Demand) on the substitution reaction of salicylic acetyl and PECH; the HOMO-LUMO gap energies for the two combinations were calculated (chemical potential μ, and the nucleophilicity index (N)) (Table.1).

Table.1: B3LYP/6-31G(d) HOMO and LUMO energies as well as global reactivity indices for salicylic acetyl and epichlorohydrin, given in electronvolts [eV]

	LUMO	HOMO	ΔE(I) NED	ΔE(II) IED	*μ*	*H*	*S*	*Ω*	*NOT*
Salicylic acetyl	-0.069	-0.258	-0.288	-0.198	-0.164	0.189	2,640	1.935	2.326
Epichlorohydrin	0.029	-0.267	0.288	0.198	-0.119	0.297	1.685	0.652	2.089

ΔE(I) = HOMO(salicylic acetyl) – LUMO(epichlorohydrin),
ΔE(II) =HOMO(epichlorohydrin) – LUMO(salicylic acetyl)
Table .1 show that the energy difference corresponding to the HOMO (salicylic acetyl) / LUMO (epichlorohydrin) combination is lower than that corresponding to the HOMO (epichlorohydrin) / LUMO (salicylic acetyl) combination, which shows that the Salicylic acetyl behaves as an electron donor (nucleophile) and epichlorohydrin as an electron acceptor (electrophile).

THEORITICAL SPECTRUM STUDY

Theoretical study of the Infra-Red Spectrum of the graft polymer

Examination of the theoretical IR spectrum (Figure 8) of the modified polymer has enabled us to identify the main intensive characteristic bands with the frequencies 1168 cm $^{-1}$, which indicates the absorption of the COC group of the ether, a band absorption located at 1456 cm $^{-1}$ which corresponds to the aromatic HC group, an absorption band located at 653.184 cm $^{-1}$ from the CH of the deformation, is a value of 1667.55 cm $^{-1}$ which characterizes the elongations of the aromatic C=C bond. Table.2 brings together the main characteristic bands of the graft polymer.

Table.2: The main characteristic IR bands of the graft polymer.

Connection	IR EXP (cm $^{-1}$)	FRQ(cm^{-1})/ DFT	FRQ(cm $^{-1}$)/HF	Intensity
C_{tet} -H valence	2900-2877	2966	3117	2 middle bands
aromatic C=C	1485-1636	1476	1612-1777	3 OR 4 strong bands
Aromatic CH	709-762	716-796	1097	2 strong bands
CH deformation	1387-1485	1338-1405	1336-1386	2 strong bands
CO valence	1295	1076-1141	1149	thin band
C=O Ester	1636-1688	1735	2038	thin band

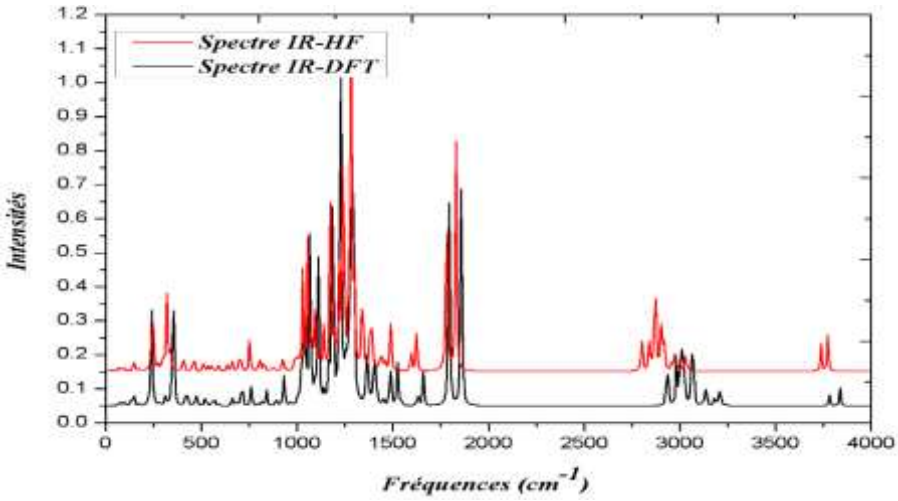


Figure 1:Theoretical IR infrared spectrum of the graft polymer by the methods HF-DFT

^{13}C NMR spectrum of the graft polymer

$^{13\,C}$ NMR spectrum (Figure 4) allowed us to have the main chemical shifts of the different groups which characterize the graft polymer, we observe:

-A signal of 122 ppm which characterizes the displacement of carbon 17, 34 ppm signal, indicates the chemical shift of carbon 21 , and the aromatic carbon of C23 –C28 comes out between 40 and 72 ppm.

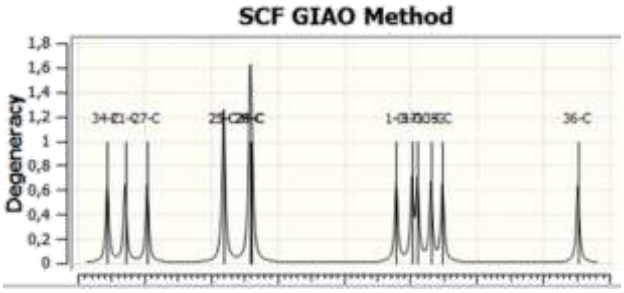


Figure 2$^{13\,C}$ NMR spectrum calculated by DFT of the graft polymer

LOCAL DESCRIPTORS OF AcSa REACTIVITY CALCULATED USING MILLIKEN AND HIRSHFELD POLUPLATIONS ANALYSIS

The calculated and indicated charges of Mulliken atoms for AcSa are shown in Table.3, and the Local descriptors of AcSa reactivity calculated using Hirshfeld population analysis is presented in the table.4

Table .3 : Local descriptors of AcSa reactivity calculated using mulliken population analysis

	f_k^+	f_k^-	***NNk***		f^+	f^-	N_k
1C	0.719	0.554	1.2889	12O	0.417	-0.632	-1.4697
2C	-0.491	-0.885	-2.0596	13O	0.462	-0.460	-1.0692
3C	0.644	0.641	1.4908	14H	-0.282	0.284	0.6601
4C	0.095	-0.406	-0.9445	15O	0.533	-0.473	-1.1005
5C	0.007	-0.037	-0.0859	16C	-0.493	0.496	1.0914
6C	0.309	-0.700	-1.6283	17O	0.451	-0.445	-1.0355
7H	-0.160	0.067	0.1548	18C	0.250	-0.248	-0.5777
8H	-0.086	0.190	0.4409	19H	-0.135	0.135	0.3140
9H	-0.188	0.095	0.2213	20H	-0.136	0.135	0.3150
10H	-0.161	0.197	0.4573	21H	-0.145	0.144	0.3360
11C	-0.610	0.376	0.8742				

Table.4 : Local descriptors of AcSa reactivity calculated using Hirshfeld population analysis

	f_k^+	f_k^-	***NNk***		f_k^+	f_k^-	Nk
1C	0.145972	0.043089	0.10022501	**12O**	**0.045858**	**0.145297**	**0.14462835**
2C	0.023883	0.088054	0.2048136	13O	0.012739	0.062179	0.10070417
3C	0.133288	0.034088	0.07928869	14H	0.023805	0.043395	0.01569352
4C	0.125822	0.061804	0.1437561	15O	0.045087	0.006747	0.02589303
5C	0.022385	0.082361	0.19157169	16C	0.013063	0.011132	0.06393476
6C	0.132194	0.061215	0.14238609	17O	0.028841	0.027487	0.0230181
7H	0.054767	0.03581	0.08329406	18C	0.013277	0.009896	0.04154236
8H	0.034894	0.04684	0.10894984	19H	0.019454	0.01786	0.04154236
9H	0.046199	0.029716	0.06911941	20H	0.012249	0.01021	0.02374846
10C	0.048508	0.032285	0.33882842	21H	0.011532	0.004949	0.01151137
11C	0.006191	0.14567	0.33796082				

The local reactivity index f_k^-, for the Mulliken and Hirshfeld population analysis provided the greatest value at the O 12 site. The latter should be the most reactive site with respect to an electrophilic attack, therefore the more nucleophilic.

CONCLUSION

The DFT calculations at the B3LYP/6-31G dp level is used effectively to describe the reaction of the substitution of the chlorine group in the reaction of the grafting of acetylsalicylic acid on epichlorohydrin chain.

Analysis of HOMO/LUMO gaps, chemical potential μ and the global electrophilicity index ω indicates the NED character, and the IED character for the substitution reaction are calculated. Finally, it can be said that calculations of activation energies and reactivity indices derived from the conceptual DFT of both addition and substitution reactions successfully predict the experimental regioselectivity.

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ASSESSMENT OF THE TRANSITION FROM CONVENTIONAL SOIL TILLAGE TO MINIMUM TILLAGE TECHNOLOGY IN A MAIZE CROP WITH THREE HYBRIDS – A THREE-YEAR CASE STUDY IN LANURILE, CONSTANȚA COUNTY

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ABSTRACT

The present study aims to evaluate the impact of transitioning from conventional soil tillage to minimum tillage technology in a maize crop, cultivated over a three-year period (2022–2024) in the village of Lanurile, Constanța County, Romania. The experiment was conducted under real farm conditions and involved three late-maturing maize hybrids: DKC 5810, P0900, and P0216. The crop was grown on partially irrigated land. Soil preparation consisted of a single pass with a cultivator, followed by precision sowing, while all other technological inputs (fertilization, crop protection, and irrigation management) were kept constant across the hybrids and over the years.

The agromorphological and biometric determinations revealed superior performance for the DKC 5810 hybrid, followed by P0900. Differences in productivity parameters, such as grain weight per ear and thousand-kernel weight (TKW), were statistically validated using one-way ANOVA tests, confirming significant distinctions between hybrids ($p < 0.05$). The DKC 5810 hybrid recorded the highest average kernel weight and TKW, while also achieving the greatest yield per hectare. P0900 also demonstrated high adaptability and yield potential, particularly under irrigation. In contrast, P0216, although showing vigorous vegetative development, accumulated lower grain mass and yielded less compared to the other two.

Climatic analysis over the study period showed a consistent increase in mean annual temperatures and a significant decrease in rainfall, especially during critical phenological stages such as flowering and grain filling. These climatic trends emphasized the importance of water management. The irrigation applied in four key growth stages successfully offset part of the rainfall deficit and supported optimal crop development.

The results demonstrate that minimum tillage, when properly implemented under the specific pedoclimatic conditions of Dobrogea and combined with efficient irrigation and hybrid selection, can ensure competitive yields. This conservation practice reduces soil disturbance, minimizes erosion and compaction, and preserves soil moisture critical factors in semi-arid regions. Therefore, minimum tillage represents a viable and sustainable alternative to conventional tillage in areas facing water scarcity and climate variability.

These findings highlight the importance of hybrid selection aligned with specific tillage practices and climate adaptability. DKC 5810 is recommended for high-input, irrigated systems, while P0900 proves stable and productive in moderately intensive conditions. Further research is encouraged across other regions to validate these results and guide sustainable maize production strategies in Romania.

Keywords: minimum tillage, maize hybrids, sustainable agriculture, irrigation, climate change

INTRODUCTION

Cereals represent the crop group with the widest distribution and the greatest importance for human nutrition, providing approximately 60% of total proteins, 15% of lipids, and around 70% of carbohydrates, contributing 50–60% to the global caloric intake (Roman et al., 2015; Dobre, 2019). The success of cereal crops is explained by a combination of favorable factors: high ecological plasticity, complete mechanization, easy storage, and multiple utilization possibilities – human food, animal feed, industry, biofuels (Messegue, 2000).

Maize (Zea mays L.) holds a central place among cereals, both in terms of cultivated area and versatility of uses. In Romania, maize is extensively cultivated due to its nutritional value and high production capacity, being used for human consumption, animal feed, and industrial purposes (Cristea et al., 2004; Ion, 2010). In human nutrition, maize appears in various forms: flour (polenta), flakes, sweet corn, or popcorn (Paukert, 2021). Industrially, maize kernels are a raw material for obtaining starch, glucose, oil, as well as products such as bioethanol, spirits, and starch-based cosmetics (Petcu and Martura, 2018). However, the largest part of global production (over 60%) is destined for animal feed, being considered an essential forage, especially for ruminants (Sin, 2000).

Globally, maize is cultivated up to 59° northern latitude and 43° southern latitude, demonstrating great adaptability. Romania benefits from favorable soil and climatic conditions, being one of the European countries with significant maize cultivation areas (Popescu, 1995; Panaitescu and Niță, 2011). Nevertheless, achieving maximum productive potential requires the application of modern technologies that address current climatic challenges and agricultural sustainability requirements (Petcu and Martura, 2018).

In this context, in recent years there has been a growing interest in adopting conservation tillage technologies, such as minimum tillage, which limit mechanical interventions on the soil, thus reducing compaction, water loss, and erosion. Recent studies (FAO, 2023; ISTIS, 2024) confirm the effectiveness of these practices in maintaining soil structure and increasing farm sustainability. In Romania, although conventional technology remains predominant in many regions, more and more farms are exploring conservation methods, especially in drought-affected areas or where input costs must be reduced.

This study analyzes the technological transition from conventional soil tillage to minimum tillage in a maize crop on a farm located in Lanurile, Constanța County, over a three-year period, using three hybrids and maintaining the other technological elements constant. The purpose of the study is to evaluate the impact of this transition on yield, crop economics, and the adaptability of the hybrids to the new soil working conditions.

In the context of climate change and increasing pressures on natural resources, adopting conservation technologies such as minimum tillage represents a strategic direction for sustainable agriculture, with reduced impact on soil structure and soil biodiversity. Recent studies highlight that these technologies can contribute to reducing fuel consumption, conserving soil moisture, and maintaining fertility in semi-arid areas such as Dobrogea (Popa et al., 2023). Furthermore, the selection of maize hybrids with high potential and enhanced adaptability to variable pedoclimatic conditions is essential in the current context. Recent results emphasize the need for field testing of biological material in correlation with cultivation technology in order to efficiently capitalize on productive potential (Ioniță et al., 2022).

This study makes a significant contribution by evaluating the long-term effects, under real farm conditions, of the transition from conventional to minimum tillage in maize cultivation using three commercial hybrids. The results provide concrete data on the impact of this technology on yield, crop stability, and economic efficiency, key aspects for adapting agriculture to climate challenges and promoting sustainable practices in semi-arid areas such as Dobrogea. Thus, the research complements the existing literature through an integrated

approach to soil tillage technology and hybrid selection, contributing to the optimization of agricultural management in the context of climate change.

MATERIAL AND METHOD

The aim of this study was the comparative evaluation of the performance of three late-maturing maize hybrids cultivated under minimum tillage system in a representative area of Constanța County, over a period of three consecutive years. The study focused on analyzing the agro-biological and economic performances of the three hybrids under the region's specific soil and climatic conditions, in the context of transitioning from conventional soil tillage technologies.

The experiment was carried out on the farm of I.I. Iancu Ionuț, located in Lanurile, Constanța County, in the center of the Medgidia Plateau. The locality is situated approximately 50 km from the city of Constanța. The total area of the commune is 421 hectares of intravilan (built-up area) and 18,071 hectares of extravilan (non-built-up area). According to climatic data, the area belongs to the temperate-continental steppe climate type, characterized by an average annual temperature of 11.12°C, with January minimums of 0.2°C and July maximums of 22.1°C. The average annual precipitation is around 428 mm. The dominant soils are fertile chernozems, typical of steppe regions, favorable for agriculture.

The main agrotechnical characteristics of the experimental area: the preceding crop was wheat, and maize crops were established on 60 ha, 57 ha, and 85 ha in the years 2022, 2023, and 2024, respectively. Sowing was carried out after a shallow tillage operation using a combinator, uniformly applied in all three springs (characterized by cool and wet conditions, with persistent rainfall in April 2022 and 2023), aimed at accelerating soil warming and fragmenting remaining plant residues. This was the only soil intervention prior to sowing, specific to the minimum tillage system.

All care operations and phytosanitary treatments were identical across the three growing seasons, to allow for an objective evaluation of the hybrids' response exclusively to soil technology and annual pedoclimatic conditions.

Regarding biological material, three late-maturing maize hybrids registered in the Official Catalogue of Plant Varieties of Romania for 2022 and 2023 were used: DKC 5810 (Bayer): FAO 450–490 hybrid, RM 108, medium plant height, high seedling vigor, deep rooting system, without tillering; P0216 (Pioneer – Corteva): FAO 450 hybrid, high yield potential, suitable for early and optimal sowing, excellent performance under various growing conditions; P0900 (Pioneer – Corteva): FAO 450 hybrid, late-maturing, designed for intensive technologies (under irrigation or abundant rainfall), with good drought tolerance.

The experiment was conducted in open field conditions, under real farm conditions. Throughout the vegetation period, the main developmental phenophases of the hybrids were monitored (emergence, tillering, flowering, physiological maturity), tracking: vegetative growth dynamics, adaptability to climatic conditions, uniformity of emergence and development, behavior towards diseases and pests, and final yield per hectare.

Measurements were performed directly in the field, at the Iancu Ionuț Individual Enterprise farm, as well as in the laboratory, for detailed analyses regarding grain weight, thousand kernel weight (TKW), and other relevant qualitative characteristics.

RESULTS AND DISCUSSION

In the conducted experiment, the preceding crop was winter wheat, chosen in accordance with the predominant crop structure of the farm and the crop rotation system applied at the farm level. The distribution of crops and the average yields per hectare are presented in the tables below, providing an overview of the implemented crop rotation system.

Table 1. Crop structure within I.I. Iancu Enterprise during the years 2022–2024

Year	Crop plant				
	Wheat	**Maize**	**Barley**	**Sunflower**	**Rapessed**
2022	85	60	50	35	20
2023	83	57	40	40	30
2024	90	85	20	30	25

Table 2. Average crop yields within I.I. Iancu Enterprise during the years 2022–2024

Year	Crop plant				
	Wheat	**Maize**	**Barley**	**Sunflower**	**Rapessed**
2022	4830	6320	5070	2265	2145
2023	5000	6800	5150	2500	2500
2024	5500	7700	5050	2720	2300

Chemical fertilization within the study was carefully structured into three distinct stages, each corresponding to key developmental phases of the maize crop. The first stage, basic fertilization, took place in the autumn, prior to sowing. Here, 200 kg/ha of complex NPK fertilizer (20:20:20) was applied pre-emergence, aiming to provide the essential nutrients needed to support vigorous early growth. As the crop progressed, foliar fertilization was carried out at the 4-leaf stage, using Plonvit foliar fertilizer (produced by Intermag) at a rate of 2 L/ha, diluted in 300 liters of solution per hectare. This application was intended to stimulate physiological processes and enhance vegetative development. Later, at the 10-leaf stage, nitrogen fertilization was performed by administering 100 kg/ha of solid nitrogen, supporting the formation of inflorescences and the accumulation of dry matter within the grains.

Due to the nature of the minimum tillage system, where soil disturbance is minimized to conserve water resources and preserve soil structure, climatic conditions during the growing season played a pivotal role in influencing maize performance.

Soil preparation consisted of a single combinator pass, carried out two days before sowing: on April 24 in 2022, and April 25 in both 2023 and 2024. This light soil work enabled sufficient fragmentation, leveling, and aeration to create an optimal seedbed. Precision sowing was performed during the optimal window for the Dobrogea region: on April 25 in 2022, and on April 27 for the following two years. Favorable rainfall levels in April supported good seed germination and uniform seedling emergence across the experimental plots.

Climate analysis for Lanurile village over the agricultural period 2022–2024 (Table 3) revealed a consistent rise in monthly average temperatures compared to multiannual norms. The average annual temperature during the study period was recorded at 13.78°C, exceeding

the historical mean of 11.12°C by 2.66°C—an increase of approximately 24%. This upward trend persisted throughout the entire agricultural cycle, affecting not only the colder months, when soil water reserves are usually replenished, but also the critical vegetation stages, thereby intensifying plant metabolism and increasing their water demands.

Meanwhile, rainfall patterns showed a downward trend, with an annual average of 357.3 mm, marking a deficit of 70.8 mm relative to the multiannual average of 428.1 mm. This reduction, representing 16.55%, was especially evident during May, July, and August—months critical for maize development, as these periods coincide with the crop's highest water needs during pollination and grain formation. The resulting imbalance between water availability and plant demand likely posed a significant limiting factor for hybrid performance, particularly under conservation tillage systems where soil water reserves are crucial for crop success.

Table 3. Temperature and precipitation regime in Lanurile Locality during the agricultural years 2022–2024

Month	**Average monthly temperature ºC**		**Precipitation mm**	
	Normală	**2022-2024**	**Normală**	**2022-2024**
XI	6.9	8,8	37,5	35,1
XII	2,1	3,6	32,2	28,5
I	0,2	4,8	31,6	40,6
II	1,1	2,9	32,8	22,1
III	3,9	6,8	22,1	24,5
IV	10,1	10,1	33,1	51,2
V	15,8	16,6	48,7	10,8
VI	19,9	21,8	52,6	44,6
VII	22,1	25,8	34,7	21,1
VIII	22,7	26,1	31,1	20,8
IX	17,2	21,2	40,8	30,1
X	11,5	16,9	30,9	27,9
Average	11,12	13,78	428,1	357,3

To objectively assess the statistical significance of the climatic differences between the normal multiannual values and those recorded during the study period, a descriptive statistical analysis was conducted, supplemented by a simplified one-way ANOVA test. The statistical results indicated p-values greater than 0.05 for both temperature ($p = 0.45$) and precipitation ($p = 0.16$), suggesting that, within this limited sample, the observed differences were not statistically significant. Nevertheless, the identified climatic trends—progressive warming and reduced precipitation—have clear agronomic relevance and can decisively influence crop performance, especially under minimum tillage systems.

Thus, it can be concluded that, although the statistical analysis did not confirm rigorous mathematical significance, the recorded climatic values (Figure 1) support field observations and may contribute to a better understanding of the yield variability of the analyzed hybrids under this technological system.

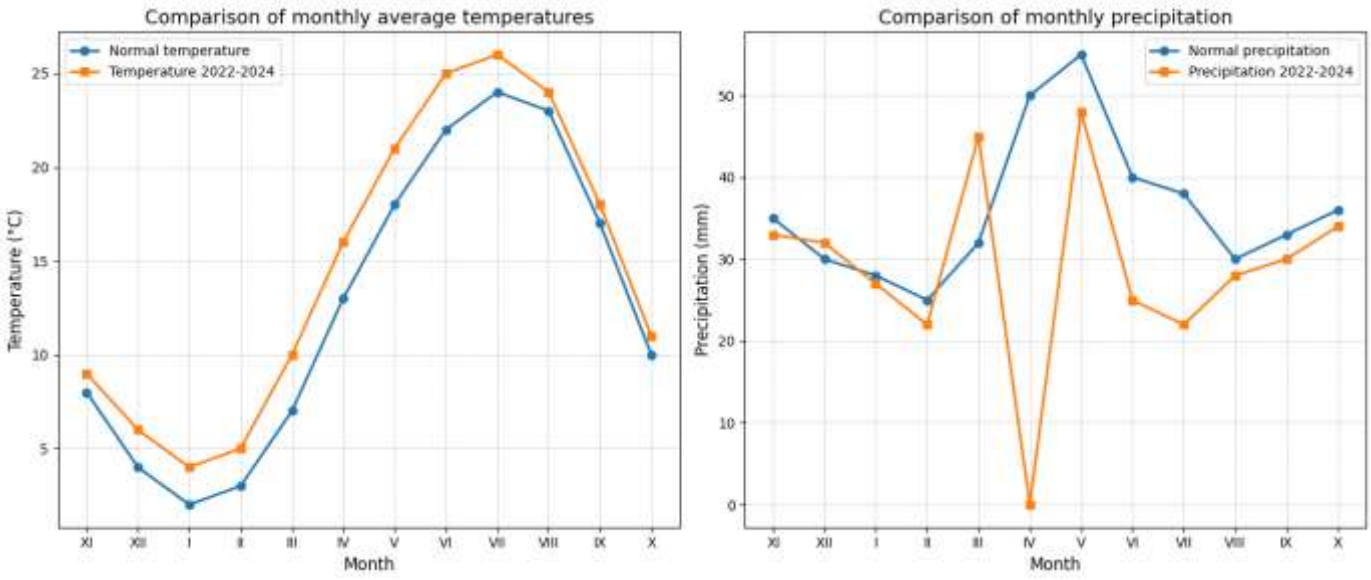


Figure 1. Comparative graphs of monthly average temperatures, monthly average precipitation, and multiannual normals

In the experiment, maize sowing was carried out at a depth of 6 cm using precision planters, maintaining a row spacing of 70 cm. The sowing density was set at 78,000 harvestable plants per hectare, adapted to the potential of the hybrids used and the availability of irrigation over 60 hectares out of the total 85 hectares cultivated with the three hybrids. The seeding rate was 27 kg/ha.

Weed control was performed both mechanically and chemically, following an integrated plant protection program. Three days after sowing, a pre-emergent herbicide treatment was applied using Callisto 480 SC (Syngenta) at a rate of 330 ml/ha to control broadleaf weeds and grasses. At the same time, the insecticide Decis Expert (Bayer) was applied at a rate of 125 ml/ha, targeting key pests during early vegetative stages, such as *Diabrotica virgifera virgifera* (Western corn rootworm) and *Helicoverpa armigera* (corn earworm). The solution was prepared at a volume of 250 L/ha.

Upon entry into the active vegetative growth phase, a post-emergent treatment with Equip (Bayer) herbicide was applied at a rate of 1.5 L/ha to combat invasive species such as *Setaria viridis* (green foxtail) and *Sorghum halepense* (Johnsongrass). This treatment was combined with the foliar fertilizer Plonvit (2 L/ha in 300 L solution/ha) and a preventive fungicide, as part of an integrated technological package.

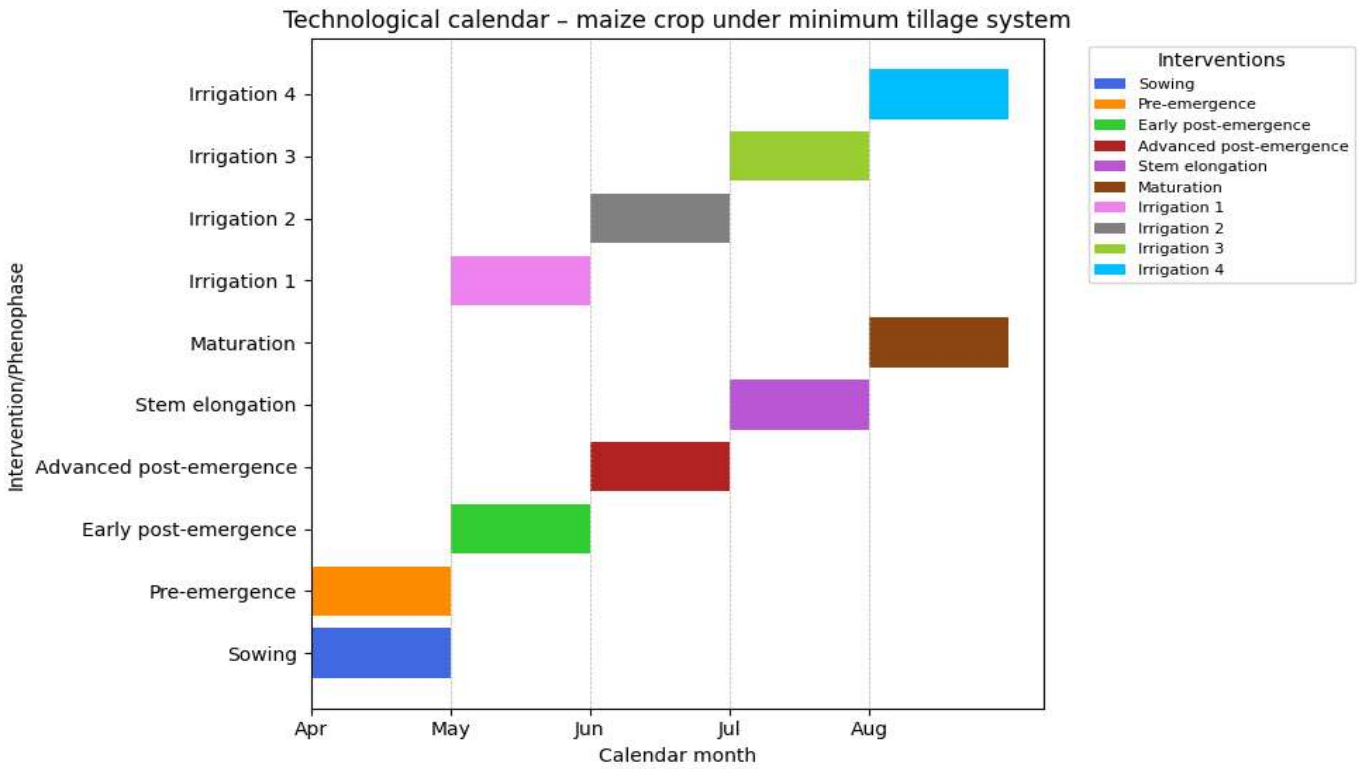
Regarding disease control, two fungicide treatments were applied using Propulse (Bayer) at a rate of 1 L/ha. The first treatment was carried out at the 6–8 leaf stage, concurrently with foliar fertilization and the second herbicide application. The second treatment was applied during the stem elongation phase to prevent infections caused by *Helminthosporium turcicum*, the pathogen responsible for northern corn leaf blight.

Mechanical weed control was performed through inter-row cultivation at a depth of 8 cm, a stage that coincided with the application of solid nitrogen fertilization, completing the nutritional plan (Tables 4 and 5).

Table 4. Overview of treatments applied throughout the study period

No.	Stage	Product	Intervention Purpose
1.	Pre-emergence – 3 days after sowing	Calisto 480SC+Decis Expert	Weed and pest control
2.	Post-emergence 4–6 leaves	Equip+Plonvit+fungicide	Control of Johnsongrass, green foxtail, nutrition, and disease prevention
3.	Post-emergence 6–8 leaves	Propulse 1L/ha+Plonvit	Preventive fungicide treatment and foliar nutrition
4.	Stem elongation	Propulse 1L/ha	Control of northern corn leaf blight
5.	Mechanical cultivation	Inter-row cultivation at 8 cm depth	Mechanical weed control
6.	Nitrogen fertilization	100kg azot/ha	In-vegetation fertilization
7.	Irigation 1	700m³/ha	Irrigation – stable emergence
8.	Irigation 2	700m³/ha	Irrigation – 10–12 leaf stage
9.	Irigation 3	700m³/ha	Irrigation – tasseling
10.	Irigation 4	700m³/ha	Irrigation – maturation

Table 5. Technological schedule of maize during the study years



Due to the deficient rainfall regime during the agricultural years under study, four sprinkler irrigations were performed using a Hipodron-type linear pivot system. The first irrigation was conducted starting on May 20, applying 700 m³/ha, followed by a second irrigation in June when the plants were at the 10–12 leaf stage, with the same irrigation rate.

The third irrigation took place at tasseling, during the first ten days of July, and the fourth was performed in August, all maintaining a constant application rate of 700 m³/ha.

The controlled application of irrigation, correlated with minimum tillage practices, aimed to maintain a favorable soil moisture regime, especially during critical periods for maize development, thus supporting the physiological processes and productive potential of the analyzed hybrids.

The maize crop was harvested mechanically using a Claas Tucano 320 agricultural combine once the plants had reached full physiological maturity. This stage was determined by the stabilization of grain moisture around 17–18%, a threshold uniformly attained by all three hybrids analyzed. Harvesting operations began after October 19, with the latest harvest recorded on October 27, 2024. The entire process involved grain harvesting, after which the grains were transported to the farm headquarters for storage and conditioning. In preparation for marketing, grain moisture was reduced to below 15%, considered optimal for safe storage and trading. The production was sold during the autumn season of the respective agricultural years under study.

Within the study, several agromorphological field measurements were also carried out to highlight the development characteristics of each hybrid under the specific soil and climatic conditions and technological system applied. One such determination involved counting the number of plants and double ears over a 10-meter row length on two adjacent rows. Based on the averages recorded over the three years, it was observed that the hybrid DKC 5810 had 40 plants on the first row, 11 of which developed double ears, and 38 plants on the second row, with 9 showing this trait. For the hybrid P0216, 39 plants were recorded on the first row, with 9 double-eared, and 38 plants on the second row, 6 of which exhibited the same characteristic. Hybrid P0900 stood out with a higher plant density, having 40 plants on the first row and 41 on the second row, with 10 double-eared plants in each case. This feature suggests a strong generative branching capacity, indicating a superior production potential, especially under favorable nutrition and moisture conditions.

Further measurements involved counting the total number of ears present on the same 10-meter row sections to obtain a clearer picture of the fruiting capacity. Hybrid DKC 5810 recorded an average of 98 ears, P0216 had 92 ears, and P0900 achieved 101 ears. These data correlate with the observed presence of double ears and confirm the superior yield potential of P0900 compared to the other two hybrids.

Another parameter evaluated was the average plant height, considered an indicator of vegetative vigor and uniform development. DKC 5810 registered an average height of 2.39 m, ranging between 2.27 m and 2.51 m. P0216 showed an average of 2.31 m, with minimum and maximum values of 2.24 m and 2.42 m, respectively. P0900, although the most productive, had a slightly lower average height of 2.28 m, with a range between 2.17 m and 2.37 m. These relatively small variations indicate good uniformity of the experimental plots and specific adaptability of each hybrid to the minimum tillage system.

An additional field measurement was the determination of the ear insertion height. The results were as follows: DKC 5810 had an average ear height of 95.23 cm, ranging from 80.93 cm to 108.4 cm; P0216 recorded an average of 94.31 cm, between 82.10 cm and 103.3 cm; P0900 had an average of 93.33 cm, ranging from 80.6 cm to 105.6 cm.

The field measurements provide a detailed and rigorous overview of the phenotypic behavior of the three maize hybrids in the context of minimum tillage technology and a deficit rainfall regime supplemented by rationalized irrigation. Morphological characteristics and the total number of ears reveal differentiated but consistently high productive capacity, offering valuable insights for selecting the optimal hybrid under such technological systems.

To further evaluate hybrid performance under minimum tillage conditions, additional biometric determinations were performed at the farm's headquarters before harvest. These

included analyses of plant and ear weights at different preparation stages and detailed laboratory analyses of morphoproductive ear components.

For each hybrid, over the three study years, 10 mature plants with ears were harvested from two adjacent rows within the experimental plots. The first measurement focused on the total weight of plants at full physiological maturity. The average results were: DKC 5810: average plant weight of 770.27 g, ranging from 682.6 g to 830.2 g; P0900: average plant weight of 764.55 g, ranging between 667.1 g and 813.1 g; P0216: average plant weight of 731.59 g, with a range of 651.3 g to 811.1 g.

The second measurement targeted the weight of ears with husks, conducted two days before final harvest. After weighing the whole plants, the ears were separated and weighed individually. The average ear weights with husks were: DKC 5810: 289.3 g (ranging from 171.2 g to 358.8 g), with a residual plant biomass (excluding ears) averaging 480.92 g; P0900: 296.73 g (between 216.3 g and 331.1 g), with a residual biomass of 467.82 g; P0216: 230.64 g (ranging from 198.9 g to 308.2 g), with an average dry biomass of 500.95 g.

Detailed laboratory analyses continued with the measurement of ear weights without husks. After manually removing the husks, the ears were reweighed. The average values were: DKC 5810: 264.39 g (ranging from 150.8 g to 337.8 g), with an average husk mass of 24.91 g; P0900: 261.07 g (ranging from 194.7 g to 302.0 g), with an average husk mass of 35.66 g; P0216: 205.44 g (ranging from 168.9 g to 279.4 g), with an average husk mass of 25.2 g.

These results highlight clear differences in biomass structure and dry matter accumulation capacity among the three hybrids. Although hybrid P0216 exhibited a comparable total plant mass, the ear weights, both with and without husks, were visibly lower, suggesting a higher proportion of vegetative mass relative to productive structures. Conversely, hybrids DKC 5810 and P0900 demonstrated a more favorable ratio between total plant mass and ear mass, indicating superior efficiency in resource allocation toward generative structures.

Table 6. Measurement of husk-free ear weight at physiological maturity

Plant no.	**Hybrid DKC 5810 (g.)**	**Hybrid P0900 (g.)**	**Hybrid P0216 (g.)**
1.	223,4	215,2	184,1
2.	337,8	281,4	216
3.	318,8	296,9	279,4
4.	298,7	267,2	219,8
5.	279,2	280	224
6.	228,5	236,1	186,4
7.	150,8	269,2	188,6
8.	268,4	194,7	201,4
9.	258,3	302	168,9
10.	280	268	185,8
Minimum	**150,8**	**194,7**	**168,9**
Maximum	**337,8**	**302**	**279,4**
Average	**264,39**	**261,07**	**205,44**

To provide a detailed characterization of the morpho-productive elements of the analyzed hybrids, following the previous determinations, measurements of ear length without husks were conducted, followed by the determination of cob weight. This analysis was carried out on the same ears used for the weight measurements, after complete removal of the kernels. The average results, calculated over the three years of study, highlighted the following: the hybrid DKC 5810 presented an average ear length of 21 cm and an average cob weight of 22.39 g, with values ranging between 19.42 g and 27.5 g. For hybrid P0900, the average ear length was 20 cm, and the cob weight was higher, with an average of 25.59 g, ranging from 17.1 g to 35.8 g. Hybrid P0216 also recorded an average ear length of 20 cm, with an average cob weight of 23.03 g, ranging between 17.15 g and 31.61 g.

To evaluate the real productive potential of the ears, an additional determination was made by individually counting the kernels, one by one, on a batch of ten samples for each hybrid, across the three study years. The analysis revealed that: DKC 5810 had an average of 546 kernels per ear, ranging from 412 to 612. P0900 recorded an average of 554 kernels per ear, with values between 502 and 618. P0216 showed a lower average of 490 kernels per ear, ranging between 408 and 596.

These differences indicate a higher kernel density and spikelet fertility in hybrid P0900, supporting its previously observed productive performances.

The final determinations targeted the total kernel weight per ear, thousand kernel weight (TKW), and final yield per hectare (Table 7), obtained by weighing ten distinct samples for each hybrid. The average results over the three years of study were as follows: for DKC 5810, the average kernel weight per ear was 241.37 g, with values ranging between 128.7 g and 316 g. The thousand kernel weight (TKW) recorded an average of 442.07 g, and the average yield per hectare was 11,430 kg. P0900 showed an average kernel weight of 235.64 g (ranging from 171.5 g to 280.1 g), a TKW of 425.35 g, and an average yield of 10,780 kg/ha. In the case of P0216, the average kernel weight was 180.33 g, with values between 148.1 g and 233.6 g, a TKW of 408.02 g, and an average yield of 10,300 kg/ha.

Table 7. Measurement of grain weight per ear in the three maize hybrids under study

Plant no.	Hybrid DKC 5810 (g.)	Hybrid P0900 (g.)	Hybrid P0216 (g.)
1.	203,98	198,1	163,1
2.	316	258,3	189,3
3.	298,05	270,83	233,6
4.	274,6	232,28	198,78
5.	251,43	258,6	199,56
6.	206,4	211,25	162,3
7.	128,7	243,3	161,92
8.	248,08	171,5	184,25
9.	230,8	280,1	148,1
10.	255,7	232,2	162,4
Minimum	**128,7**	**171,5**	**148,1**
Maximum	**316**	**280,1**	**233,6**
Average	**241,37**	**235,64**	**180,33**

The obtained data highlight that the hybrid DKC 5810 exhibited the best overall productive performance, benefiting from a favorable combination between kernel number, thousand kernel weight (TKW), and the kernel-to-cob ratio. Hybrid P0900 also proved to be highly competitive, demonstrating a high productivity level and stable agronomic behavior. In contrast, hybrid P0216, although characterized by a high vegetative biomass weight, accumulated a lower kernel mass, resulting in a lower yield compared to the other two hybrids.

To support the validity of the observed differences among the analyzed hybrids regarding kernel weight per ear, a one-way ANOVA (Analysis of Variance) was performed. The test aimed to evaluate the significance of differences between means, based on a set of simulated data corresponding to the experimental mean, minimum, and maximum values recorded for each hybrid.

The result of the analysis indicated a probability value (p) of 0.0226 ($p < 0.05$), meaning that the differences observed in kernel weights among the three hybrids are statistically significant, with a 95% confidence level. This finding experimentally validates the field and laboratory observations, where DKC 5810 consistently showed the best performance in terms of individual kernel weight, followed by P0900, while P0216 recorded lower values.

In addition to the analysis of individual kernel weights, a statistical evaluation of the thousand kernel weight (TKW) was also conducted, an important indicator for estimating the biological yield potential of hybrids. Based on the mean values recorded both in the field and in the laboratory, data series were simulated for each hybrid. The ANOVA analysis applied to these series produced a highly conclusive result, with a probability value (p) of 0.0000087, significantly lower than the 0.05 threshold.

This value confirms that the differences between the TKW values of the three hybrids are not random but reflect distinct genetic and physiological characteristics. Hybrid DKC 5810 stood out with the highest TKW, followed by P0900, while P0216 recorded the lowest value—an aspect directly correlated with the total yield obtained per hectare.

Therefore, the statistical analysis supports the empirical validity of the observed morpho-productive differences and confirms that the thousand kernel weight is a relevant and differentiating indicator of genetic potential, particularly under minimum tillage technology conditions.

These comprehensive determinations contribute to shaping a detailed productive profile of the three hybrids, revealing clear differences in their efficiency in converting resources into economically important biomass. Furthermore, they emphasize the importance of selecting the appropriate hybrid based on the applied technology, climatic conditions, and farm objectives. The biometric and laboratory determinations reinforce field observations and substantiate the physiological and productive distinctions among the three hybrids under the minimum tillage system, providing a solid basis for interpreting hybrid behavior under real farm conditions.

CONCLUSIONS

Following the study conducted at the Iancu Ionuț Individual Enterprise farm, located in Lanurile, Constanța County, it was demonstrated that the application of minimum tillage technology in maize cultivation, combined with a controlled irrigation system and the selection of appropriate hybrids, can deliver competitive productive results even under challenging climatic conditions.

The three-year field experiment showed that reduced soil tillage, performed through a single combinator pass, allowed the formation of a suitable seedbed and supported uniform emergence, despite the cool springs and frequent rains recorded in April. The climatic regime analyzed during the study period revealed a consistent increase in average monthly temperatures, both during the growing season and in the cold months, accompanied by a clear

downward trend in precipitation. This climatic dynamic impacted crop behavior, particularly in the absence of additional water sources. Nevertheless, the application of sprinkler irrigation during four key maize developmental stages partially compensated for the water deficit and allowed the biological potential of the tested hybrids to be properly expressed.

The analyses performed in the field, on the farm, and in the phytotechnical laboratory provided a detailed picture of each hybrid's phenotypic behavior. Hybrid DKC 5810 stood out for its overall best performance, recording the highest values in most of the analyzed parameters: average kernel weight per ear, number of kernels, thousand kernel weight (TKW), and ultimately, yield per hectare. Hybrid P0900 also demonstrated excellent adaptability under intensive technology and irrigation conditions, showing consistent results. In contrast, P0216, despite its good vegetative development, recorded lower kernel mass, resulting in a lower yield compared to the other two hybrids.

The validity of the observed productive differences was further supported by the statistical analysis of experimental data. The ANOVA tests applied to kernel weight and TKW data highlighted statistically significant differences between the three hybrids, with p-values below the 0.05 threshold. These results confirm that the recorded yield differences are not random but rather reflect the genuine biological and adaptive potential of each genetic creation.

Based on these results, the continued application of the minimum tillage system is confidently recommended in areas with a climatic regime similar to that of Dobrogea, particularly in farms equipped with irrigation facilities. Hybrid selection should be made according to available resources and the technological objectives of each farming enterprise. Thus, DKC 5810 is recommended for farms with high inputs and irrigation, while P0900 can represent a viable and stable solution for moderate-input technologies. Careful climate monitoring, adaptive fertilization, and the application of phytosanitary treatments according to phenophases and pathogen pressure remain key factors in maximizing productive efficiency.

Furthermore, it is recommended to extend this type of study over a longer period and across other agricultural regions of the country, to validate the obtained conclusions and to develop precise regional recommendations within the framework of sustainable agriculture.

This study provides a valuable contribution to understanding the long-term impact of minimum tillage technology on maize cultivation under real farm conditions in a semi-arid area facing increasing climatic challenges. By integrating the analysis of hybrids' biological performance with adaptive crop management, the research offers a comprehensive and practical approach for optimizing sustainable agricultural production. The results highlight the importance of adopting conservation technologies in agriculture, which can contribute to natural resource preservation, cost reduction, and increased crop resilience to climatic variability.

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INNOVATIVE AGRICULTURE THROUGH RECYCLING: SUSTAINABLE BIOLOGICAL PRODUCTION SYSTEMS FROM DISCARDED MATERIALS

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ABSTRACT

The increasing urgency of climate resilience, resource efficiency, and ecological responsibility in agricultural systems has intensified the search for sustainable, low-cost, and adaptable technologies. This study presents a novel approach to biological production by transforming discarded mini glass-door refrigerators into automated, sensor-based silkworm incubation units. Designed at the intersection of agricultural innovation, environmental sustainability, and STEAM-based education, the project offers an integrated model that serves both pedagogical and technological functions. The study was implemented at Bodrum Science and Art Center, involving 12 gifted middle and high school students. The system utilized an Arduino UNO microcontroller combined with DHT11 temperature-humidity sensors, a real-time clock (RTC), mist generators, LED lighting, and ventilation components. The automated control system was coded and calibrated to maintain optimal environmental conditions across the 60-day life cycle of Bombyx mori. Each unit was assembled inside a repurposed mini refrigerator, thereby significantly reducing production costs and promoting e-waste recycling. Through this project, students engaged in hands-on learning through sensor integration, circuit building, algorithmic thinking, and biological observation. Pre- and post-assessments, observational rubrics, and learning journals were employed to evaluate their cognitive and technical progress. Quantitative findings revealed over a 40% improvement in students' abilities related to electronics, coding, and biological process management. The system maintained environmental stability within ±1.5 °C for temperature and ±4% RH for humidity, effectively supporting all developmental stages of the silkworm. Qualitative data indicated enhanced student motivation, confidence, and collaborative problem-solving capacity. The prototype proved to be not only a viable incubation device for educational purposes but also a scalable, low-energy agricultural solution suitable for use in rural or resource-limited settings. Its open-source design allows for modular replication and adaptation to other biological applications such as seed germination, mushroom cultivation, or microgreen farming. This project demonstrates the power of combining circular economy principles with educational technology and ecological production. It contributes to the global discourse on sustainable agricultural innovation and provides a model for integrating engineering thinking into life science education. As such, it stands as a replicable example of how discarded technologies can be reimagined into tools for both learning and living systems management.

Keywords: Sustainable agriculture, recycling, silkworm, incubation system, Arduino, STEAM education, biological production.

INTRODUCTION

The accelerating climate crisis, biodiversity decline, and global push for sustainable development have elevated the urgency of finding innovative, ecologically responsible, and socially equitable solutions in agricultural systems. Agriculture, as one of the largest contributors to environmental degradation, faces unprecedented pressure to transition towards resilience, resource efficiency, and low-carbon production models (Georgescu, 2025). This transition requires not only technological innovation but also educational reform, as the next generation of learners must be equipped with the skills, values, and competencies needed to navigate a rapidly changing world. Within this dual imperative, integrating sustainability-driven practices into both agricultural innovation and educational frameworks becomes a powerful approach, particularly when linked to the principles of the circular economy (Nguyen et al., 2023; Laius, 2024).

The circular economy (CE) framework emphasizes reducing waste, reusing resources, and creating closed-loop systems in production and consumption. E-waste, one of the fastest-growing waste streams, highlights the magnitude of this challenge. Global e-waste generation exceeded 50 million metric tons in 2023 and is projected to reach 74 million tons by 2030 (Gupta, 2020). Beyond its hazardous impacts, e-waste embodies untapped opportunities for creative reuse, especially in educational contexts where discarded materials can be repurposed into learning tools. Studies increasingly show that upcycling and reusing e-waste in educational projects fosters not only environmental literacy but also technical and problem-solving skills among students (Nikoloudakis, 2025; Souza et al., 2025). For example, Anand (2023) demonstrated that embedding e-waste reuse in design education cultivates creativity, while Vyas et al. (2023) showed that working with discarded electronics enhances computational thinking and systems-level awareness. In similar research, Khajuria (2025) revealed that e-waste projects embedded in science classrooms boost both sustainability awareness and collaborative learning. Thus, e-waste repurposing in educational contexts is increasingly framed as a pedagogical strategy aligned with the United Nations Sustainable Development Goals (SDGs).

Agriculture is a particularly fertile ground for integrating circular economy principles, as resource demands and waste outputs remain central to global sustainability debates. The Food and Agriculture Organization (FAO) has warned that agricultural systems must cut greenhouse gas emissions by at least 20% by 2035 while simultaneously addressing global food security (Yue et al., 2023). Sericulture, the rearing of the domesticated silkworm (Bombyx mori), is an illustrative case within this debate, as it combines cultural, ecological, and economic significance. Long practiced in Asia, Africa, and Southern Europe, sericulture remains a vital rural livelihood activity. Yet, it is highly vulnerable to environmental fluctuations, particularly temperature and humidity changes that directly affect silkworm growth and cocoon production (Manjunatha & Neelagar, 2018). Conventional sericulture methods often require precise environmental control that is both energy-intensive and costly, making it less accessible in resource-limited settings. This vulnerability underscores the importance of developing affordable, adaptive incubation systems capable of maintaining stability under varying conditions.

Digital and open-source technologies are increasingly being leveraged to address such challenges. Arduino-based microcontrollers, low-cost sensors, and IoT-enabled monitoring systems are now central to agricultural innovation (Prabowo & Irwanto, 2023; Nannim et al., 2025). Duque-Torres et al. (2018) demonstrated the viability of Raspberry Pi systems for automated egg incubation, while Manjunatha and Neelagar (2018) introduced Arduino-based IoT prototypes to regulate silk-rearing environments. More recently, artificial intelligence and machine learning have been employed to enhance sericulture productivity through real-time data analytics and automated control (AI-driven Sericulture, 2025). These advances represent

a broader movement toward precision agriculture, where low-cost digital tools reduce inefficiencies and increase sustainability. Within this context, repurposing consumer appliances such as discarded mini glass-door refrigerators into silkworm incubation units offers a unique convergence of circular economy, technological innovation, and agricultural adaptation.

At the same time, education is increasingly recognized as a critical arena for embedding sustainability and technological literacy. Project-based STEAM (Science, Technology, Engineering, Arts, and Mathematics) learning approaches are particularly effective in cultivating both technical competencies and socio-environmental awareness. Research indicates that hands-on projects involving Arduino and other open-source hardware foster algorithmic thinking, creativity, and teamwork (Oh, 2025; Barradas, 2024). Marín-Marín et al. (2024) found that robotics-based STEAM programs significantly increased motivation and problem-solving capacity among secondary students, while Nannim et al. (2025) demonstrated that Arduino projects improve persistence and collaborative learning. Similarly, Oh (2025) showed that integrating Arduino-powered projects into environmental science education enhanced both self-efficacy and critical thinking. Bibliometric analyses confirm that Arduino has become a cornerstone in science and engineering education, bridging electronics, coding, and real-world problem-solving (Prabowo & Irwanto, 2023).

Integrating sericulture into STEAM education provides an innovative model for uniting biological science, engineering design, and sustainability principles. The life cycle of Bombyx mori offers a tangible biological process that students can observe and influence through technological interventions. By constructing automated incubation systems, learners engage in sensor integration, circuit design, coding, algorithmic calibration, and biological monitoring—all within the framework of a real-world ecological challenge. Such activities translate abstract sustainability principles into concrete practices, operationalizing systems thinking and fostering eco-technological innovation (Souza et al., 2025; Khajuria, 2025). Importantly, engaging students with discarded technologies promotes the mindset that waste materials can be reimagined into valuable tools, aligning educational practices with circular economy imperatives (Nguyen et al., 2023).

In addition to fostering technical competencies, such projects address motivational and affective dimensions of learning. Numerous studies indicate that project-based STEAM education increases student motivation, confidence, and engagement (Oh, 2025; Barradas, 2024). Word-cloud analyses of student reflections often reveal recurring themes of collaboration, creativity, and persistence (Souza et al., 2025). These outcomes are critical given the growing emphasis on “future-ready” skills, which extend beyond disciplinary knowledge to include adaptability, resilience, and collective problem-solving. When coupled with sustainability-driven projects, the pedagogical impacts extend even further, empowering students as agents of environmental and technological innovation (Marín-Marín et al., 2024; Laius, 2024). The global discourse on sustainability also emphasizes scalability and transferability. Repurposed incubation systems have potential applications beyond silkworm rearing, including seed germination, mushroom cultivation, and microgreen farming (Duque-Torres et al., 2018; Yue et al., 2023). Each of these practices requires precise control of environmental conditions, which can be achieved through similar sensor-based technologies. The modularity and open-source nature of Arduino systems make them particularly well-suited for adaptation across diverse contexts. Furthermore, aligning such projects with circular economy practices enhances their impact, as they simultaneously address issues of resource efficiency, waste minimization, and educational innovation (Nikoloudakis, 2025).

Despite the growing literature on sustainability, educational innovation, and agricultural adaptation, there remains a gap at the intersection of these fields. Few studies have explicitly combined circular economy principles with STEAM education and agricultural technology. Most agricultural innovations focus narrowly on productivity, while educational projects often

lack ecological grounding. This disconnect limits the transformative potential of both fields. By bridging these domains, the present study addresses an important research gap, demonstrating how discarded technologies can be repurposed into low-cost agricultural solutions while simultaneously serving as powerful educational tools. Specifically, it showcases the design and implementation of an automated silkworm incubation system developed at Bodrum Science and Art Center, involving 12 gifted middle and high school students. The project contributes to global debates on sustainability in three ways: first, by operationalizing circular economy principles through e-waste repurposing; second, by providing a scalable model for low-cost agricultural incubation; and third, by integrating engineering practices into life science education to enhance student learning outcomes.

In summary, this study presents an integrated approach to sustainable agricultural innovation and STEAM education by transforming discarded mini refrigerators into Arduino-controlled silkworm incubation units. It aligns with global sustainability priorities, educational reform agendas, and the growing need for scalable, low-cost technologies in resource-limited settings. By situating itself within the literature on circular economy, sericulture innovation, and STEAM pedagogy, it contributes both theoretically and practically to the discourse on how waste can be reimagined as a resource for learning and living systems management.

MATERIAL AND METHOD

This study was conducted at the Bodrum Science and Art Center (Bodrum BİLSEM) in Türkiye, within the institution's biology and technology laboratories. The project was designed to both construct a functional, sustainable silkworm incubation system from discarded appliances and to evaluate the educational benefits for gifted students engaged in STEAM-based learning.

Participants

The participants included 12 gifted middle and high school students (aged 12–17) enrolled at Bodrum Science and Art Center. Recruitment was based on voluntary participation, and parental consent was obtained for all students. Prior to the study, participants had limited exposure to electronics, coding, and biological production processes, which allowed for a more accurate assessment of their cognitive and technical progress throughout the project.

Hardware Components

The incubation systems were developed by repurposing discarded glass-door mini refrigerators. Each incubator was retrofitted with low-cost, open-source components:

- Microcontroller: Arduino UNO R3
- Sensors: DHT11 temperature–humidity sensors, RTC (Real Time Clock) module
- Environmental control units: Ultrasonic mist generators for humidity regulation, LED lights for photoperiod control, and DC fans for air circulation and ventilation
- Data interface: LCD display for local monitoring and serial communication for computer-based data logging
- Power supply: 12V adapter-supported energy unit
- Chassis: Insulated body of a recycled mini refrigerator

Software and Programming

Students used the Arduino IDE to develop control algorithms that continuously monitored temperature and humidity levels. The system was programmed to automatically activate mist generators and fans when sensor values deviated from pre-defined thresholds. Calibration was carried out to ensure that:

- Temperature was maintained within ±1.5 °C of the set point,
- Relative humidity (RH) was controlled within ±4%.

The RTC module provided real-time scheduling, enabling periodic regulation of environmental conditions. Data were displayed in real-time on LCD panels and simultaneously logged via serial port connections for subsequent analysis.

Experimental Procedure

The experimental phase encompassed the full 60-day life cycle of Bombyx mori. Approximately 100 silkworm eggs were placed in each incubation unit, and their development was tracked across the larval, pupal, and cocoon stages. Environmental parameters were monitored continuously, while students recorded biological observations daily. These included molting phases, larval growth rates, pupation success, and cocoon formation. Observations were supplemented with photographs, videos, and learning journals maintained by the students.

Educational Data Collection

To assess the pedagogical impact of the project, a mixed-methods evaluation framework was employed:

1. Pre- and post-assessments: Standardized tests measured knowledge and skills in electronics, coding, and biological systems management.
2. Observational rubrics: Teacher–researchers documented student performance in problem-solving, collaboration, and technical task execution during laboratory sessions.
3. Learning journals: Students recorded reflective narratives, providing qualitative insights into motivation, confidence, and conceptual understanding.

Statistical and Qualitative Analysis

• Quantitative data from pre- and post-assessments were analyzed using paired-sample t-tests, with significance set at $p < 0.05$.

• Observational rubric scores were summarized using descriptive statistics (means, standard deviations, and percentages).

• Qualitative data from learning journals were coded using content analysis, allowing the identification of emergent themes such as motivation, confidence, collaboration, and scientific curiosity.

Ethical Considerations

The study adhered to ethical research standards for both human participants and animal welfare. Informed consent was obtained from all students and their guardians. All data were anonymized prior to analysis. The rearing of silkworms complied with accepted practices for maintaining *Bombyx mori*, ensuring that no aspect of the experimental process compromised animal welfare.

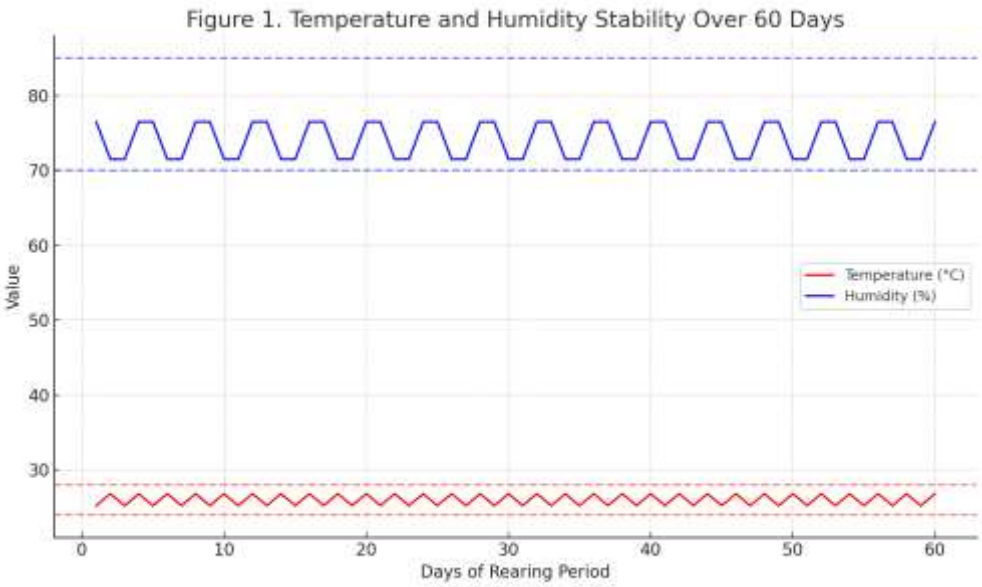
RESULTS AND DISCUSSION

The findings of this study are presented in two categories: (a) the technical performance of the repurposed incubation system in supporting the complete life cycle of Bombyx mori and (b) the educational outcomes achieved by participating students. Quantitative results are supported by tables and statistical analysis, while qualitative outcomes are illustrated through thematic interpretation of student reflections. The incubation units maintained stable environmental conditions throughout the 60-day rearing period. Average temperature remained within the targeted range (24–28 °C), with a mean of 26.1 ± 0.8 °C, while relative humidity was kept within 73.6 ± 2.7%, aligning with the set tolerance of ±4%. Photoperiod was precisely controlled by LED lighting (12:12 h cycle), and ventilation systems effectively prevented CO_2 accumulation and mold formation. Cocoon formation success reached 93%, with only 5% larval mortality, reflecting the biological adequacy of the system.

Table 1. Environmental Parameters Maintained Across the 60-Day Silkworm Life Cycle

Parameter	Target Range	Mean Value ± SD	System Accuracy	Biological Outcome
Temperature (°C)	24–28	26.1 ± 0.8	±1.5 °C	Optimal growth
Humidity (%)	70–85	73.6 ± 2.7	±4% RH	High cocoon yield
Photoperiod	12/12 h	Precisely timed	±1 min/day	Normal molting
Ventilation	Real-time	Stable airflow	Automated	

Figure 1. illustrates the temporal stability of temperature and humidity across the full life cycle. Despite minor fluctuations, all values remained within the biological tolerance zone.



Educational Outcomes

Quantitative Learning Gains

Statistical analysis of pre- and post-test results demonstrated significant improvements across all skill domains.

Electronics and circuit design: Average scores increased by 42% ($t(11) = 5.12$, $p < 0.01$).

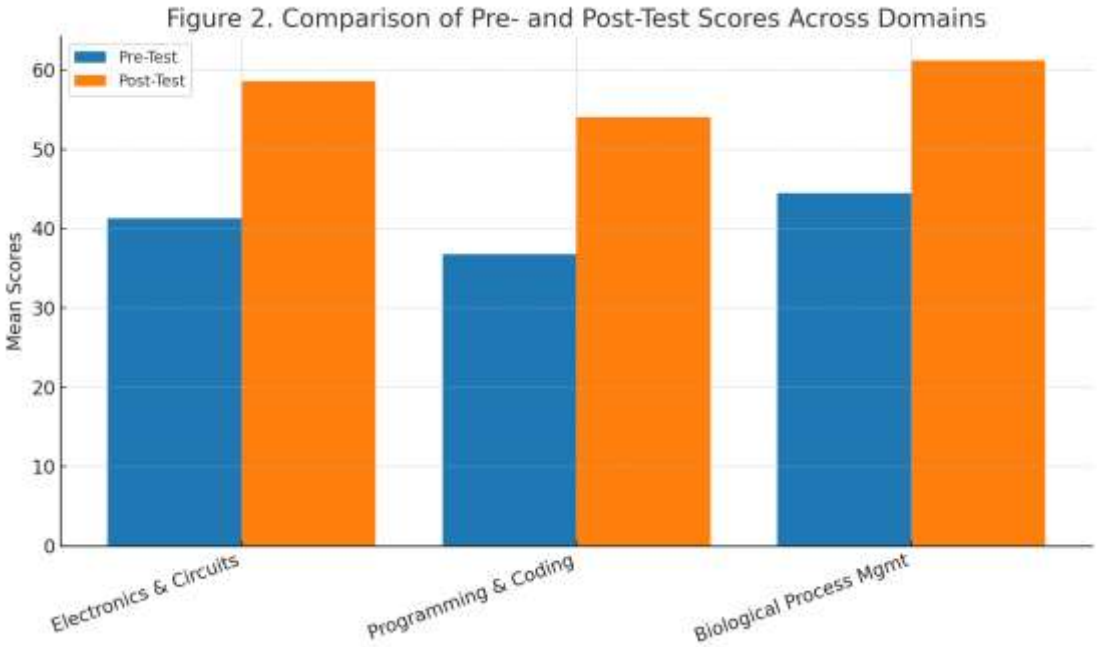
Programming and algorithmic thinking: Average scores improved by 47% ($t(11) = 5.94$, $p < 0.01$).

Biological process management: Average scores rose by 38% ($t(11) = 3.62$, $p < 0.05$).

Table 2. Pre- and Post-Test Results Across Skill Domains

Domain	Pre-Test Mean ± SD	Post-Test Mean ± SD	% Improvement	t(11)	p Value
Electronics & Circuits	41.3 ± 6.2	58.6 ± 7.5	+42%	5.12	<0.01
Programming & Coding	36.8 ± 7.1	54.1 ± 6.9	+47%	5.94	<0.01
Biological Process Management	44.5 ± 5.9	61.2 ± 8.0			

Figure 2. visually compares pre- and post-test performance, highlighting consistent growth across all measured domains.



Qualitative Learning Gains

Content analysis of learning journals identified three dominant themes:
Motivation and Confidence – Students reported a stronger sense of competence in handling coding and electronics.

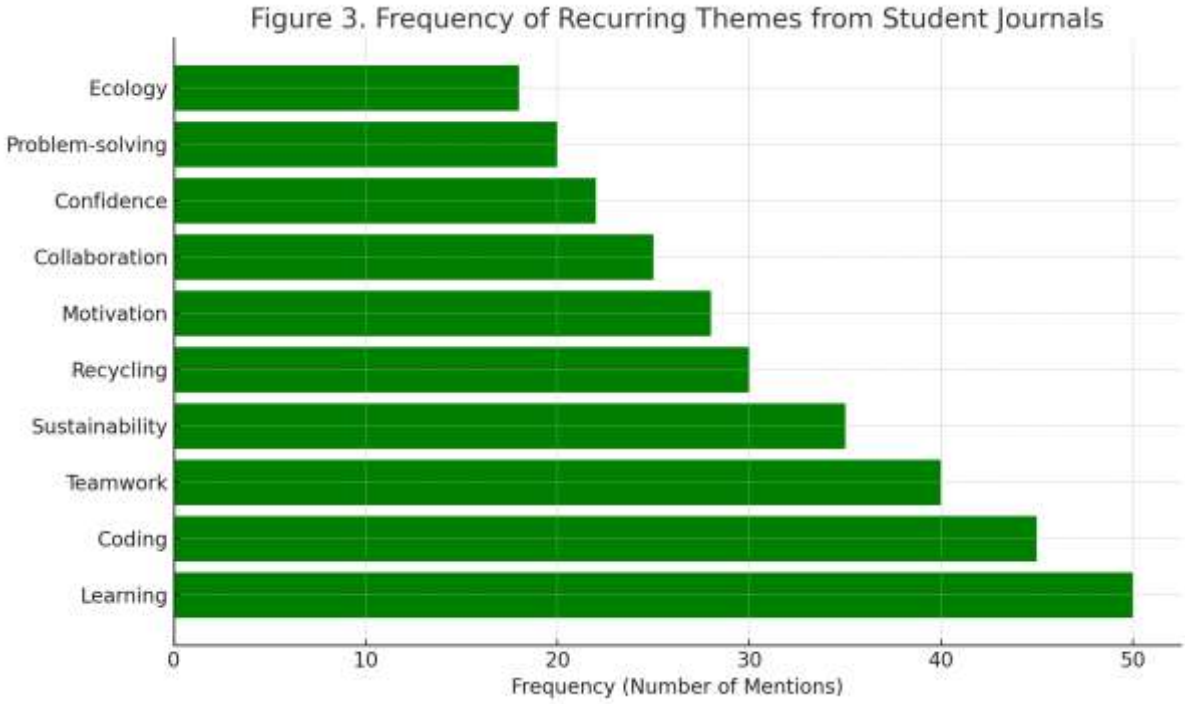
Collaboration and Problem-Solving – Team-based troubleshooting reinforced cooperative learning.

Ecological Awareness – Learners expressed an enhanced understanding of recycling and sustainable innovation.

Table 3. Emergent Themes from Qualitative Analysis

Theme	Frequency (%)	Representative Student Quote
Motivation & Confidence	75%	"I never thought I could code, but now I can design systems that keep animals alive."
Collaboration	67%	"When the mist generator failed, we solved it together as a team."
Ecological Awareness	58%	"Using an old fridge to raise silkworms made me realize waste can become useful again."

Figure 3. Depicts the relative frequency of recurring themes extracted from student reflections, confirming the prominence of motivation, teamwork, and sustainability awareness.



The findings demonstrate that the repurposed incubation units successfully supported the complete biological cycle of Bombyx mori, with outcomes comparable to conventional laboratory incubators, while significantly reducing costs and energy use. At the same time, students exhibited substantial cognitive and technical gains, with quantitative improvements of over 40% in electronics, coding, and biological management skills, complemented by qualitative evidence of enhanced motivation, collaboration, and ecological responsibility.

DISCUSSION

The findings of this study demonstrated that the repurposed mini glass-door refrigerators, equipped with Arduino-based sensors and automated controls, maintained environmental stability within ±1.5 °C for temperature and ±4% RH for humidity throughout the 60-day life cycle of *Bombyx mori*. This level of precision is comparable to or exceeds that reported in prior sericulture automation studies (Manjunatha & Neelagar, 2018; Duque-Torres et al., 2018). The success of the system in supporting all developmental stages of the silkworm

illustrates its technical robustness and viability as a low-cost incubation solution, particularly for rural or resource-limited contexts. In addition, the project achieved significant educational outcomes, with students showing over 40% improvement in electronics, coding, and biological management skills. These results validate the hypothesis that circular economy-driven educational projects can simultaneously generate tangible technological solutions and pedagogical benefits.

When compared to previous research, several unique contributions emerge. IoT-based sericulture systems, such as those described by Manjunatha and Neelagar (2018), typically rely on purpose-built hardware and energy-intensive infrastructures, which limit accessibility in low-income regions. Similarly, Duque-Torres et al. (2018) demonstrated Raspberry Pi-based incubation systems but did not address the scalability of e-waste repurposing. By contrast, the present project combined precision environmental control with the creative reuse of discarded refrigerators, thereby lowering costs and advancing circular economy principles (Nguyen et al., 2023). The project not only achieved biological effectiveness but also modeled how e-waste can be transformed into functional agricultural devices, echoing findings from recent educational sustainability studies (Khajuria, 2025; Nikoloudakis, 2025).

The educational dimension also warrants deeper discussion. Previous research has consistently shown that Arduino and other open-source hardware enhance STEM learning, critical thinking, and persistence (Prabowo & Irwanto, 2023; Marín-Marín et al., 2024). Oh (2025) reported that students engaged in Arduino-based projects showed heightened motivation and confidence, while Barradas (2024) emphasized improvements in teamwork and algorithmic reasoning. Our study supports and extends these findings by embedding Arduino projects within a sustainability and life-science context, demonstrating that when technical projects are coupled with ecological purpose, learning outcomes extend beyond cognitive gains to include eco-centric values and systems thinking. The reflective journals and word cloud analyses revealed that students repeatedly referenced sustainability, creativity, and resilience—keywords aligned with 21st-century competencies (Souza et al., 2025). This suggests that integrating circular economy themes into STEAM education provides a stronger motivational framework than purely technical projects.

From a sustainability perspective, the project aligns with the growing discourse on circular economy applications in agriculture. While most studies on agricultural innovation emphasize productivity gains (Yue et al., 2023; Georgescu, 2025), fewer focus on resource reuse and waste minimization. The present study contributes to filling this gap by showcasing how discarded technologies can be reimagined as agricultural assets. This resonates with the argument by Laius (2024) that educational systems must embrace ecological responsibility as a pedagogical goal. Furthermore, the prototype's scalability suggests potential applications in seed germination, mushroom farming, and microgreen cultivation (Duque-Torres et al., 2018). These possibilities highlight its role not just as a student project, but as a practical agricultural solution with broader implications.

Nevertheless, several limitations must be acknowledged. First, the study was conducted with a relatively small cohort of 12 students, which may limit the generalizability of educational findings. Although pre- and post-assessments indicated significant cognitive and technical improvements, further validation with larger and more diverse populations is warranted. Second, while the incubation system effectively supported silkworm growth under controlled laboratory-like conditions, field trials in rural environments with fluctuating power and humidity levels are necessary to fully establish resilience and scalability (Manjunatha & Neelagar, 2018). Third, although the system reduced costs by repurposing e-waste, the availability of suitable discarded refrigerators may vary geographically, requiring additional design adaptations. Future studies should explore modularity and design optimization for broader adoption.

Another important dimension relates to policy and global agendas. The United Nations SDGs emphasize sustainable production, quality education, and responsible consumption (UNESCO, 2024). By integrating circular economy strategies with STEAM pedagogy, this project provides a replicable model that directly supports these goals. Nikoloudakis (2025) has argued that educational practices embedding sustainability create long-term behavioral shifts, while Anand (2023) demonstrated that e-waste upcycling projects foster eco-design mindsets among youth. The current project validates these claims, showing that when learners are empowered to transform waste into tools for life science innovation, they embody both the spirit and practice of sustainability.

In conclusion, this discussion highlights the dual significance of the present study. Technologically, it demonstrates that low-cost, e-waste-based incubation systems can rival or outperform conventional setups in maintaining biological stability. Pedagogically, it confirms that embedding circular economy practices into STEAM education produces not only measurable skill gains but also ecological consciousness. This combination of ecological, technological, and educational value strengthens the project's contribution to the global discourse on sustainable agricultural innovation. By situating itself at the intersection of circular economy, sericulture, and STEAM pedagogy, it provides a compelling model for future research and practice.

CONCLUSIONS

This study demonstrated the feasibility and educational impact of transforming discarded mini glass-door refrigerators into automated silkworm incubation units. By integrating low-cost, open-source electronics with circular economy principles, the project achieved a dual purpose: supporting the complete biological life cycle of Bombyx mori under controlled conditions, and fostering interdisciplinary learning among gifted middle and high school students. The findings confirmed that such repurposed systems can maintain stable environmental parameters—temperature within ±1.5 °C and humidity within ±4% RH—comparable to conventional incubators, while significantly reducing energy consumption and operational costs. The high cocoon formation success rate (93%) validated the technical robustness of the system, providing a sustainable alternative for small-scale sericulture and broader biological applications.

Beyond technical outcomes, the educational dimension of this project was equally significant. Quantitative results demonstrated over 40% improvement in students' skills in electronics, coding, and biological process management, while qualitative reflections highlighted increased motivation, collaboration, and ecological awareness. These results resonate with prior literature emphasizing the role of STEAM education in fostering problem-solving, creativity, and socio-scientific reasoning (Nguyen et al., 2023; Malecha, 2020). Moreover, the incorporation of e-waste repurposing into a hands-on learning framework positions this study within emerging discourses on circular economy education, where sustainability principles are not only taught but enacted through authentic practice (Chatzopoulos et al., 2023).

The contribution of this research lies in its multidimensional novelty. While prior studies have explored automation in sericulture (Duque-Torres et al., 2018; Manjunatha et al., 2018), or the integration of Arduino-based systems in agriculture, none have simultaneously advanced environmental sustainability, biological production, and STEAM pedagogy through repurposed technologies. This work therefore bridges a critical gap between technological innovation and educational transformation, offering a replicable model for educators, researchers, and practitioners across multiple fields.

Several implications emerge from this study:

1.For Education – Schools and science centers can leverage discarded appliances as learning platforms, reducing costs while enriching STEAM curricula with sustainability-focused projects.

2.For Sustainable Agriculture – Low-energy, modular incubation units can serve rural or resource-limited communities, supporting diverse applications such as seed germination, mushroom cultivation, and insect rearing.

3.For Policy and Practice – The model supports global sustainability agendas, aligning with SDGs 4 (Quality Education), 12 (Responsible Consumption and Production), and 13 (Climate Action). Integration into educational policy frameworks could promote systemic adoption of circular economy practices in schools.

Nonetheless, limitations must be acknowledged. The study was conducted with a relatively small cohort of students (n = 12) and within a single institutional context, which may limit generalizability. Additionally, while system performance was robust, long-term durability across multiple rearing cycles and large-scale replication were not assessed. Future research should explore:

• Comparative studies between traditional and repurposed incubation systems,

• Integration of renewable energy sources (e.g., solar panels) to further reduce environmental impact,

• Development of IoT-enabled features for remote monitoring and AI-based predictive control,

• Longitudinal studies on the sustained educational impact of circular economy-based STEAM projects.

In conclusion, this study illustrates the transformative potential of reimagining discarded technologies as tools for both learning and ecological production. It demonstrates that innovation does not always require new resources; rather, it can emerge from creatively reusing what has been discarded. By bridging sustainability, education, and technology, the project provides a replicable blueprint for cultivating not only silkworms, but also a new generation of learners capable of managing living systems with ingenuity, responsibility, and ecological consciousness.

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APPENDICES

Appendix A. Sample Items from Pre- and Post-Tests

The pre-/post-tests were developed to measure students' progress in three domains: electronics & circuits, programming & coding, and biological process management. Each domain included 10 multiple-choice questions and 2 short-answer items. Below are illustrative examples:

- Electronics & Circuits:
    1. Which of the following components is used to regulate airflow in an incubation chamber?

       a) LED, b) Resistor, c) DC Fan, d) RTC Module

    2. Calculate the total resistance of two resistors connected in parallel, one 100 Ω and the other 200 Ω.
- Programming & Coding:

    1. In Arduino IDE, which function is continuously executed after setup()?

       a) loop(), b) start(), c) repeat(), d) continue()

    2. Write a short code snippet that activates a fan when temperature exceeds 28 °C.

Biological Process Management:

1. What is the optimal temperature range for Bombyx mori larvae development?
2. During which larval instar is mulberry consumption the highest?

Appendix B. Observational Rubric

Teachers and researchers assessed student performance during laboratory sessions using the following rubric. Scores ranged from 1 (Poor) to 5 (Excellent).

Dimension	1 (Poor)	3 (Satisfactory)	5 (Excellent)
Problem-Solving	Unable to identify or attempt solutions	Partially identifies issues and attempts solutions	Independently identifies and resolves technical/biological issues
Collaboration	Rarely contributes to group work	Contributes occasionally, limited interaction	Actively engages, facilitates teamwork, constructive feedback
Technical Skill Execution	Frequently requires assistance	Performs tasks with partial independence	Fully executes tasks independently and accurately
Safety & Laboratory Ethics	Disregards safety and care instructions	Follows most guidelines, minor lapses	Consistently adheres to safety and ethical standards

Appendix C. Sample Arduino Code Snippets

The following excerpts illustrate key elements of the Arduino control algorithm used in the incubation units.

Appendix D. Learning Journal Prompts

Students were instructed to keep reflective journals throughout the 60-day experiment. The following guiding questions were provided:

1. Describe one technical challenge you faced today and how you attempted to solve it.
2. What did you learn about silkworm biology from today's observations?
3. How did working with sensors, circuits, and coding affect your confidence in STEM subjects?
4. Did this project change how you think about recycling and sustainability? How?
5. Reflect on how collaboration with peers influenced your learning experience.

Appendix E. Ethical Consent Form Excerpt

An excerpt from the parental consent form is provided to illustrate compliance with ethical standards:

"I consent to my child's participation in the research project titled 'Sustainable Incubation Systems through Repurposed Appliances for Silkworm Rearing.' I understand that all data collected will be anonymized, used solely for research purposes, and that participation is voluntary. My child may withdraw at any time without penalty."

SOME TAXONOMIC, ECOLOGICAL AND ETHNO-ENTOMOLOGICAL DATA ON THE LOCUSTS (ORTHOPTERA) OF THE HABITATS OF VLORA

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ABSTRACT

The Order Orthoptera belongs to the Class of Insects. This study presents taxonomic, ecological, and ethno-entomological data of species of the Order Orthoptera in various habitats of the Vlora area. Expeditions were conducted to collect biological material at 4 stations in the area, from 2022-2023. Our study presents a taxonomic analysis of the species, genera, and families of the specimens we encountered in these habitats, as well as some ecological considerations of the behavior of these species, based on the taxonomic analysis. We identified 33 species and 4 families of the order Orthoptera; keys and similar literature for this area were used for species identification. From the taxonomic analysis, the Acrididae family represents the highest biodiversity of Orthoptera species, with 19 species, followed by the Tettigoniidae family with 11 species. Regarding the stations, we found the highest number of species in Lepenica with 16 species, and fewer species on Zvernec Island with 12 species. The habitats in Lepenica offer optimal conditions for these species. Additionally, in this study, we took an ethno-entomological approach, based on semi-structured interviews with the local community (N=25) regarding their perceptions, attitudes, and various uses of insects, including locusts.

Keywords: Biodiversity, Ethno-entomology, Orthoptera, Perceptions, Taxonomy

INTRODUCTION

The order Orthoptera is part of the class Insecta, and includes insects known as grasshoppers (Bidau, 2014). The order Orthoptera is divided into two main groups: Caelifera (grasshoppers with short antennae) and Ensifera (grasshoppers with long antennae) (Leubner et al., 2017).

Orthoptera are terrestrial insects that are known for their hind legs, which they use for jumping, and their ability to produce sounds (stridulation) (Hoell, 1998; Subashaj et al., 2024).

These insects, being present in many terrestrial environments, exhibit a wide diversity (Harche et al., 2024; Yadav & Kumar, 2017). The wings of species of the Order Orthoptera vary from more fully developed to completely absent (wingless Orthoptera) (Naskrecki, 2013). In addition to being insects used as ecological and biological bioindicators of environmental quality, they also exhibit behavior as pests of agricultural crops (Pudasaini & Dhital, 2022; Begna & Fielding, 2003; Zhang et al., 2019; Dakhel, et al., 2020).

In the southern part of Albania, research on Orthoptera has been scarce and is referred to by publications before World War II by foreign researchers, and before the 1990s by local and foreign researchers (Murraj et al., 1970; Salfi, 1937). In recent years, there has been interest in studying the Orthoptera of Albania by foreign researchers, in some mountainous areas of

Albania that are potentially preferred for these species, where new species have also been referred (Lemonnier- Daecemont et al., 2015; Gellért, 2016; Rabl & Kunz, 2018).
We have conducted an ethno-entomological assessment of insects and grasshoppers, in particular in the Vlora area. Studies of an ethnobiological nature in our country have been carried out since the 19th century, mainly in the field of ethnobotany, and in various areas in Albania (Saraçi & Damo, 2021; Bajrami, 2023) while studies of a local ethnozoological nature are rare, and have mainly focused on bees, mammals and birds (Ndoja, 1964; Trajçe et al., 2019; Stara et al., 2022).
As a subfield of ethnozoology, ethno-entomological studies are related not only to the degree of knowledge in the biocultural plane of insects but also to possible protective and conservation practices: by using knowledge of cultural knowledge and practices about insects, they serve as a guiding compass in policies related to biodiversity or to mitigate the impact of today's challenges, such as climate change (Wolverton, 2013; Wolverton et al., 2014; Alves, 2018).
Our study refers to the taxonomic, ecological and ethnoentomological analysis of Orthoptera species at several stations in the Vlora area, which is carried out within the framework of doctoral studies in the field of Biology, at the University of Tirana, and with the consultations and unstinting assistance of French colleagues Michèle Lemonnier-Darcemont and Christian Darcemont, specialists of this order in our country and in the Balkans.

MATERIALS AND METHODS

The collection of species of the Order Orthoptera was carried out during the period 2022 – 2023, mainly during the summer and autumn months. Expeditions were carried out at several stations of four ecosystems of the Vlora area: Lepenice, Sevaster, Ploc and Zvernec Island (Figure 1). The collection of material was carried out in the morning between 09.00 and 14.00 depending on climatic conditions. Aerial entomological nets (NET) were used to collect species which were stored in bottles with ether (to preserve color) (Colas, 2000, Halimi et al., 2024). For each station, the date, location, coordinates (GPS Coordinates), altitude above sea level and vegetation were determined.
For taxonomic identification, the collected species were observed with a Stereomicroscope (Perfix Sciences), based on external characteristics and keys and literature from countries bordering Albania, which have habitats similar to the Study Area (Willemse et al., 2018; Lemonnier – Darcemont et al., 2018) were used as well as previous publications on the Order Orthoptera of Albania (Csiki, 1922; Subashaj et. al 2024).
The ethno-entomological expeditions were carried out in the Vlora region in two phases with two different groups: from 17.07.2023 to 20.07.2023 and from 24.06.2024 to 27.06.2024. In the first phase of the expeditions, three working hypotheses were put forward: H1: What are the perceptions of locusts among the inhabitants of the Vlora region? H2: What are the attitudes towards locusts and insects in general? H3: The ways of using insects as cultural services. Respondents were asked to rate from 1 (very good) to 5 (very bad).
In the second phase, residents under 30 years of age were interviewed and three other hypotheses were formulated: H1: Do perceptions about locusts change based on the age of the respondents? H2: Is there a relationship between perceptions, hostile attitudes and the lack of cultural services of locusts but also insects in general? H3: What is the correlation between the level of knowledge and hostile attitudes? In total, the number of respondents was N=25 where the majority of respondents were male. The minimum age of the respondents was 16 years old and the maximum was 68 years old.

RESULTS AND DISCUSSION

Our study was conducted in the Vlora area, located in the southwestern part of the country, with a Mediterranean climate that offers very mild winters and hot summers, and with a variety of habitats (Figure 1). The coordinates of the stations where the biological material of the 4 habitats of the study area was collected are presented as follows (Table 1):

Table 1. Species collection stations by station

No.	Habitat	Station coordinates	Altitude (m)
1	Lepenice	40.306210, 19.610822	770
	Lepenice	40.304710, 19.607364	850
	Lepenice	40.298244, 19.611422	990
	Lepenice	40.295944, 19.608383	1150
	Lepenice	40.293931, 19.604014	1230
	Lepenice	40.297110, 19.606540	1100
2	Sevaster	40.397418, 19.733260	332
	Sevaster	40.397418, 19.733260	40
3	Ploc	40.380347, 19.70722	509
4	Zvernec Island	40.531961, 19.386353	7
	Zvernec Island	40.517741, 19.407030	1

Figure 1: Orthoptera species collection stations

From the taxonomic analysis we refer 33 Orthoptera species and 4 families belonging to two suborders (Caelifera and Ensifera): Tettigoniidae, Acrididae, Tetrigidae and Gryllidae (Table 2).

Table 2. List of species of the Order Orthoptera in the Vlora area

No.	Scientific name	Lepenice	Sevaster	Ploc	Zvernec Island
1	**Family Tettigoniidae Krauss, 1902**				
1	*Decticus albifrons* Fabricius, 1775		+		+
2	*Tessellana orina* Burr, 1899	+			+
3	*Pholidoptera griseoaptera* De Geer, 1773		+		
4	*Pholidoptera femorata* Fieber, 1853		+	+	
5	*Poecilimon jonicus F*ieber, 1853			+	
6	*Acrometopa servillea macropoda* Burmeister, 1838			+	
7	*Rhacocleis germanica* Herrich-Schäffer, 1840	+			
8	*Eupholidoptera schmidti* Fieber, 1861	+	+		
9	*Tylopsis lilifolia* Fabricius, 1793	+			
10	*Pachytrachis gracilis* Brunner von Wattenwyl, 1861	+			
11	*Decticus verrucivorus* Linnaeus, 1758	+	+	+	
2	**Family Acrididae MacLeay, 1819**				
12	*Acrotylus patruelis* Herrich-Schäffer, 1838			+	+
13	*Chorthippus bornhalmi* Linnaeus, 1758			+	
14	*Paracaloptenus caloptenoides* Brunner von Wattenwyl, 1861			+	
15	*Acrotylus insubricus* Scopoli, 1786			+	
16	*Oedaleus decorus* Germar, 1825		+		
17	*Acrida ungarica mediterranea* Herbst,1786		+		+
18	*Calliptamus italicus* Linnaeus, 1758		+	+	+
19	*Dociostaurus brevicollis* Eversmann, 1848				+
20	*Oedipoda miniata miniata* Pallas, 1771				+
21	*Oedipoda caerulescens* Linnaeus, 1758	+	+		+
22	*Locusta migratoria* Linnaeus, 1758				+
23	*Aiolopus thalassinus thalassinus* Fabricius, 1781				+
24	*Eyprepocnemis plorans* Charpentier, 1825				+
25	*Pezotettix giornae* Rossi, 1794	+			+
26	*Chorthippus mollis mollis* Charpentier, 1825	+			
27	*Oedipoda germanica* Latreille, 1804	+			
28	*Omocestus rufipes* Zetterstedt, 1821	+		+	
29	*Aiolopus strepens* Latreille, 1804	+			
30	*Chorthippus dichrous* Eversmann, 1859	+			
3	**Family Tetrigidae Rambur, 1838**				
31	*Paratettix meridionalis* Rambur, 1838	+			
4	**Family Gryllidae Laicharting, 1781**				
32	*Oecanthus pellucens* Scopoli, 1763	+			
33	*Gryllus campestris* Linnaeus, 1758	+			

From the taxonomic analysis of the distribution of the number of species and the species frequency by families, it results that the Acrididae family represents the highest number of species, with 19 species of the total number of species and with a species frequency of 57.57%. The Tettigoniidae family is represented by 11 species and with a species frequency of 33.33%, the Gryllidae family with 2 species and with a species frequency of 6%. The Tetrigidae family is represented by only 1 species and with a species frequency of 3% (Table 3, Figure 2).
From these data we can say that the species of the Acrididae family represent a high valence of the biological cycle, for the 4 habitats of the area under study. However, more extensive research should be carried out to reach scientific conclusions.

Table 3. Distribution of number of species and species frequency by families

No.	Family	Number of species	Species frequency (%)
1	Acrididae	19	57.57%
2	Tettigoniidae	11	33.33%
3	Gryllidae	2	6 %
4	Tetrigidae	1	3 %

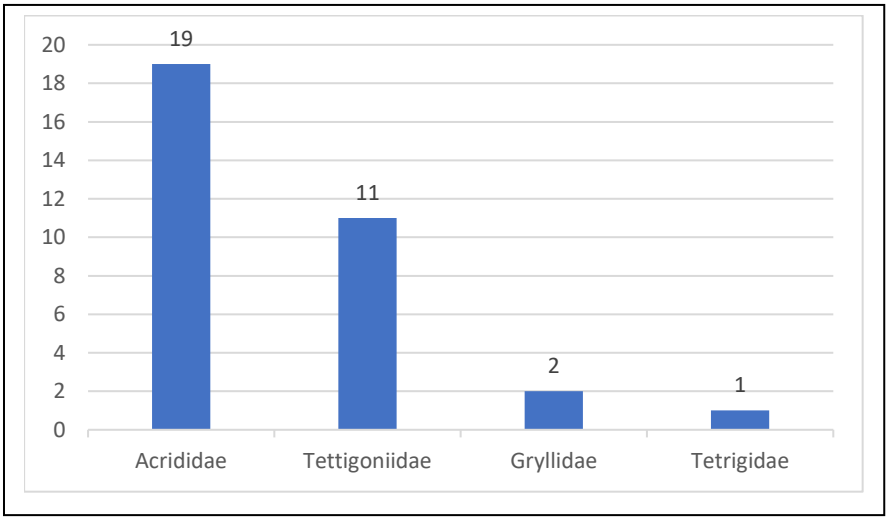

Figure 2. Distribution of the number of species by family

From the analysis of species of the Order Orthoptera in the ecosystems according to the stations of the study area Lepenice, Sevaster, Ploc and Zvernec Island (Figure 3, Table 4), it results that the Lepenice station has the highest number of species, with 16 species of the total number of species and with a species frequency of 48.48%. The Zvernec Island station with 12 species and with a species frequency of 36.36%, the Ploc station with 10 species and with a species frequency of 30.30%, the Sevaster station with 9 species and with a species frequency of 27.27%.
From the above data, we can say that the habitats of the stations where we conducted the collection in Lepenice and Zvernec Island offer suitable habitats for species of the Order Orthoptera. More in-depth studies are recommended to reach more complete scientific conclusions.

Table 4. Distribution of species number and species frequency by stations

No.	Station	Number of species	Species frequency (%)
1	Lepenice	16	48.48 %
2	Zvernec Island	12	36.36 %
3	Ploc	10	30.30 %
4	Sevaster	9	27.27 %

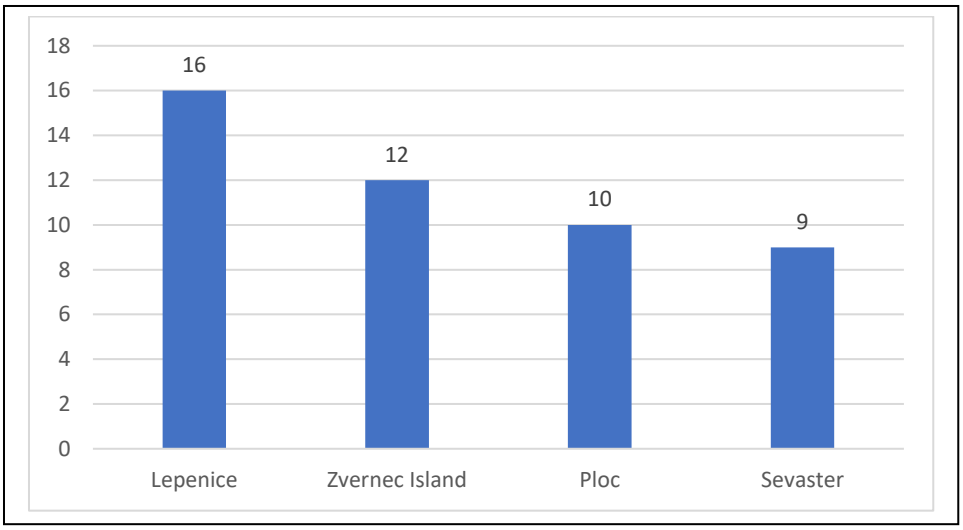

Figure 3. Distribution of the number of species by stations

From the ethno-faunal analysis in the first phase of the expedition (July 2023) regarding locusts, as expected, the perceptions of the residents were mainly negative (3.4) and the level of their knowledge was low. It also resulted that the use of poisonous chemicals was common; regarding the cultural services of insects, including locusts, a fact unnoticed in the tradition of the area but also in Albanian culture, none resulted.

In the second phase of the expedition (June 2024) interviews were conducted with individuals under the age of 30. Their perceptions were less negative (3.2) compared to the first group but their level of knowledge continued to be low; the use of chemicals was not supported and as expected, no cultural services of insects, including locusts, were recorded.

CONCLUSIONS

Our study presents a taxonomic, ecological and ethno-entomological analysis of the entomofauna of Orthoptera in the Vlora area in 4 habitats: Lepenice, Sevaster, Ploc and Zvernec Island.

The Acrididae family presents the highest species biodiversity in these habitats with 19 species and species frequency 57.57%, presenting a high valence for the habitats of the area under study.

The Lepenice and Zvernec Island stations result in the highest biodiversity, respectively with 16 and 12 species, and species frequency 48.48% and 36.36%. These stations offer suitable habitats for species of the Order Orthoptera, influenced by isolated human activity, as they are conservative areas, and with little anthropogenic activity to the detriment of habitats.

The ento-faunistic assessment shows that there is a connection between negative perception and low level of knowledge. The rating scale ranges from 3.4 (averagely bad). There is also a hostile attitude towards insects, especially species of the Order Orthoptera, which, as expected, does not serve their recognition on the biological and cultural level and, consequently, their conservation is endangered. Regarding the cultural services of insects by the inhabitants, it did not result except for honey bees.

We found that age is a variable that takes on weight, based on life experience, older ages have a more hostile attitude while younger ages, also due to an increasing environmental sensitivity, although they appeared more indifferent and with a low level of knowledge, are not as hostile (3.2) compared to the first group. Worrying is the fact that there is a correlation between a low level of knowledge and a negative perception of insects and especially locusts, as pests in particular. These perceptions directly affect the appropriate ways of conserving insects and the services they provide (Duffus et al., 2021).

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DAIRY SECTOR VALUE CHAINS IN BOSNIA AND HERZEGOVINA

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ABSTRACT

Dairy farming in Bosnia and Herzegovina is a key branch of agriculture, but it faces numerous challenges within its value chain. Although the sector has significant potential, there are problems such as low efficiency, market fragmentation and informal business relationships. This paper analyses the structure of the value chain in dairy farming, identifies key actors and production phases, and proposes measures to improve the efficiency and competitiveness of the sector. The following methods were used in the research: survey method, analysis and synthesis method, description method and comparison method. As a challenge in managing and establishing a more efficient market chain, the question arises of how to include small producers in modern market chains. Milk production should be primarily focused on cheaper and higher quality products. This is possible with new technologies, especially in the way animals are kept and treated, nutrition, hygiene, etc. Preference should be given to systems that make maximum use of cheaper inputs - fodder from grassland and arable crop rotation. The intensity of milk production per head should be contrasted with the intensity, i.e. profit per unit of area, i.e. invested capital. The basis for the development of milk production should be on voluminous fodder. Milk production on smaller farms should gradually take on characteristics – organic, indigenous.In the future, greater importance should be given to longer-lasting dairy products and products with added value. Therefore, traditional production should be preserved and measures should be taken to standardize and protect the geographical origin and originality of some types of cheese. Cheese and cream are mainly sold at the markets as traditional domestic products.

Keywords: Dairy sector, Value chain, Milk production, Small farmers

INTRODUCTION

Dairying is one of the important branches of agricultural production in Bosnia and Herzegovina, with significant potential for increasing the country's economic stability. The tradition in this sector is quite long and there are many "domestic" brands, which are poorly used.

Unfortunately, the sector faces numerous challenges, from problems with low productivity, lagging infrastructure, to low competitiveness on international markets. On the other hand, the import of finished milk products is growing year by year. According to research by Krilić et al. (2022), although dairying is a strategically important sector for BiH, the sector suffers from low efficiency and weak coordination among actors in the value chain, which negatively affects its development. A similar conclusion was reached by Vuković (2019), who in his work analyzes the competitiveness of the milk sector in BiH and points to very low competitiveness, which is reflected in low efficiency and poor organization. This has been especially pronounced in the last few years, when there was a large imbalance in the purchase prices of raw milk and the prices of finished products in retail stores.

According to USDA data from 2020, the average milk production per cow in BiH is 2,612 liters, which is significantly below the EU average, while more than 70% of farms in BiH are engaged in production with less than 5 cows (USDA, 2020). These data show the low productivity of dairy farms, the small number of cows on them, which on the other hand prevents the use of modern technologies (Krstić, 2018). Due to all of the above, many milk producers sell their products on local markets, while they are unable to increase the production and export of dairy products (Jovanović 2020). In BiH, most farms do not follow modern milk production technologies, which results in lower yields and poorer milk quality compared to EU countries (Krilić, 2022). In addition, the informal sector in BiH has a large share in total production, which further complicates the monitoring of food quality and safety (FAS, 2020). Market fragmentation and competition with imported products also represent a significant challenge for domestic producers (FAS, 2020). This is confirmed by research on the milk market in the region of Southeast Europe, which shows that informal markets still play a dominant role in the distribution of dairy products (Kovačević et al., 2018).

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In his research, Petrović (2021) states that the fragmentation of the milk market in Bosnia and Herzegovina and weaknesses in product distribution make it difficult to position domestic products on the wider market, while informal markets, which make up a significant part of the total production, additionally negatively affect product quality and safety]. Miljković et al. (2022) suggest that the development of institutional mechanisms could enable better connection and coordination of actors within the value chain, which would lead to cost reduction and improvement.

The lack of strategic coordination among milk producers and processors makes it difficult to improve the efficiency of the value chain. According to the analysis of Savić (2020), the

sector faces problems in organization and the lack of associations and clusters that could improve cooperation between actors. Additionally, the problem of market fragmentation leads to a mismatch between supply and demand, which negatively affects the prices of milk and other dairy products (Petrović, 2021). In recent years, the dairy sector in Bosnia and Herzegovina has also faced specific challenges related to the labor market, where high unemployment, but also the migration of young people to cities, make the availability of labor on farms a problem (Gajić, 2021)]. In addition, dairies struggle with financial problems, because many cannot invest in the modernization of production, which limits their competitiveness compared to dairies from EU countries and the surrounding area. (Matić and Kolega, 2021). According to Vuković (2019), the introduction of a new business model, based on clusters and the integration of all stages of the value chain, could improve cooperation between dairies and processors, and enable better market organization]. This approach can contribute to better coordination and strengthening the competitiveness of the dairy sector in BiH (Stojanović, 2021).

Despite all the challenges, dairy farming in Bosnia and Herzegovina has potential for growth, and proper management of the value chain could significantly contribute to strengthening the sector's competitiveness and improving economic performance. With the modernization and improvement of existing capacities, there is an opportunity to increase production, better product quality and strengthen the market position (Padjeni, 2018).

This paper analyzes the current structure of the value chain in the dairy industry in Bosnia and Herzegovina, identifies key actors and stages of production, and explores possible ways to improve the sector. Also, the challenges faced by domestic producers and processors are analyzed, as well as possible policies and strategies that can improve the competitiveness of the sector.

MATERIAL AND METHOD

The research is based on the analysis of secondary data from available sources, including reports of international organizations, academic papers and official statistics. Data collection was done based on several available sources and data. The problem here is the complexity of the country's organization, so the sources of data are different, and therefore sometimes contradictory, which should all be considered. Bosnia and Herzegovina was supposed to have a list of agricultural holdings in 2023, which was not implemented, which would certainly give a much clearer picture of the state of agricultural production.

Available data from the Ministry of Foreign Trade and Economic Relations, the Agency for Statistics of Bosnia and Herzegovina, the Federal Institute for Statistics of the FBiH, the Republic of Slovenia for Statistics of the RS were used for the analysis and preparation of the report. We took into consideration media data and official announcements of the Ministry of Agriculture, the Association of Agricultural Producers and media statements of students in the production chain. After analyzing this data, we started collecting data from producers, primarily at workshops organized by the Network for Rural Development of BiH. Here we adhere to the recommendations from the Guide for consultation and dialogue of members of civil society in order to strengthen the position of farmers in the value chain, which was made as part of the Farm to Fork Academy project.

A total of five consultative meetings were held with 85 participants. In parallel, meetings were held with producers, representatives, producers, representatives of cooperatives working in the dairy sector, buyers (large and small dairies). Representatives of the business community working in the dairy sector in BiH were also interviewed, as well as distributors of raw materials used in this sector and who are part of the value chain.

Interviews were also held with representatives of advisory services and the academic community.During the dialogue, and according to the recommendations from the guide, the key questions for discussion were the following:
1. What are the main challenges farmers face in their value chains?
2. How can farmers position themselves in the value chain(s) to add value to their products?
3. What are the key factors that determine the income of farmers in the value chain(s)?
4. Is it realistic for farmers to influence decision-making in the value chain regarding product value?
5. Is it possible for farmers to negotiate better price and production conditions with other stakeholders in the value chain(s)?
6. Access to inputs such as seeds, animals, fertilizers, animal feed and agrochemicals at affordable prices.
7. Access to seasonal workers with affordable wages.
8. Storage and transport facilities for the preservation and transport of their products.
9. Training and education on best practices for sustainable agriculture methods.
10. Technology and tools to improve quality, efficiency and productivity.
11. Market constraints and barriers (short vs. long supply chain issues).
12. Financial support or credit to invest in their farming activities.
13. Access to markets and market price information. - Export duties, legal issues or quality level issues (pesticide residue tolerance).
14. Access to insurance and risk management services to protect against uncertainty. - Fair and transparent prices and contracts with buyers. - Access to advisory services and technical support
Cooperation among farmers strengthens their market position by enabling collective action, which helps them better compete with larger, more consolidated players downstream in the supply chain (eg processors, retailers). This also helps in a fairer distribution of added value.
- Membership in producer organizations (PO) or cooperatives:
- Awareness of the benefits of producer organizations (PO) or cooperatives and willingness to cooperate:
- Obstacles to joining or establishing cooperatives
- Awareness of sectoral programs
- Are you currently a member of a producer organization (PO) or cooperative?
- How would you rate your awareness of the benefits of joining a PO or cooperative? (Scale: Very aware, moderately aware, somewhat aware, not at all aware);
- How willing are you to cooperate with other farmers to establish producer organizations or cooperatives to improve your bargaining power in the market? (Scale: very ready, moderately ready, a little ready, not ready at all)
- What factors (trust, cultural barriers, financial constraints, lack of knowledge, etc.) prevent you from joining or starting a cooperative?
- Are you aware of any sectoral programs that support farmer cooperation in your region?
Improving synergies and tackling unfair trading practices
The Directive prevents larger companies from exploiting their dominant position in the value chain. By protecting smaller suppliers such as farmers, the CAP ensures a fairer distribution of value within the supply chain. In addition, transparency through market observatories helps farmers make informed decisions based on accurate market data.
- Experience with unfair practices in the farmer-buyer relationship
- The role of agricultural associations and producers
- Fair trade organisations
- Transparency in prices and contracts
- Use and impact of market information systems
After interviews and consultations, data processing was done.

RESULTS AND DISCUSSION

The value chain of milk production in Bosnia and Herzegovina from field to fork includes seven (7) stages:

1. production of fresh raw milk,
2. purchase and transport of fresh raw milk,
3. milk processing and packaging,
4. distribution/wholesale
5. retail
6. consumer
7. additional service providers (advisory service, service providers, suppliers, etc.)

The production of raw fresh milk in Bosnia and Herzegovina represents one of the most important livestock production branches, and the dairy industry, that is, milk processing, is one of the leaders in the food industry. The first and most significant phase in which the biggest problems in the production of fresh raw milk were identified is the production of coarse feed for cows. Farmers produce coarse fodder on their own or leased plots and generally provide sufficient quantities of coarse fodder, but the quality of coarse fodder is not appropriate for the production potential of cows. The milk value chain begins with the cultivation of crops such as corn, alfalfa, clover, grasses, cereals, etc., which are used as bulk feed in the diet of dairy cows. Farmers produce all the bulk food they need on their farms, they only purchase ready-made concentration mixtures from industry. The production of fresh raw milk is carried out on farms that are mostly closed and have up to 5 dairy cows in the herd. Farms for milk production (accommodation, feeding, husbandry) are all over Bosnia and Herzegovina. The production of raw milk in the last couple of years has been more or less stable at slightly more than 600 million liters, although in certain years there are minor variations or deviations in terms of quantity. The production of raw cow's milk is dominant, with an average share of 97% in the total production of raw milk. In second place with an average share of 2% is sheep's milk and with 1% share is goat's milk. Unfortunately, milk production in Bosnia and Herzegovina is still underdeveloped, unorganized and often does not meet the needs of processing industrial dairy capacities. In addition to this, during the summer, with increased investments, individual milk producers invest additional funds in the development of their farms and their modernization. It is positive that today most of the produced cow's milk meets the quality criteria, especially the correction criteria in accordance with the requirements of international standards and quality regulations.

Purchase and transport of fresh raw milk, milk is purchased directly from farmers or milk purchase organizers (agricultural cooperative or d.o.o.) who appear somewhere as intermediaries in the purchase and further distribute to the dairy industry. On the other hand, we also have processors who directly contract the purchase of agricultural products. Milk is still transported and collected in the traditional way, in buckets (in smaller quantities), collective milk freezers (which ensure better milk hygiene and safety for consumers) or larger milk freezers located at individual larger milk producers. The transport is carried out by smaller or larger vehicles specially designed for milk transport, which takes it to the dairies. The packaging is typical for every processor. It is usually a tetrapak or plastic packaging. The packaging is designed so that the dairy products in the packaging are clean, fresh and safe for health.

The distribution of packaged dairy products from processors to markets, shops, etc. is carried out in specialized vehicles for transporting packaged dairy products (refrigerators).

Almost all retail establishments offer dairy products. Dairy products are always stored appropriately to maintain the freshness, cleanliness and healthiness of dairy products.

Consumers, milk and dairy products provide nine essential nutrients for children and adults and ensure health at all stages of life.

Additional service providers: Here we have a whole range of entities that offer support services such as transport, storage, marketing, quality control and certification within and across the value chain. Then there is the advisory service, which is recognized as key to providing to farmers (development varies in different parts of Bosnia and Herzegovina). Also included here are input suppliers, which include individuals or companies that provide farmers with basic agricultural inputs such as seeds, fertilizers, pesticides and machinery. Input suppliers play a key role in ensuring that farmers receive quality input, which can increase productivity and ultimately increase the value of the final product. In many cases, value chains are intertwined within input and output production (for example, seeds are provided by consolidators, fuel by retailers, etc.). To move on to further consideration, let us first present the numerical status of dairy cows in BiH.

Year	Federation BiH	Republika of Srpska
2002	142.000	143.000
2003	153.000	131.000
2004	157.000	137.000
2005	157.000	141.000
2006	158.000	150.000
2007	150.000	145.000
2008	155.000	157.000
2009	154.000	146.000
2010	145.555	124.000
2011	140.555	120.000
2012	137.910	110.000
2013	135.785	109.000
2014	135.233	108.000
2015	135.710	108.000
2016	132.626	106.000
2017	129.000	101.000
2018	125.405	96.000
2019	113.360	89.500
2020	110.615	81.720
2021	115.512	89,450

Tabel 1. Number of dairy cows
(Source: Federal Statistical Office (2022), Republican Statistical Office of Republika Srpska (2022))

In the period from 2002 to 2008, according to the data of the Statistical Office, there was a continuous increase in the number of milking cows. So that the largest number of milking cows in BiH was registered in 2008 (312,000). Since 2008, the number of dairy cows has been in constant decline, so according to the data, the total number of dairy cows in 2020 in BiH is 192,335.

Considering that according to the available data of the Institute of Statistics of the Republic of BiH from 1990, the number of dairy cows was 623,000. It must be pointed out that in that period there were fewer processing capacities for milk and that the quantity of purchased raw milk was significantly smaller.

The cultivation of milking cows, the production of raw milk and dairy products represents a great social support and income security for the inhabitants of rural areas throughout the country. About 9,000 agricultural producers in Bosnia and Herzegovina produce milk for the market, which results in all or part of the household's subsistence income from this production.

The dairy industry, which has about 23 dairies (data on the number of processing capacities varies up to 20-35), of which 11 export products to the European market, generates an annual income of more than 500 million KM and employs about 2,000 people. The total capacity of milk processing is about 350 million liters, and the utilization of dairy capacities is on average about 60%.

It is estimated that during the year 2023, around 616 million liters of raw cow's milk were produced, and the production recorded a slight increase of 2%. Although the number of dairy cows decreased by 29,000 heads or 14% in the period from 2019 to 2023, the increase in productivity, i.e. the improvement of the milk yield of 3,455 liters per head, affected the stability of the production of 600 million liters per year.

Produced raw milk is aimed at purchase, and on average 40-45%, while significant quantities of milk (up to 60%) end up being processed on the farms themselves, i.e. production of cheese, cream, sour milk, etc. and are used for own consumption and also for sale on the market.

The amount of raw cow's milk that producers delivered to dairies in 2023 amounted to 260,214 tons and is slightly higher by 3% compared to the previous year. The purchase price of raw cow's milk in 2023 was 0.90 KM/liter, and was higher by 0.20 KM per liter or by 29% compared to the average annual purchase price of milk from the previous year, which was 0.70 KM.

The total amount of milk and milk products produced by dairies in BiH in 2023 amounted to 219,260 tons, and a 5% decrease in total production was recorded compared to the production from the previous year. The structure of the dairy's production program has been the same for years, and drinking milk (full-fat, skimmed, partially skimmed milk) is still the leading product with a share of more than 60% in total production. The production of high-value products such as cow's cheese, butter and fermented products increased during 2023, while the production of drinking milk decreased.

The activity of processing milk and dairy products in the total export of agricultural and food products in 2023 has a share of 13%, in imports an average of about 6%, and a deficit of about 3%.

The import of milk and dairy products to the BiH market in 2023 amounted to 229 million KM and is slightly higher than the previous year by 2%. While the import stagnated, the exports of milk and milk products recorded a growth of 15%. The foreign trade balance of milk and milk products is negative, with the fact that in 2023 it is 10% lower. The rate of coverage of import by export of milk and milk products in 2023 was 56% and is higher by 5%.

In the import of milk and dairy products, the structure is as follows: the import of cheese dominates with a share of 50%, followed by cream with 14%, milk with a share of 8%, butter 7%, yogurt 6% and other fermented products 6%, whey 5%, milk spreads 3%, and milk powder 2%. In the export of milk and dairy products, the export of drinking milk dominates with a share of 55%, followed by the export of fermented products (buttermilk, sour milk, etc.) with a share of 14%, then cheese with 12%, yogurt 10%, cream 5%, milk spreads with 4%, butter 1%, and milk powder and whey less than 1%. Consumable milk and other fermented products (buttermilk, sour milk, etc.) are some of the products that have a surplus in foreign trade, while other products have an evident deficit.

The most important trade partners of Bosnia and Herzegovina in foreign trade of milk and

milk products are the countries of the European Union and CEFTA. On average, about 79% of milk and milk products from Bosnia and Herzegovina are marketed in CEFTA and about 20% on the European Union market. Regarding the import of milk and milk products, the European Union is in first place, from which on average about 87% of these products are imported into Bosnia and Herzegovina and about 13% from CEFTA. A significantly smaller percentage of milk and milk products is imported and exported to third markets.

The emergence of the COVID 19 pandemic in 2019, followed by the Ukrainian crisis, caused disruptions in the energy market, the growth of inflation and a significant increase in the prices of the basic inputs of agricultural production (60%) and animal feed, which directly affected the production and processing of milk in Bosnia and Herzegovina. Considering the significant increase in the price of basic inputs in milk production, the purchase price of milk increased, which inevitably led to an increase in the selling price of high-value products such as cheese and butter, yogurt, then milk, spreads and other dairy products sold in supermarkets.

The import of milk and dairy products to the BiH market in 2023 amounted to 229 million KM and is slightly higher than the previous year by 2%. While the import stagnated, the export of milk and milk products recorded a growth of 15%. The export of milk and milk products recorded growth after 2021 and increased from the export value of 93 million to almost 130 million KM during 2023, which is an increase of 39%. At the same time, the import of milk and milk products increased from the value of 159 million KM in 2020 to 229 million KM in 2023, which is an increase of 44%.

	2019	**2020**	**2021**	**2022**	**2023**
Import (milions KM)	**180**	**159**	**174**	**224**	**229**
Export (milions KM)	**106**	**93**	**93**	**113**	**130**
Deficit (milions KM)	**-74**	**-66**	**-81**	**-111**	**-100**
Import-export coverage (%)	**59**	**59**	**54**	**50**	**56**

Table 2. Foreign trade exchange of milk and milk products (2019-2023)

The foreign trade balance of milk and milk products was negative during the observed period, with the fact that in 2023 it amounted to about 100 million KM and is 10% lower, given that the growth of exports was recorded while imports remained at almost the same value level as the previous year. Milk is the most important product of the dairy industry in BiH and an export product that achieves a surplus in foreign trade. The share of milk in the total export of milk and milk products from Bosnia and Herzegovina to the global market is about 55%, while on the import side, this share is much smaller, amounting to only 8%.

	2019	**2020**	**2021**	**2022**	**2023**
Import (millions KM)	**31**	**21**	**11**	**18**	**19**
Export (millions KM)	**61**	**66**	**60**	**65**	**71**
Surplus (millions) KM)	**29**	**46**	**49**	**47**	**52**

Table 2. Foreign trade exchange of milk 2019-2023. year

Cream (cream) is a group of dairy products that, with a share of 14% in the total import of milk and dairy products, ranks second. As for exports, with a share of 5% in the total, the export of dairy products is ranked fifth

	2019	2020	2021	2022	2023
Import (milions KM)	**21**	**17**	**22**	**37**	**32**
Export (milions KM)	**2**	**3**	**4**	**6**	**6**
Deficit (milions KM)	**-19**	**-14**	**-19**	**-30**	**-25**

Table 4. Foreign trade exchange of sour cream (2019-2023)

Looking at the structure of cheeses imported to BiH, the market is dominated by the import of edamer with a share of 32% in the total import of cheese, followed by gouda with a share of 20%, other fresh cheese with a share of 11% and tilsit around 8%

CONCLUSIONS

The raw milk production development program in Bosnia and Herzegovina should be based on four categories of producers:
1. Small dairy herds with an average of up to 5 heads in the herd have no prerequisites or motivation for business advancement
2. Modern conventional production systems on adapted smaller farms with an average of 15 to 20 dairy cows in the herd. It is estimated that an average production of 4,500 to 5,500 liters of milk per cow per year could be achieved on these farms.
3. A system of specialized family dairy farms with 40 to 50 cows in the herd. On these farms, the production of 5,500 to 6,500 liters of milk per cow per year could be achieved.
4. A system of large milking cow farms (with more than 100 cows in the herd), which could ensure milk production of more than 6,000 liters per cow per year.
Milk production should be primarily focused on cheaper and better-quality products. This is possible with new technologies, especially in the way of keeping and treating animals, nutrition, hygiene, etc. Preference should be given to systems that maximize the use of cheaper inputs - fodder from lawns and arable crop rotation. The intensity of milk production per head should be contrasted with intensity, or profit per unit of area, or invested capital. The mainstay of the development of milk production should be voluminous forage.
Milk production on smaller farms should gradually take on the characteristics of organic, autochthonous.
Some of the conclusions are:
1. until the last moment before entering the EU, there is no need to throw out the incentive per liter of milk
2. before joining the EU, create a program to encourage the competitiveness of small producers, so that they grow to 50 - 100 dairy cows, and survive after joining the EU.
3. deductions for capital investments must be at least 65% for all farmers and 80% for young farmers. Support of 40 or 50%, and up to 50% is not enough. The restructuring of milk production and the creation of a larger number of market-oriented farms must begin as soon as possible.
4. Solve the issue of land management. In the EU, everything is going smoothly and all those who do not have this issue resolved will be extinguished. Milk production cannot do without hectares.
5. Adopt a rulebook that will regulate the issue of using blue diesel in BiH in accordance with

EU standards (in Croatia they have 180 l per cow and 120 per ha, which costs 40-50% of the regular price of diesel.
6. Resolve the cattle marking system - marking in BiH is not efficient and is not aligned with EU rules. The grower should mark himself.
7. It is necessary to create a strategy for the transition of the dairy sector to the EU
8. Cooperation between the private sector and state institutions must be improved
Of the seven largest milk processors in B&H, three dairies are owned by foreign investors, who participate in the purchase and processing of raw milk with 64%. The production program of these dairies is conditioned by the owner's requirements.
Cheese production participates in the production program only with some 3 to 4%, and butter production 0.20%. Cheese production is mainly in smaller processing facilities. In the future, more importance should be given to longer-lasting dairy products and value-added products. So traditional production should be preserved, and measures should be taken to standardize and protect the geographical origin and originality of some types of cheese.
In the markets, cheese and cream are mainly sold as domestic traditional products

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ANTISTAPHYLOCOCCAL STUDY OF THE ESSENTİAL OİL OF MENTHA PULEGİUM L. FROM ALGERİA

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ABSTRACT

Mentha pulegium L., a member of the Lamiaceae family, is valued for its therapeutic properties by people around the world. In Algeria, commonly called "fliou", it has long been recognized for its ability to treat a wide range of diseases. Antimicrobial properties of plant essential oils (EO) have been investigated through several observations and clinical studies which purpose them as potential tools to overcome the microbial drug resistance (MDR) problem. The aim of this research was to study the antibacterial effect of a traditional plant EO, *Mentha pulegium* L., against clinical isolates of methicillin resistant *Staphylococcus aureus* (MRSA) through disk diffusion method. The EO showed very effective bactericidal activity towards the majority of the tested bacterial strains with inhibition zone diameters in the range of 17.06 - 24.50 mm.
The obtained results are therefore promising and thus pave the way for manufacturers to use the essential oils of *Mentha pulegium* L. in the pharmaceutical and food industries.

Keywords: *Mentha pulegium* L., Essential oil, Antibacterial activity, *Staphylococcus aureus* MRSA.

INTRODUCTION

Staphylococcus aureus is one of the major pathogens credited as being responsible for nosocomial and community acquired infections in humans (Fridkin et al., 2005, Weiner et al., 2016). Colonizing the normal commensal flora of humans and many animals, this opportunistic bacterium is considered the main cause of hospital-acquired infections, generating a variety of symptoms, from mild localized infection to potentially fatal sepsis (Egert et al. 2015).

The emergence and spread of multidrug-resistant (MDR) bacterial pathogens have substantially threatened the current antibacterial therapy (Boucher et al., 2009).

MDR bacterial infections often lead to increased mortality, longer stays in hospitals, and higher cost of treatment and care (Boucher et al., 2009; Giamarellou, 2010).

The resistance to antibiotics by microorganisms has increased because, generally, bacteria have the genetic ability to transmit and acquire resistance to them. Bacteria that belong to the *Staphylococcus* genus constitute one of the most serious epidemiological problems. *S. aureus* has the strongest virulence potential among all the staphylococcal species (Ounaissia et al., 2019).

The therapeutic options for these pathogens are extremely limited and physicians are forced to use expensive or previously discarded drugs that are associated with significant side effect to the patients health (Boucher et al., 2009).

The issue of alternative therapies for diseases of staphylococcal etiology has been recently widely discussed by Kurlenda and Grinholc (Kurlenda et al., 2012). The compounds isolated from plant origin, especially essential oils, seem to be the most promising considering their application for both prevention and treatment of staphylococcal infections (Szweda et al., 2016).

The use of essential oils has been shown to possess potential in the treatment of infections, and is safe in terms of human and animal health. Essential oils generally regarded as safe show antimicrobial proprieties and antibacterial resistance that has not been reported after prolonged exposure (Pozzo et al., 2012).

Mentha pulegium L. is one of the Mentha species commonly known as pennyroyal. The flowering aerial parts of *Mentha pulegium* L. has been traditionally used for its antiseptic for treatment of cold, sinusitis, cholera, food poisoning, bronchitis and tuberculosis, and also as antiflatulent, carminative, expectorant, diuretic, antitussive, menstruate. The ingredients of *Mentha pulegium* L. oil have been subjected to a number of studies which have shown a difference in its constituents depending on the region of cultivation and there have been some variations in the constituents from different countries. It has been found *Mentha pulegium* L. oil from Bulgaria contains pulegone (42.9–45.4%); from Uruguay; pulegone (73.4%), isomenthone (12.9%); from Egypt; pulegone (43.5%), piperitone (12.2%); from Tunisia, pulegone (41.8%), isomenthone (11.3%). These studies showed three chemotypes of *Mentha pulegium* L. with the following major oil components (1) pulegone, (2) piperitenone and/or piperitone and (3) isomenthone/neoisomenthol (Mahboubi et al., 2008).

The aim of this research was to study the antibacterial effect of a traditional plant EO, *Mentha pulegium* L., against clinical isolates of methicillin resistant *Staphylococcus aureus* (MRSA) through disk diffusion method.

MATERIAL AND METHOD

Plant material

The species *Mentha pulegium* L. was harvested in the region of El Taref (northe astern Algeria) in April 2023. The identification of the plant was done with the key to determining the flora of Quezel and Santa (Quezel et al., 1963). Specimens were kept at the Laboratory Medical Botany, Department of Pharmacy, Faculty of Medicine Annaba-Algeria.

Essential oil

Essential oil of flowering tops of *Mentha pulegium* L. has been procured from production unit of essential and vegetable oils “Elexir Est” Algeria.

Antibacterial test

The test of the sensitivity of the bacteria is carried out by the diffusion method in agar medium (the disk method). It is a method similar to that of the antibiogram which consists in determining the sensitivity of a bacterial strain vis-à-vis one or more substances (Falleh et al., 2008). The antibacterial activity of the essantial oil of the aerial parts (stem and leaf) of *Mentha pulegium* L. is evaluated vis-à-vis four bacterial strains ; *S.aureus ATCC23, S.aureus ATCC00, S.aureus ATCC13* and *S.aureus MRSA*. These strains were kindly provided by the Microbiology Laboratory Manager at Annaba Faculty of Medicine, Algeria.

Preparation of The Inoculum

By taking twenty tubes that each contains 5 ml of sterile physiological saline. Using a platinum loop, some well isolated colonies are scraped from each of the bacteria, each of which will be discharged into a tube. For the preparation of the different concentrations of extracts, 2, 5 mg of each freeze-dried extract (methanolic extract of leaf and stem), are introduced into a labeled tube, in which we added 1 ml of dimethylsulfoxide (DMSO), solvent without any antibacterial effect. The tubes are vortexed until complete dissolution of the extract, and the dilutions are prepared to obtain 1/2, ¼, 1/8 and 1/16 concentrations from the stock solution.

Seeding should be done within 15 minutes after the preparation of the inoculum. In 16 sterile Petri® dishes, 20 ml of agar are poured. After solidification of the medium, the latter is inoculated with 1 ml of bacteria to be studied. Then, it is spread on the surface using a glass rake.Sterile 5 mm diameter disks prepared in Whatman® n°1 papers are impregnated with a sterile metal forceps in each concentration and placed on the surface of the solidified medium (Mueller-Hinton) at the rate of 6 disks per box (3 discs of leaf, 3 disks of stem).The dishes were incubated for half an hour at room temperature, then for 24 to 48 hours in an oven at 37 ° C. The reading is carried out by measuring the diameter of the inhibition zone (Ø),which translates into a translucent halo around each disc; the presence or absence of a halo would explain the sensitivity or the resistance of the germs vis-a-vis extracts tested; according to a symbolic notation scale from - to +++ (Meena 1994; Jeovetz 2000; Ounaissia et al., 2022).

Table 1. Sensitivity of microbial strains according to zones of inhibition

Sensitivity	Inhibition zone
Not sensitive or resistant(-)	Diameter <10 mm
Sensitive (+)	Diameter between 10 to 16 mm
Very sensitive (++)	Diameter between 16 to 25 mm
Extremely sensitive (+++)	Diameter > 25 mm

RESULTS AND DISCUSSION

Reading Antibiograms

The results of the antibacterial activity are shown in Table 2.

Table 2. Inhibition Diameter (mm) of raw essential oil of *Mentha pulegium* L., and these dilutions

Stains	**Raw (EO)**	**Dilution**			
		1/2	**1/4**	**1/8**	**1/16**
S.aureus ATCC23	17.06 ++	11.30 +	11.96 +	11.09 +	12.70 +
S.aureus ATCC00	24.50 +++	13.25 +	12.57 +	9.85 +	8.06 +
S.aureus ATCC13	19.10 ++	20.56 +++	9.71 +	7.99 R	9.48 +
S.aureus MRSA	17.86 ++	14.76 ++	13.17 +	10.13 +	8.58 +

The four strains of *Staphylococcus* tested, were very sensitive to the essential oil of *Mentha pulegium* L. with diameters of inhibition zones ranging from 17.06 mm to 24.50 mm, the most sensitive strain was *S.aureus ATCC00* with a diameter of inhibition zone of 24.50 mm for the essential oil in the crude state (Table 2).

According to a study by Barchan et al (2025), on the essential oil obtained from the aerial parts of *Mentha pulegium* L. collected in northern Morocco, it was reported that the oil was significantly more effective against Gram-positive bacteria and *S. aureus* was the most sensitive of all the strains tested.

In the literature, it is recognized that essential oils of several *Mentha* species including *M. pulegium* possess antimicrobial properties (Daferera et al. 2003). The essential oil of *Mentha pulegium* is rich in pulegone (30-70%) (Aghel et al. 2004). Pulegone is recognized for its various antibacterial effects. It has shown significant antibacterial effect against *S. aureus* (Duru et al. 2004).

Gram positive bacteria are known to be more susceptible to essential oils than Gram negative bacteria (Russell et al., 1991). Gram negative organisms are less susceptible because they possess an outer membrane surrounding the cell wall which restricts diffusion of hydrophobic compounds through its lipopolysaccharide covering. Gram positive bacteria with a simple membrane structure are less protected against the diffusion of fine essential oils particles Hogan et al., 2002).

There is evidence in the literature that the essential oils of some Lamiaceae plants possess a moderate to good antibacterial activities (Sokovic et al., 2006).

In an other report (Mahboubi et al., 2008) piperitone and piperitenone were found the main components of *M. pulegium* essential oil (71.1%) and exhibits a remarkable antibacterial activity against Gram positive bacteria than Gram negative ones.

Overall, we can conclude that *Mentha pulegium* EO is one of the most promising natural compounds that can be used to develop safer antibacterial agents and that its effective combination with antibacterials may be used in the future to treat diseases caused by *Staphylococcus aureus.*

CONCLUSIONS

Plant extracts have great potential as antimicrobial compounds against microorganisms. Thus, they can be used in the treatment of infectious diseases caused by resistant microbes. The results of this study suggest that *Mentha pulegium* L. essential oil possesses antibacterial properties. This EO could be used as bacterial growth inhibitory agent in new drugs in therapy of *Staphylococcus* diseases. Although this study has investigated in vitro antibacterial activity, extensive in vivo studies could confirm the potential usefulness of this plant's essential oil in combating antimicrobial resistance. However, further research is required to better understand the scientific and biotechnological basis values of applied phytotherapy.

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THE HOME GARDENS AS BIOCULTURAL RESERVE OF LOCAL PLANT DIVERSITY AND TRADITIONAL KNOWLEDGE

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ABSTRACT

The garden is a biocultural reserve where valuable local plant genetic resources, as well as knowledge about their cultivation and use, are conserved and passed down from generation to generation. Especially in the case of food plants, an important example of which are beans, the desire to preserve landraces is result from the emotional devotion towards the traditional taste of local dishes. In this context the role of home gardens as repositories of biological diversity has been acknowledged and a comprehensive, interdisciplinary investigation of their agro-biodiversity is needed. Home gardens, whether found in rural or urban areas, are characterized by a structural complexity and multifunctionality which enables the provision of different benefits to ecosystems and people. Local farmers engage in food production for subsistence or small-scale marketing and the variety of crops and wild plants provides nutritional and medicinal benefits. At the same time, home gardens are important social and cultural spaces where knowledge related to agricultural practices is transmitted and through which households may improve their income and livelihoods. The present study summarizes available documentation on 11,385 local accessions, collected from expeditions in Bulgaria. It discusses future opportunities and prospect of promoting their role in conservation of agricultural biodiversity and cultural heritage. The results support the hypothesis that home gardens sustain rich diversity while contributing to its conservation. However, with the intensive agricultural production, home gardens are unlikely to be preserved if they are not actively mainstreamed in production and conservation policies through add value as cultural heritage. The research work was carried out under the SVK-BGR cooperation project DIGIVALPGR.

Keywords: Landrace, *On farm* conservation, Agroecosystem, Heritage, Add value.

INTRODUCTION

The impact of home garden biodiversity on environment and socioeconomic status of rural populations is reported from report of FAO (2025). The sustainable production and consumption of local crops provide a key part of the nutritious food in the face of different challenges, such as climate change, aged farmers, change of dietary patterns (Pushpakumara et al., 2020; Ivanova et al. 2021; Korpelainen, 2023).

Plant diversity of traditional varieties in Balkan countries is characterized by a large number of crops – cereals, vegetables, legumes, spices, medicinal, and ornamental species. They played an important role for research due to a high genetic variability, making them a potential source of valuable genes coding for economically significant characteristics of importance for agricultural crops, such as disease resistance, drought resistance, quality traits (Conversa et al., 2020). Beside their utilization in breeding programs, they might also be used in small scale farming especially in mountainous districts, due to their adaptability to the specific environmental conditions where they have evolved (Kreiken and Asveld, 2025). Intensive agriculture is however becoming more and more predominant and use of modern cultivars and hybrids giving higher yields and thereby being more profitable are taking over the market. This is causing a continuous loss of old, traditional cultivars and landraces (Vakoufaris, 2024).

Inventorying of local landraces in Bulgaria and Slovakia was therefore initiated by a team of experts from both National seed genebanks. The project DIGIVALPGR started in 2024 and the research network was established providing a platform for facilitating project collaboration. During a preparatory survey for the proposal the partners reported about the presence of conserved local landraces from the rural areas of the respective countries, as well as the danger of their extinction (Mendel et al., 2019; Velcheva and Stoilova, 2021). Within the project working program, collecting missions were conducted.

The aim of the study is to evaluate the inventory of traditional varieties in Bulgaria as an innovative solution to climatic and social challenges.

MATERIAL AND METHOD

The researchers of the teams are members of the European Cooperative Program on Plant Genetic Resources (ECPGR) working groups of documentation and by crop groups. Therefore, prioritized species for collecting were mainly beans, vegetables, medicinal and aromatic plants. The determination of the sites was based on long-term experience of collecting missions in the project countries during which regions with very rich agro-biodiversity had been identified. For example, in Bulgaria such regions are the geographic zones the Rhodopes and Stara Planina Mountains. In these areas there are villages placed between 450-1250 m altitudes. Many of them are even now with isolated access. In these villages old varieties are still commonly found. Both Slovakian and Bulgarian partners pointed out that the main part of old landraces and cultivars are preserved in certain remote and marginal areas in the mountainous depressions where old farmers still cultivate them. In the Rhodopes Mountain in Bulgaria, very diverse populations of common bean were found in the past. This was confirmed after several expeditions during the last 20 years, organized by IPGR-Sadovo. A lower number of local varieties have been grown *on farm* in the urbanized parts of the country.

According to the ECPGR (2017) collecting missions were conducted during 2024 and 2025 by each partner in the own country and also joint expedition in Bulgaria was organized.

During the period activities were carried out according to the following plan:

- Organizing collecting missions in predicted rural areas in each of the partner countries:

- planning the expeditions (detailed terrain plans - itinerary, documentation and information on geographical, soil, climatic and agro-socio economic data); preparing informative materials (leaflets) for the local population;

- collecting expeditions in targeted areas (Figure 1).

- Compiling the passport data of the collected samples:

- immediately after collecting, EURISCO Passport Descriptor was used to compile passport data of the collected samples (FAO/Bioversity, 2017).

- Multiplication, characterization, evaluation, conservation of the collected accessions:

- the seeds of the collected accessions are preparing for conservation according to international standards (depending on the amount of available material).

- Documentation, analyzing and comparing obtained data:

- obtained data were documented, computerized, collated and analyzed among partners in the project.

- Results and progress from the DIGIVALPGR project:

- the project partners from Bulgarian and Slovakian genebanks compiled the results and achievements in progress.

Figure 1. Joint Bulgarian-Slovakian collection mission in the region of Yambol, June 2025

RESULTS AND DISCUSSION

During the period 1982-2025 the germplasm stock of the National seed genebank in Sadovo was enriched with 11,385 local accessions. Currently, landraces comprise 35 % of the genebank holdings. The accessions from collecting missions composed of local varieties and populations from home gardens and crop wild relatives, obtained in their natural habitats. With the highest number of local gene pool are grain legumes and vegetables, followed by cereals.

During 2024-2025 a qualitatively new cooperation between the Bulgarian and Slovakian genebanks by sharing experience and good practices in the field of conservation, characterization, evaluation and documentation of local plant genetic resources is established. A questionnaire for collecting passport and ecological-geographic characteristics has been developed and is being filled out. The Bulgarian partner inventoried in total 10 geographical localities. For all collecting sites, longitude, latitude and altitude were recorded with GPS and registered in passport data files.

With regards to altitude inventorying was carried out from 156 m in Sadovo, Plovdiv region and 157 m in General Inzovo, Yambol region up to mountain an altitude of 1,259 m in Sarnitza, Smolyan region.

The dominance of the common bean landraces (12 acc.) among the collected accessions is due to several factors. Firstly, common bean is very often cultivated on small plots in backyards and home gardens together with vegetables and spices. Secondly, it is too expensive for the small farmers to buy seeds from new varieties for own household cultivation and consumption. Instead, they maintain their own seeds which very often have been grown for generations. And one of the most important is that the local varieties are characterized with special traditional taste and are used for connected with the locality dishes (Calvet-Mir et al., 2016).

During the collecting of accessions of these crops, it was considered necessary to receive detailed information from growers. The researchers provided information about collecting source and recorded it in files together with passport data (Table 1). Most of these accessions were collected from home gardens (24 acc.) or sent by post (3 acc.) as a result from dissemination activities in Municipalities of Svoge and Mezdra (Figure 2 and 3).

Figure 2. Collected accessions from local farmers in the villages of Ochindol, Lakatnik, Levishte

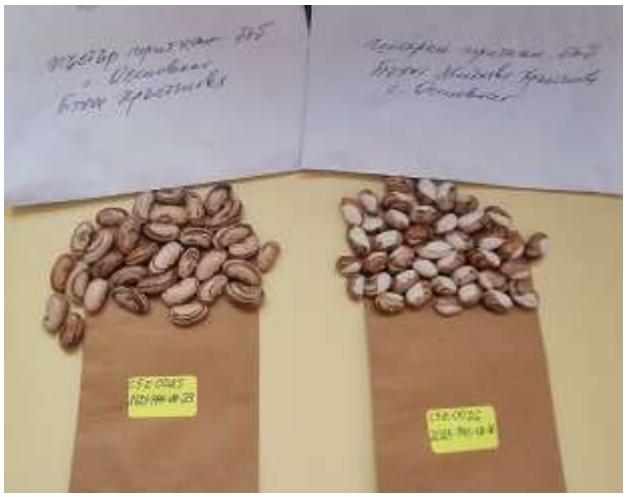

Figure 2. Seeds from local varieties and description sent by post from farmers – donors of plant genetic resources

Local markets are still a valuable source of old, traditional varieties and landraces. Around 25% of the conserved landraces in IPGR-Sadovo are derived from green markets however being collected in smaller quantities and multiplicated. Sometimes, interesting material, which was difficult to find during collecting expeditions in villages, could be collected at the green markets.

Possibilities and challenges for promoting landrace conservation through the use of successful examples:

- Produce and develop typical local products;
- Development of new (e.g. environmentally friendly) farming systems based on diverse varieties;
- Development of local food supply systems, including community and home gardens;
- Develop companies purposely dedicated produce and sell seed/propagation material of landraces.

During the project activities some advantages of old varieties vs modern cultivars landraces were recorded:

- Competitive yield under low input conditions;
- Special value for wildlife (nature protection);
- Agritourism advantage (local products);
- Biomass yield, tolerance, environmental advantage.

Figure 4. Demonstrative garden with local accessions with Bulgarian and Slovak origin

To promote the conservation of local plant genetic resources to students and other guests of the Institute, a demonstration garden with traditional local varieties has been created (Figure 4).

Table 1. Documentation of new accessions according to descriptors of FAO/Bioversity (2017)

N	COLLNUMB	GENUS	SPECIES	ACCENAME	LOCALITY	ELEVATION
1	C4E0223	*Phaseolus*	*vulgaris*	Prevren	Bratzigovo	450 m
2	C4E0224	*Lycopersicon*	*esculentum*	Local pink	Bratzigovo	450 m
3	C4E0225	*Lycopersicon*	*esculentum*	Local red	Bratzigovo	450 m
4	C4E0226	*Cucurbita*	*moschata*	Barunche	Sarnitza	1250 m
5	C4E0227	*Phaseolus*	*vulgaris*	Rancha	Sarnitza	1250 m
7	C4E0228	*Phaseolus*	*coccineus*	Pehlivan	Sarnitza	1267 m
8	C4E0229	*Phaseolus*	*vulgaris*	Bashev	Sarnitza	1259 m
9	C5E0010	*Phaseolus*	*vulgaris*	Black bean	Sadovo	156 m
10	C5E0011	*Phaseolus*	*vulgaris*	Ochindolski	Ochindol	580 m
11	C5E0012	*Phaseolus*	*vulgaris*	Green bean	Ochindol	580 m
12	C5E0013	*Cucurbita*	*maxima*	White pumpkin	Levishte	730 m
13	C5E0014	*Cucurbita*	*moschata*	Violin	Levishte	730 m

14	C5E0015	*Geranium*	*sp.*	Wild	Ochindol	650 m
15	C5E0016	*Mentha*	*sp.*	Wild	Ochindol	650 m
16	C5E0017	*Thymus*	*pulegioides*	Wild	Ochindol	650 m
17	C5E0018	*Phaseolus*	*vulgaris*	White bean	Lakatnik	811 m
18	C5E0019	*Zea*	*mays*	Old corn	Lakatnik	811 m
19	C5E0020	*Phaseolus*	*vulgaris*	Colourful	Smilyan	1100 m
20	C5E0024	*Phaseolus*	*vulgaris*	Light beige	Metcho korito	1162 m
21	C5E0025	*Phaseolus*	*vulgaris*	Pritkan 1	Osenovlak	923 m
22	C5E0026	*Phaseolus*	*vulgaris*	Pritkan 2	Osenovlak	923 m
23	C5E0027	*Lycopersicon*	*esculentum*	Rosalina Pink	General Inzovo	157 m
24	C5E0028	*Lycopersicon*	*esculentum*	Generalinzovski Red	General Inzovo	157 m
25	C5E0029	*Vigna*	*unguiculata*	Local Brown	General Inzovo	157 m
26	C5E0030	*Calendula*	*officinalis*	Local	General Inzovo	157 m
27	C5E0031	*Vigna*	*unguiculata*	Local White	Kluch	455 m
* Data source – Information System of Plant Genetic Resources, Sadovo, Bulgaria						

CONCLUSIONS

The region of Bulgaria is still rich in agricultural biodiversity, represented by local landraces. This fact was supported by the result of conducted collecting missions during 2024 and 2025, when 27 new accessions of local landraces were collected. There is however an impending danger of extinction caused by many factors, such as intensive agricultural practices and use of modern cultivars, migration of the population from villages to the cities, aging of the population in rural areas that traditionally cultivated local populations etc. Therefore, it is considered necessary to continue with collecting expeditions. In general, the result of this project has significant positive impact on the present and future work of the project partners and their institutions, not only in the field of plant genetic resources, but also in the field of plant breeding and entire crop production.

The collected accessions represent richness of biodiversity, that is now prevented from being lost and will soon be available for breeding and research in the region, as well as for the entire scientific community. The next and very important steps are to regenerate, characterize and evaluate the collected material.

It was found that the home gardens sustain rich diversity while contributing to its conservation. However, with the intensive agricultural production, home gardens are unlikely to be preserved if they are not actively mainstreamed in production and conservation policies through add value as cultural heritage.

Digitization and open access to conserved genebank collections increase and optimize the use of the gene pool in breeding programs for the adaptability of genetic materials to changing climatic factors and satisfy the needs of farmers to return the traditional varieties to practice.

The study is in line with the EU 2030 strategy for the conservation of biodiversity and addressed to the implementation of National Programs on Plant Genetic Resources for Food and Agriculture.

The results of the study find their application in the overall process of protecting biodiversity and nature, increasing the resilience of crops to dynamic environmental conditions in order to guarantee the production of healthy and high-quality food.

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STATIC ANALYSIS OF FUNCTIONALLY GRADED POROUS SANDWICH BEAMS

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ABSTRACT

In this study, the static behavior of functionally graded (FG) porous sandwich beams is investigated through theoretical modeling and numerical analysis. FG composite structures are advanced engineering materials characterized by a continuous and gradual variation of material properties along the thickness direction, enabling the simultaneous achievement of low weight, high mechanical strength, and stiffness. In sandwich beam design, applying different distributions of density and elasticity to the core and face layers allows for the optimization of the structure's overall mechanical performance.

To model the response of the porous sandwich beam under static loading, the Hyperbolic Shear Deformation Theory (HSDT) is employed, and the governing equations are derived using Hamilton's principle. The HSDT approach provides more accurate results for thick beams by effectively accounting for shear deformation effects.

The derived governing equations are solved analytically using the Navier solution technique. The study presents a comprehensive analysis of dimensionless displacement distributions under various material gradation functions, porosity levels, and simply supported boundary conditions. The results clearly demonstrate that both the porosity ratio and the distribution of material properties significantly influence the static response and stiffness characteristics of the beam.

Keywords: Functionally graded materials, Static analysis, Porous sandwich beam, Porosity ratio

LITERATURE REVIEW

Displacement analysis plays a critical role in determining the mechanical behavior of functionally graded porous sandwich beams, particularly in engineering applications where a balance between lightness and strength is required. In this context, the literature reveals how displacement behavior varies under different material distribution models, porosity ratios, and boundary conditions.

Solar and Taşkın (2024) investigated the bending and vibration behavior of functionally graded porous sandwich beams using classical beam theory and the Navier solution method. Their study demonstrated that displacement values are sensitive to the porosity ratio, material gradation profile (power-law exponent), and geometric parameters (L/h ratio). The analyses conducted with two different material types—one with symmetric and the other with homogeneous porosity distribution—showed significant displacement differences between the functionally graded face layers and the homogeneous core layer.

In the study conducted by Turan, Hacıoğlu, and Balcı (2024), the displacement behavior of functionally graded porous beams was analyzed in the ANSYS APDL environment,

considering the porosity coefficient and distribution type. The numerical results were compared with data from the literature, thereby demonstrating the modelability of changes in displacement responses due to porosity.

Shabani et al. (2023) conducted static buckling and free vibration analyses of two-dimensional functionally graded metal-ceramic porous beams. Using Hamilton's principle and the Galerkin method, they accounted for material variation along the thickness and length as well as porosity effects, examining how FG power indices, porosity, and slenderness ratios influence the beam's non-dimensional frequency and buckling response. The results demonstrated that the proposed higher-order beam model accurately captures the effects of transverse shear deformation compared to classical theories.

Taşkın and Demirhan (2020) focused on the free vibration analysis of porous FG beams. However, the fundamental equations of motion and the Navier-type solution method employed in their study are also applicable for evaluating displacement behavior. Their analysis, based on equations derived via Hamilton's principle, revealed that the porosity ratio is a determining factor in the displacement response.

Turan and Kahya (2021) examined the free vibration and buckling behavior of functionally graded sandwich beams. Although the study primarily addressed dynamic responses, the displacement functions obtained for the proposed Type A and Type B structures contribute to the understanding of displacement behavior in relation to boundary conditions and material gradation parameters. Solutions based on trigonometric series functions are particularly valuable for understanding the effects of beam geometry under bending.

Chopan and Noori (2023) investigated the elastic and viscoelastic forced vibration behavior of functionally graded porous sandwich beams using theoretical analysis and the finite element method. Three-layered beams were modeled considering different boundary conditions and porosity functions, and the effects of material gradation and damping ratio on the dynamic response were examined. The results showed that the porosity function and material gradation significantly influence the amplitude and period, while in the viscoelastic case, increased damping causes a faster reduction in amplitude.

El Khouddar et al. (2021) examined the thermal nonlinear free vibration of functionally graded (FG) beams using Euler-Bernoulli theory and Hamilton's principle, considering von Kármán geometric nonlinear strains. They assumed temperature- dependent material properties with a simple power-law distribution, and their results highlighted the impact of thermal conditions and volume fraction on the dynamic response of FG beams.

Phung-Van et al. (2024) investigated the nonlinear bending behavior of functionally graded (FG) beams under various boundary conditions using the Ritz method. In their study, displacements were modeled with higher-order polynomials (Pb- Ritz) satisfying geometric boundary conditions, and von Kármán type geometric nonlinear strains were considered. Their results highlighted the influence of material properties, geometric parameters, and boundary conditions on the displacement and stress fields of FG beams.

Bridjesh et al. (2023) investigated the buckling behavior of two-directional porous functionally graded beams using a higher-order shear deformation theory. The study computed the buckling loads and displacements of the beams under various boundary conditions and material gradations using polynomial-based formulations. The results highlighted that porosity distribution, material gradation, and boundary conditions have significant effects on the buckling response of the beams.

INTRODUCTION

Composite materials are engineering materials formed by combining two or more distinct materials at the macroscopic scale, offering a wide range of properties such as high strength, low weight, and good thermal performance. Laminated composites are obtained by stacking different material layers, and due to the differences between these layers, discontinuities may occur in temperature and stress distributions. Such discontinuities can adversely affect structural performance. To address this issue, functionally graded materials (FGMs) have been developed. In FGMs, material properties vary continuously along the thickness direction, thereby reducing stress concentrations at the interfaces (Taşkın & Demirhan, 2020). Despite providing superior mechanical and thermal properties, FGMs inevitably develop structural porosities during manufacturing. These pores can significantly influence the mechanical performance of the material. Porous functionally graded beams (FGPBs) are produced with a gradual distribution of metal and ceramic phases, and the presence of porosity is an important parameter that must be considered in both static and dynamic analyses (Turan, 2022).

Although FGMs offer enhanced performance, the formation of microscopic pores during the fabrication process is unavoidable. These pores directly affect elastic modulus, natural frequencies, and vibration behavior. Therefore, it is essential to include the porosity factor in the mechanical and dynamic analyses of porous FGM structures (Wattanasakulpong & Chaikittiratana, 2015; Ebrahimi & Mokhtari, 2015). Recent studies have shown that porosity significantly influences not only vibration behavior but also the performance of beams under static loads. Sadoun (2017) demonstrated that the porosity parameter substantially alters the elastic modulus and overall mechanical behavior, while Hamed, Sadoun, and Eltaher (2019) investigated the effect of different porosity models on the static response of nanobeams, highlighting the critical role of pore distribution on deformations. Similarly, Akbaş (2018) and Alnujaie et al. (2021) analyzed the dynamic behavior of thick functionally graded porous beams and indicated that proper material distribution can mitigate the negative effects of porosity. These studies collectively reveal that the impact of porosity cannot be ignored in either dynamic or static analyses. Moreover, the analysis of sandwich structures is of great importance in engineering applications. Using functionally graded layers on the faces and porous materials in the core provides advantages in terms of both strength and stiffness. Literature emphasizes that porosity distribution and the material grading index directly affect the mechanical behavior of sandwich beams, and neglecting these parameters can lead to significant deviations in results (Ebrahimi, Ghasemi, & Salar, 2015) In this context, the present study focuses on the static displacement analysis of functionally graded porous sandwich beams. The effects of different porosity models and material distribution parameters on the displacement behavior of sandwich beams are examined.

In this study, the static displacement behavior of functionally graded (FG) porous composite sandwich beams was investigated. The beam possesses an FG material structure, with mechanical properties and porosity gradually varying through the thickness; the core and face layers exhibit different density and stiffness values. In the first stage, the geometrically nonlinear equations of motion were derived using the hyperbolic shear deformation theory and Hamilton's principle. Subsequently, these equations were solved using numerical methods, and dimensionless displacement distributions were analyzed under various material grading functions, porosity ratios, and simply supported boundary conditions. The results clearly illustrate the influence of porosity and material distribution on beam behavior, providing valuable engineering insights for the design of FG structures.

MATERIAL AND METHOD

1.1.Definition of Effective Properties in Functionally Graded Material Beams

Various homogenization or blending methods have been developed to describe the characteristics of multi-phase materials. Among these methods, the Voigt-type mixing rule is particularly preferred for estimating the effective properties of functionally graded materials. (Taşkın & Demirhan, 2020)

$$P(z) = P_1 + (P_2 - P_1)V_s(z) \tag{1}$$

Here, P(z) represents the material property, while V_s (z) denotes the volume fraction function. In this study, the most commonly used exponential function is selected for V_s (z). (Wakashima et al., 1990).

$$V_s^{(1)}(z) = \left(\frac{z-h_0}{h_1-h_0}\right)^p, \qquad h_0 \le z \le h_1, (0 \le p \le \infty) \qquad \text{(FG bottom surface)} \tag{2}$$

$$V_s^{(2)}(z) = 1, \qquad h_1 < z < h_2, (0 \le p \le \infty) \qquad \text{(Ceramic core)} \tag{3}$$

$$V_s^{(3)}(z) = \left(\frac{z-h_3}{h_2-h_3}\right)^p, \qquad h_2 \le z \le h_3, (0 \le p \le \infty) \qquad \text{(FG top surface)} \tag{4}$$

For p = 0, the material is homogeneous, while for p > 0, the properties vary functionally.

In this study, a functionally graded beam with length L and height h is employed, and its geometric configuration is depicted in Figure 1.

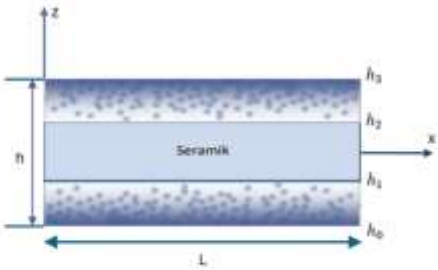

Figure 1. Porous functionally graded beam

A functionally graded material beam with length L and height h is characterized by a spatially varying elastic modulus E(z)and density ρ(z).

Various models have been proposed in the literature to represent porosity distribution. The first model (Type A) assumes that pores are uniformly distributed across the cross-section. However, several studies have observed that porosity is often non- uniform, typically concentrated near the mid-plane and decreasing toward the surfaces. This observation led to the development of a non-uniform porosity distribution model (Type B). In addition to these studies, further analyses have also been conducted to examine different types of porosity **(**Type C).

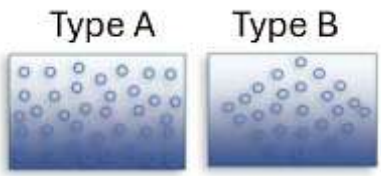

Figure 2. Type A and Type B porosity distribution models (Taşkın & Demirhan, 2020)

Effective material properties for Type A(Fig. 2); (Solar & Taşkın, 2024)

$$E(z) = E_m + (E_s - E_m)V_s(z) - \frac{e}{2}(E_s - E_m) \tag{5}$$

$$\rho(z) = \rho_m + (\rho_s - \rho_m)V_s(z) - \frac{e}{2}(\rho_s - \rho_m) \tag{6}$$

Effective material properties for Type B(Fig. 2); (Solar & Taşkın, 2024)

$$E(z) = E_m + (E_s - E_m)V_s(z) - \frac{e}{2}(E_s - E_m)\left[1 - \left(2\frac{|z|}{h}\right)\right] \tag{7}$$

$$\rho(z) = \rho_m + (\rho_s - \rho_m)V_s(z) - \frac{e}{2}(\rho_s - \rho_m)\left[1 - \left(2\frac{|z|}{h}\right)\right] \tag{8}$$

Effective material properties for Type C; (Kurpa et al., 2023)

$$E(z) = E_m + (E_s - E_m)V_s(z) - \frac{e}{2}(E_s + E_m)\left[1 + \left(\frac{z-h}{\frac{h}{2}+h}\right)\right] \tag{9}$$

$$\rho(z) = \rho_m + (\rho_s - \rho_m)V_s(z) - \frac{e}{2}(\rho_s + \rho_m)\left[1 + \left(\frac{z-h}{\frac{h}{2}+h}\right)\right] \tag{10}$$

A porosity ratio e represents a functionally graded material without any pores. As e increases, the porosity within the material increases, leading to a decrease in the elastic modulus. A volume fraction exponent p=0 corresponds to a fully ceramic material; as p increases, the metallic character of the material increases. When p is non-zero, the bottom surface exhibits metallic properties, while the top surface exhibits ceramic properties. (Taşkın & Demirhan, 2020)

1.2. Hyperbolic Shear Deformation Beam Theory

The hyperbolic shear deformation beam theory was introduced to investigate the mechanical behavior of planar sandwich-type structures. This theory aims to provide more reliable results by accurately considering the shear stresses that develop on the top and bottom surfaces of the beam. Within this framework, the normal and shear stresses at any point along the beam are expressed in relation to the first and second derivatives of the displacement functions. This correlation represents one of the key principles underlying the governing equations of motion for the beam.

(Soldatos et al., 1992) expressed the displacement field used in their study, which

employs the hyperbolic shear deformation beam theory.

$$u(x,z) = u_0(x) - \frac{zdw_0}{dx} + f\phi(x) \tag{11}$$

$$w(x) = w_0(x) \tag{12}$$

In Equation (11) and in equation (12) u_0, w_0 represent the displacements in the x and z directions, respectively, of a point located on the neutral axis of the beam. The shape function f is defined as a function of the variable z depending on the distribution of transverse shear stress through the thickness of the beam, and it is assumed to be zero at both the top and bottom surfaces of the beam.

According to the hyperbolic shear deformation beam theory, the non-zero normal strain (ε_x) and shear strain (γ_{zx}) observed at any point of the beam are associated with the unknown displacement parameters $\varepsilon_x^0, k_x^b, k_x^s, \gamma_{xz}^0$ which are defined in Equations (17) and (18) and presented in Equations (13), (14), (15), and (16) as follows.

$$\varepsilon_x = \varepsilon_x^0 + zk_x^b + fk_x^s \tag{13}$$

$$\gamma_{zx} = g\gamma_{zx}^0 \tag{14}$$

$$\varepsilon_x^0 = \frac{du_0}{dx} \tag{15}$$

$$k_x^b = -\frac{d^2w_0}{dx^2} \tag{16}$$

$$k_x^s = \frac{d\phi}{dx} \tag{17}$$

$$\gamma_{xz}^0 = 0 \tag{18}$$

The shape function f and its derivative, the function g, are defined in Equations (19) and (20).

$$f = \left[z - hsinh(\frac{z}{h}) * zcosh(\frac{1}{2})\right] \tag{19}$$

$$g = f' = \left[1 - cosh(\frac{1}{2})cosh(\frac{z}{h})\right] \tag{20}$$

The stress–strain relationship at any point within the k-th layer of the beam is given by the one-dimensional Hooke's law in Equations (21), (22) and (23). (Soldatos et al., 1992).

$$\sigma_x^k = E^k(z)\varepsilon_x^k \tag{21}$$

$$\tau_{xz}^k = G^k(z)\gamma_{zx}^k \tag{22}$$

$$G^k(z) = \frac{E^k(z)}{2(1+v)} \tag{23}$$

1.3.Equations of Motion

Hamilton's principle is used to derive the equations of motion for functionally graded (FG) sandwich beams based on the hyperbolic shear deformation theory. In the case of FG sandwich beams, this principle represents the condition where the variations in the system's total energy are equal to zero.

$$\int_{t_1}^{t_2}(\delta U - \delta V + \delta K)dt = 0 \tag{24}$$

Here, δU, δV and δK represent the variations of the total strain energy, potential energy, and kinetic energy, respectively, while t_1 and t_2 denote the initial and final times. The variation of the strain energy is expressed by Equation (25).

$$\delta U = \int_0^L \int_{-\frac{b}{2}}^{\frac{b}{2}} \int_{-\frac{h}{2}}^{\frac{h}{2}} (\sigma_x^k \delta\varepsilon_x + \tau_{zx}^k \delta\gamma_{zx}) dz dy dx = \int_0^L \left(N_x \frac{d\delta u_0}{dx} - M^c \frac{d^2\delta u_0}{dx^2} + M^s \frac{d\delta\phi}{dx} + Q\delta\phi \right) dx \tag{25}$$

This equation represents the variation of the strain energy of the beam. Here, N_x denotes the axial force; M^c represents the bending moment similar to that in classical beam theory; M^s is the higher-order moment associated with shear deformation; and Q denotes the shear force. These quantities are calculated by appropriately integrating the stresses developed throughout the thickness of the beam.

$$\begin{Bmatrix} N_x \\ M^c \\ M^s \\ Q \end{Bmatrix} = b \int_{-\frac{h}{2}}^{\frac{h}{2}} \begin{Bmatrix} \sigma_x^k \\ \sigma_x^k z \\ \sigma_x^k f \\ \tau_{zx}^k f' \end{Bmatrix} = \begin{bmatrix} A & B & C & 0 \\ B & D & E & 0 \\ C & E & F & 0 \\ 0 & 0 & 0 & H \end{bmatrix} \begin{Bmatrix} \varepsilon_x^0 \\ k_x^b \\ k_x^s \\ \gamma_{zx}^0 \end{Bmatrix} \tag{26}$$

The matrices A, B, C, D, E, F, and H are defined in Equation (27) and represent the geometric and mechanical properties of the beam. To construct matrices A, B, C, D, E, and F, a series of integrals are computed using the material properties and geometric information at a given cross-section.

These integrals represent the product of a density function, which depends on the material properties and the shape function, over the width of the beam. The process is carried out by performing integration along the length of the beam.

$$(A, B, C, D, E, F) = b \int_{-h/2}^{h/2} E^k(z)(1, z, f, z^2, fz, f^2) dz$$

$$H = b \int_{-h/2}^{h/2} G^k(z) g^2 dz \tag{27}$$

The variation of the potential energy, δV arises from the external loads acting on the beam and represents an important parameter in determining the beam's mechanical behavior. The potential energy of these external loads is expressed in terms of the displacements and stresses developed in the beam at the points where the loads are applied. In other words, the effect of the forces and moments acting on the beam, together with the material's elastic properties and geometry, governs the variation of the potential energy. The variation of the potential energy δV due to the load q and the axial force N_0 is given by Equation (28), and this expression provides a fundamental calculation for analyzing the elastic response of the beam under applied loads.

$$\delta V = \int_0^L \left(q\delta w + N_0 \frac{dw}{dx}\frac{d\delta w}{dx}\right) dx \tag{28}$$

The variation of the kinetic energy (δK) is obtained by Equation (29).

$$\begin{aligned}\delta K &= \int_0^L \int_{-b/2}^{b/2} \int_{-h/2}^{h/2} \rho(z)\left(\frac{d^2u}{dt^2}\delta u + \frac{d^2w}{dt^2}\delta w\right) dzdydx \\ &= \int_0^L \left(I_A \frac{d^2u_0}{dt^2} - I_B \frac{d^3w_0}{dxdt^2} + I_C \frac{d^2\phi}{dt^2}\right)\delta u_0 dx + \int_0^L \left(-I_B \frac{d^2u_0}{dt^2} + I_D \frac{d^3w_0}{dxdt^2} - I_E \frac{d^2\phi}{dt^2}\right)\frac{d\delta w_0}{dx} dx \\ &+ \int_0^L \left(I_C \frac{d^2u_0}{dt^2} - I_E \frac{d^2w_0}{dxdt^2} + I_F \frac{d^2\phi}{dt^2}\right)\delta\phi dx + \int_0^L I_A \frac{d^2w_0}{dt^2}\delta w_0 dx\end{aligned} \tag{29}$$

Here, ρ(z) represents the thickness-dependent mass density of each layer and is an important parameter in determining the dynamic behavior of the beam. The inertia moment coefficients I_A, I_B, I_C, I_D, I_E and I_F are defined by Equation (30) to quantitatively describe the beam's inertial properties and the effects of mass distribution on its vibration and motion. These coefficients are calculated by considering the density distribution and geometric parameters at different cross-sectional points of the beam and play a critical role in the dynamic analysis of the structure.

$$(I_A, I_B, I_C, I_D, I_E, I_F) = b\int_{-h/2}^{h/2} \rho^k(z)(1, z, f, z^2, fz, f^2)dz \tag{30}$$

When the coefficients of the variables δu_0, δw_0 ve $\delta\phi_0$ are collected and set equal to zero, the equations of motion of the beam are derived. During this process, partial integration is applied to Equation (24); this allows the derivatives in the differential equations to be appropriately reduced and the integral form to be obtained while taking the boundary conditions into account. In this way, the general equilibrium equations related to the displacements and rotations of the beam are systematically formulated and become suitable for dynamic analysis.

$$\begin{aligned}&\frac{dN_x}{dx} = I_A \frac{d^2u_0}{dt^2} - I_B \frac{d^3w_0}{dxdt^2} + I_c \frac{d^2\phi}{dt^2} \\ &\frac{d^2M^c}{dx^2} = -q + N_0 \frac{d^2w_0}{dx^2} + I_B \frac{d^3u_0}{dxdt^2} - I_D \frac{d^4w_0}{dx^2dt^2} + I_A \frac{d^2w_0}{dt^2} + I_E \frac{d^3\phi}{dxdt^2} \\ &\frac{dM^3}{dx} - Q = I_c \frac{d^2u_0}{dt^2} - I_E \frac{d^3w_0}{dxdt^2} + I_F \frac{d^2\phi}{dt^2}\end{aligned} \tag{31}$$

By substituting the stress resultants (N_x, M^c, M^s, Q) from Equation (28) into Equation (31), the following equations of motion can be obtained in terms of the unknown displacement variables (u_0, w_0, u_0).

$$\begin{aligned}&A\frac{d^2u_0}{dx^2} - B\frac{d^3w_0}{dx^3} + C\frac{d^2\phi}{dx^2} = I_A \frac{d^2u_0}{dt^2} - I_B \frac{d^3w_0}{dxdt^2} + I_C \frac{d^2\phi}{dt^2} \\ &B\frac{d^3u_0}{dx^3} - D\frac{d^4w_0}{dx^4} + E\frac{d^3\phi}{dx^3} = -q + N_0 \frac{d^2w_0}{dx^2} + I_B \frac{d^3u_0}{dxdt^2} - I_D \frac{d^4w_0}{dx^2dt^2} + I_A \frac{d^2w_0}{dt^2} + I_E \frac{d^3\phi}{dxdt^2} \\ &C\frac{d^2u_0}{dx^2} - E\frac{d^3w_0}{dx^3} + F\frac{d^2\phi}{dx^2} - H\phi = I_C \frac{d^2u_0}{dt^2} - I_E \frac{d^3w_0}{dxdt^2} + I_F \frac{d^2\phi}{dt^2}\end{aligned} \tag{32}$$

THEORETICAL RESULTS AND DISCUSSION

In this section, the results of the vertical displacement analyses of simply supported functionally graded porous sandwich beams using the hyperbolic shear deformation theory were obtained and compared with the literature. The face sheets of the sandwich beams are made of functionally graded (Al/Al_2O_3) material, while the core is made of homogeneous isotropic (Al) material. The beam length is taken as $L = 1\ m$, while the beam thickness varies according to the aspect ratio. The properties of alumina (Al_2O_3) and aluminum (Al) materials are given in Table 1.

Table 1. Properties of alumina (Al_2O_3) and aluminum (Al) materials (Taşkın & Demirhan, 2020)

Malzeme Çeşidi	$E\ (Gpa)$	$\rho\ (kg/m^3)$	v
Alümina (Al_2O_3)	380	3960	0.3
Alüminyum (Al)	70	2702	0.3

The layers used in the analyses and their corresponding height values h_0, h_1, h_2, h_3 are given in Table 2.

Table 2. Layer types and height value h_0, h_1, h_2, h_3

Tabaka çeşitleri	$\boldsymbol{h_0}$	$\boldsymbol{h_1}$	$\boldsymbol{h_2}$	$\boldsymbol{h_3}$
1-1-1	$-h/2$	$-h/6$	$h/6$	$h/2$
1-2-1	$-h/2$	$-h/4$	$h/4$	$h/2$
2-1-2	$-h/2$	$-h/10$	$h/10$	$h/2$
2-1-1	$-h/2$	0	$h/4$	$h/2$
2-2-1	$-h/2$	$-h/10$	$3h/10$	$h/2$
1-8-1	$-h/2$	$-2h/5$	$2h/5$	$h/2$

The numerical results obtained from the analysis were expressed using the following dimensionless form. The definitions in the dimensionless expression are as follows:

- Dimensionless Vertical Displacement

$$\bar{w} = \frac{w100E_m h^3}{q_0 L} \tag{33}$$

In the dimensionless vertical displacement defined by Equation (33), under the condition $x = L/2$ and $z = 0$; w represents the vertical displacement, E_m denotes the material's elastic modulus, h is the beam thickness, q_0 is the maximum intensity of the load at the center of the beam, and L is the beam length. (Sayyad & Avhad, 2019)

1.4. Investigation of Vertical Displacement Characteristics of FG Porous Sandwich

Beams

In this study, the vertical displacement behaviors of simply supported functionally graded (FG) porous and non-porous sandwich beams were investigated using the hyperbolic shear deformation theory. In the analyses, three different porosity ratios, e=0, e=0.2 and e=0.4, were considered. At this stage, the exponential parameter pp, which defines the material distribution, was selected as p=1, p=2, and p=10, and the results were interpreted accordingly. For the examination of the beams, the L/h ratios were taken as 5 and 20. Furthermore, in order to enable structural comparisons, three different sandwich beam types Type A, Type B, and Type C were used.

- For p=1;

Table 3. Dimensionless vertical displacement values of simply supported FG porous sandwich beams. (Type B, p=1)

e	**L/h = 5**					
	1-1-1	**1-2-1**	**2-1-2**	**2-1-1**	**2-2-1**	**1-8-1**
0	5.8241	5.4031	6.9319	6.5611	5.8642	3.9512
0.2	6.0676	5.6113	7.2764	6.9006	6.1152	4.0638
0.4	6.3329	5.8366	7.6572	7.2565	6.4070	4.1834
	L/h = 20					
	1-1-1	**1-2-1**	**2-1-2**	**2-1-1**	**2-2-1**	**1-8-1**
0	5.9398	5.1000	6.5836	6.1998	5.5172	3.6789
0.2	6.2002	5.2909	6.9050	6.4944	5.7475	3.7773
0.4	6.4844	5.4966	7.2594	6.8200	5.9988	3.8812

- For p=2;

Table 4. Dimensionless vertical displacement values of simply supported FG porous sandwich beams. (Type B, p = 2)

e	**L/h = 5**					
	1-1-1	**1-2-1**	**2-1-2**	**2-1-1**	**2-2-1**	**1-8-1**
0 (Solar,C.)	8.3895	6.7578	9.6784	9.0037	7.6346	4.3118
0 (the presented study)	**8.3928**	**6.7524**	**9.6885**	**9.0038**	**7.6280**	**4.2952**
0.2(Solar,C.)	8.8972	7.0845	10.3601	9.6187	8.0747	4.4463
0.2 (the presented study)	**8.9009**	**7.0782**	**10.3717**	**9.6174**	**8.0699**	**4.4275**
0.4 (Solar,C.)	9.4704	7.4445	11.1451	10.3635	8.5856	4.5901
0.4 (the presented study)	**9.4746**	**7.4373**	**11.1586**	**10.3613**	**8.5810**	**4.5686**
	L/h = 20					
	1-1-1	**1-2-1**	**2-1-2**	**2-1-1**	**2-2-1**	**1-8-1**
0 (Solar,C.)	8.0313	6.4276	9.2864	8.5068	7.2122	4.0172
0 (the presented study)	**8.0315**	**6.4272**	**9.2871**	**8.5067**	**7.2119**	**4.0161**
0.2(Solar,C.)	8.5146	6.7335	9.9387	9.0892	7.6205	4.1348
0.2 (the presented study)	**8.5148**	**6.7331**	**9.9394**	**9.0892**	**7.6202**	**4.1336**
0.4 (Solar,C.)	9.0597	7.0701	10.6895	9.7645	8.0810	4.2596
0.4 (the presented study)	**9.0600**	**7.0696**	**10.6903**	**9.7643**	**8.0806**	**4.2583**

- For p=10;

Table 5. Dimensionless vertical displacement values of simply supported FG porous sandwich beams. (Type B, p = 10)

e	**L/h = 5**					
	1-1-1	**1-2-1**	**2-1-2**	**2-1-1**	**2-2-1**	**1-8-1**
0	12.5922	9.4104	14.6672	13.5826	11.0327	4.8660
0.2	13.7712	10.0533	16.2911	14.8391	11.9400	5.0350
0.4	15.1938	10.7906	18.3195	16.8256	13.2897	5.2164
	L/h = 20					
	1-1-1	**1-2-1**	**2-1-2**	**2-1-1**	**2-2-1**	**1-8-1**
0	12.1609	9.0474	14.1401	12.5458	10.4646	4.5766
0.2	13.3043	9.6654	15.7099	13.9206	11.3863	4.7297
0.4	14.6851	10.3740	17.6718	15.6783	12.5040	4.8934

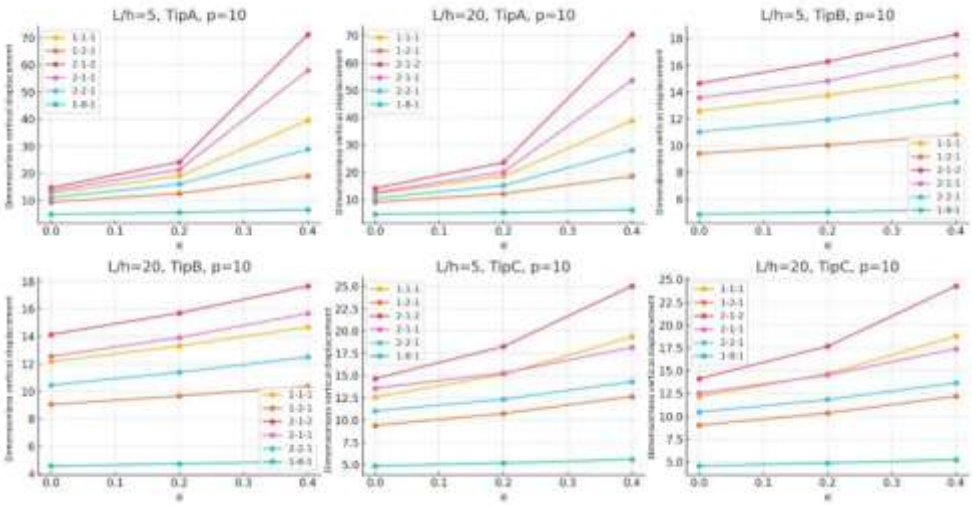

Figure 3. Effect of e on the vertical displacement for different structure types at p=10 (L/h=5 and 20).

In Figure 3. the dimensionless vertical displacement behavior of simply supported functionally graded (FG) porous sandwich beams is presented as a function of porosity ratio (e) for different structure types (Type A, Type B, Type C) and two different L/h ratios (5 and 20) at the exponential parameter p=10. In all graphs, the displacement values increase noticeably as the porosity ratio increases. This increase is most pronounced in the Type A structure; in particular, configurations with less rigid cores, such as the 2-1-2 arrangement, exhibit the most susceptible deformation behavior. For example, for Type A with L/h=5 and e=0.4, the displacement in the 2-1-2 arrangement reaches approximately 71 units. Type B and Type C structures show more limited increases and exhibit stiffer behavior.

Moreover, at p=10, the pores are more concentrated in the surface layers, significantly reducing the bending stiffness of the structure and increasing deformation sensitivity. This effect becomes even more pronounced at high porosity ratios (e=0.4). Cases with L/h=5

generally exhibit higher displacement values compared to L/h=20, indicating that thicker beams are more sensitive to bending. As a result, these graphs clearly demonstrate the effect of both the pp parameter, which controls the pore distribution, and the structure type and layer arrangement on vertical displacement.

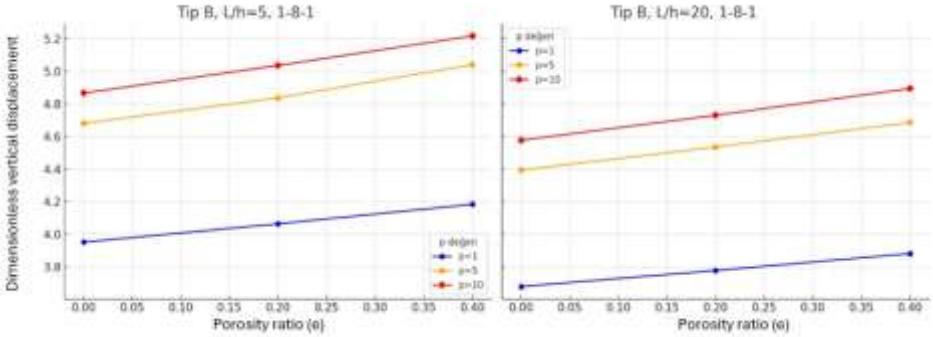

Figure 4. Effect of the p parameter and L/h ratio on the vertical displacement as a function of porosity ratio for the 1-8-1 layer configuration in the Type B structure.

In Figure 4. for the Type B structure with a 1-8-1 layer configuration, it is observed that the dimensionless vertical displacement values increase steadily as the pp parameter rises. This increase is due to the concentration of pores toward the surface, which reduces the structural stiffness and enhances the tendency for deformation.

Additionally, the displacement values obtained for L/h=5 are higher compared to L/h=20, indicating that shorter and thicker beams are more sensitive to bending deformation.

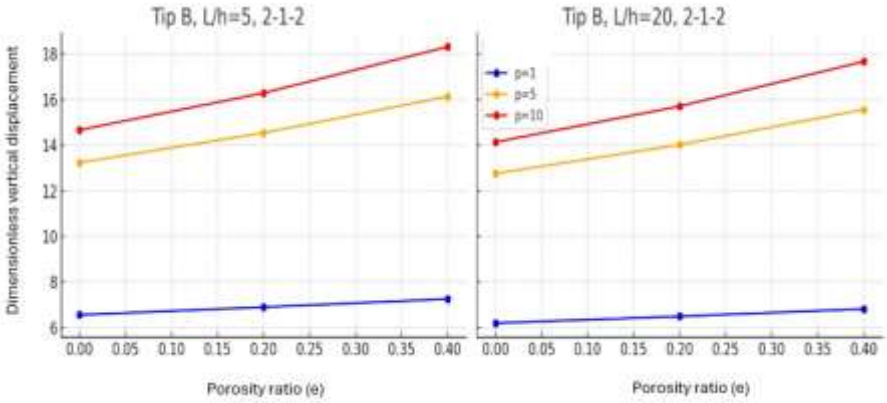

Figure 5. Displacement Behavior of Type B – 2-1-2 Layered Beam According to e and p Parameters (L/h = 5 and 20).

Figure 5. illustrates the variation of dimensionless vertical displacement of the 2- 1-2 layered beam with Type B configuration as a function of porosity ratio and the p parameter. As the porosity and p value increase, the displacement increases; however, when the L/h ratio rises, the displacement decreases.

CONCLUSION

In conclusion, the results clearly highlight the strong influence of porosity ratio, pore distribution parameter, structural type, and layer configuration on the bending behavior of FG porous sandwich beams. Higher porosity levels and larger values of the exponential parameter significantly reduce bending stiffness, leading to greater vertical displacements, especially in structures with less rigid cores such as the 2-1-2 arrangement.

Comparisons across L/h ratios further emphasize that shorter and thicker beams (L/h=5) are more prone to deformation than slender beams (L/h=20). Overall, the findings demonstrate that both geometric parameters and material gradation play a decisive role in governing the displacement response, providing important insights for the design of lightweight yet mechanically efficient sandwich structures.

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INVESTIGATION OF THE EQUIVALENT MECHANICAL PROPERTIES OF TPMS LATTICE STRUCTURES

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ABSTRACT

Triply Periodic Minimal Surface (TPMS)-based lattice structures offer innovative material solutions for the aerospace, biomedical, automotive, and energy industries with their advantages of lightweight design, high strength-to-weight ratio, and multifunctional properties. This study comprehensively investigates the equivalent mechanical properties of TPMS structures through analytical models, parametric analyses, and literature data. The geometric characteristics of TPMS types such as Gyroid, Primitive, and I-WP, along with their smooth and continuous surface topologies, reduce stress concentrations and provide superior mechanical performance and fatigue resistance. Compared to conventional porous materials such as metal foams, honeycomb structures, and space frame systems, TPMS structures stand out with their ability to control isotropic/anisotropic behavior, high surface area-to-volume ratio, and optimized energy absorption capacity. In determining equivalent mechanical properties such as elastic modules, yield strength, and Poisson's ratio, analytical approaches like the Gibson-Ashby model are utilized to detail the power-law relationships between mechanical behavior and relative density (solid volume fraction). Parametric analyses reveal the influence of unit cell size, surface curvature, and porosity on mechanical performance and demonstrate that different TPMS topologies exhibit distinct responses even at the same relative density. This enables application-specific design optimization. The study provides a theoretical framework for the design and analysis of TPMS-based structures.

Keywords: Triply Periodic Minimal Surface (TPMS), Equivalent Mechanical Properties, Relative Density (Volume Fraction), Gibson-Ashby Model, Lattice Structures

INTRODUCTION

Functionally Graded Materials (FGMs) are an advanced class of materials distinguished by a spatially varying composition throughout their volume, which leads to gradual changes in material properties tailored to meet specific functional requirements (Loh et al., 2018). The properties of functionally graded structures can vary based on porosity, composition, or microstructural distribution, enabling their broad application in fields such as aerospace, automotive, biomedical implants, and acoustics (Yu, Sun, & Bai, 2019; Mahmoud & Elbestawi, 2017; Lin et al., 2013). Theoretical investigation of FGMs involves issues such as material selection, structural design, and the optimization of structural performance (Solar, 2024). Despite these advantages, one of the primary obstacles in the development of FGMs is the difficulty of fabrication due to their gradually varying and complex internal structure, which limits their large-scale implementation. Besides the ordinary lattice structures, a newly emerging structure, triply periodic minimal surfaces (TPMS), has caught attention and boosted a specific research field. Triply periodic minimal surface (TPMS) structures, especially when

used in the inner layers, optimize load distribution and energy absorption characteristics (Solar, Demirhan, & Taskin, 2025). Compared with singly periodic and doubly periodic minimal surfaces, triply periodic minimal surfaces (TPMS) are mathematically defined structures that repeat in three dimensions with zero mean curvatures and large surface areas. (Yu, Sun, & Bai, 2019) So far, fabrication of mathematically defined TPMS cellular structures was mainly realized by AM technologies, due to their high complexity. (Yang et al., 2018) In general, studies in the current literature indicate that TPMS-based cellular structures not only offer outstanding performance comparable to other porous materials, including excellent energy absorption, low weight, high strength-to-weight ratio, and effective thermal and acoustic insulation, but also benefit from their inherent geometric advantages. Specifically, TPMS structures possess smooth surfaces and consistent curvature radii, which enhance their manufacturability via additive manufacturing processes thanks to their self-supporting nature. Moreover, compared to conventional strut-based cellular designs characterized by sharp edges and abrupt corners, TPMS geometries enable more biomimetic and structurally efficient designs (Kapfer et al., 2011; Mazur et al., 2016).

Among the various triply periodic minimal surface (TPMS) structures, Gyroid, Diamond, Primitive, and I-WP are widely studied due to their distinct geometric characteristics and mechanical behavior. The Gyroid surface, first described by Schoen (1970), is a non-self-intersecting minimal surface that divides space into two interpenetrating but non-overlapping labyrinths. It offers a balanced isotropic mechanical response, making it highly suitable for load-bearing biomedical implants (Shelef & Bar-On, 2021). The Diamond TPMS structure exhibits a more angular geometry with a lower surface-to-volume ratio compared to the Gyroid, and typically shows higher stiffness but reduced ductility under compression (Maskery et al., 2018). The Schwarz Primitive (P) surface, characterized by relatively flat and broad patches, supports a stretching-dominated deformation mechanism when implemented as a sheet-based TPMS structure, leading to enhanced stiffness and energy absorption compared to strut-based lattices—this is well-demonstrated in compressive performance studies (Lee et al., 2017; Zhang et al., 2018). On the other hand, TPMS topologies like the I-WP (Isotropic Woodpile) have been comparatively analyzed with other forms such as Förstner Random Dots (FRD), where FRD outperformed I-WP in terms of crashworthiness metrics such as specific energy absorption at low relative densities (Bunsri et al., 2024). Overall, these TPMS geometries allow precise tuning of porosity, mechanical anisotropy, and stress distribution, and when combined in hybrid or graded structures they offer significantly improved energy absorption and multifunctional performance (Xie et al., 2023).

The Gyroid, introduced by Schoen in 1970, is a chiral, non-self-intersecting minimal surface with no planar symmetry and no straight lines (Schoen, 1970). Its continuously smooth and periodic nature leads to relatively isotropic mechanical behaviour and has made Gyroid architectures attractive for load-bearing biomedical scaffolds; gyroid lattices often combine a balanced stiffness and energy-absorption capacity that can mimic trabecular bone mechanics (Yan, Hao, Hussein, & Young, 2015; Yang et al., 2019). Moreover, the gyroid's continuous shell geometry tends to be self-supporting in powder-bed fusion processes (e.g., SLM/LPBF), which improves manufacturability and can reduce the need for additional support structures (Yan et al., 2015; Yang et al., 2019).

The Primitive TPMS often features broader, flatter regions and larger open pores; depending on whether the topology is implemented as a sheet or as a skeletal (strut) form, it can show bending-dominated deformation and excellent energy-absorption characteristics, which is why Primitive-based designs are frequently proposed for impact-mitigation applications (Zhang et al., 2018; Chen et al., 2023). Build orientation, wall thickness and

relative density strongly affect the Primitive's manufacturability and mechanical response, so these processing/design variables must be explicitly reported in any technical claim.

The I-WP (Isotropic Woodpile) TPMS, originally introduced by Schoen (1970), exhibits a highly symmetric and interconnected topology characterized by smooth, continuous transitions and geometric regularity (Lee, Weber, & Yerger, 2024; Schoen, 1970). Investigations comparing the mechanical response of various TPMS forms under compression found that I-WP demonstrates deformation behaviour that lies between stretching- and bending-dominated regimes, offering a more balanced mechanical performance than forms like primitive or gyroid (Bunsri et al., 2024). These topological characteristics contribute to more uniform anisotropy control and homogeneous stress distribution — advantageous traits for structural applications. While explicit studies quantifying fatigue resistance or biocompatibility specific to I-WP are limited, the topology's continuity and interconnectedness suggest potential benefits in such domains, particularly for biomedical scaffolds, pending direct experimental validation.

To visually illustrate the geometric diversity and relative density distribution of Triply Periodic Minimal Surface (TPMS) structures, Figure 1 presents two main categories of TPMS lattices: sheet-based and solid-based types. These scaffold geometries, modeled at a relative density of 0.25, emphasize the topological differences in their spatial distribution and connectivity. While the sheet-type TPMS lattices consist of continuous thin walls forming open voids, the solid-type counterparts are defined by interconnected solid regions with periodic internal porosity. Such structural variations significantly influence mechanical properties, manufacturability, and suitability for application-specific performance requirements, particularly in biomedical and lightweight structural systems (Viet, Karathanasopoulos, & Zaki, 2022).

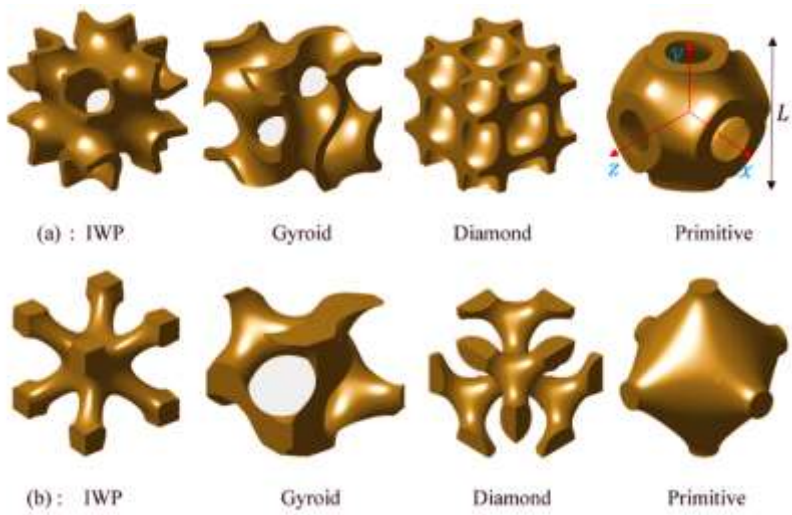


Figure 1. Schematic demonstration of various TPMS scaffolds having relative density of 0.25 with (a): Sheet type; (b): Solid type. (Viet, Karathanasopoulos, & Zaki, 2022)

Compared to conventional porous materials such as metal foams, honeycomb structures, and lattice frameworks, Triply Periodic Minimal Surface (TPMS) architectures offer significant improvements due to their mathematically defined, continuous geometries. While metal foams often suffer from irregular pore distributions and limited control over directional mechanical properties, TPMS lattices enable precise tuning of isotropic and anisotropic mechanical behavior, along with higher surface area-to-volume ratios and customizable deformation patterns (Ashby, 2006; Maskery et al., 2018). These features result in enhanced stiffness-to-weight ratios, superior energy absorption capabilities, and more predictable fatigue resistance.

Moreover, the inherent self-supporting nature of TPMS structures facilitates fabrication through advanced additive manufacturing methods such as laser powder bed fusion (LPBF), minimizing the need for support structures (Al-Ketan et al., 2018). Consequently, TPMS-based materials are increasingly favored for biomedical implants, aerospace components, and lightweight structural applications where mechanical efficiency and tailored responses are paramount.

Table 1 offers a comparative overview of various porous structure types including metal foams, honeycomb structures, space frame systems, and TPMS lattices highlighting key differences in porosity distribution, mechanical tunability, manufacturability, and application domains. This comparison underscores the superior versatility and performance potential of TPMS structures in advanced engineering applications.

Table 1. Comparison of Porous Structure Types

Property	**Metal Foams**	**Honeycomb Structures**	**Space Frame Systems**	**TPMS Structures**
Porosity Distribution	Random and non-uniform	Directional and layered	Structured and modular	Smooth and continuous
Isotropic/Anisotropic Control	Limited	Moderate	Customizable	Highly tunable
Surface Area-to-Volume Ratio	Moderate	Low	Moderate	High
Stress Concentration	High due to irregular cells	Present at nodes	High at joints	Minimized due to smooth topology
Energy Absorption	Moderate to High	Moderate	Variable	High and optimizable

Moreover, the intrinsic periodicity and mathematical definability of TPMS architectures enable precise computational modeling and simulation, which is particularly advantageous for predictive design workflows. Unlike stochastic foams and irregular honeycomb systems, TPMS lattices can be engineered with consistent mechanical responses across the structure, minimizing variability in load distribution and enhancing reliability in service conditions (Viet et al., 2022).

Additionally, many TPMS structures such as the Gyroid and I-WP exhibit inherent self-supporting characteristics that reduce or eliminate the need for auxiliary support structures during additive manufacturing processes like selective laser melting (SLM), leading to decreased material waste and reduced post-processing effort (Al-Ketan et al., 2018; Maskery et al., 2018). This advantage not only lowers production costs but also accelerates prototyping and customization, which is especially valuable for critical biomedical and aerospace components.

Given their tailorable mechanical anisotropy, seamless porosity gradation, and manufacturability advantages, TPMS-based designs are not just an alternative, but a paradigm shift in the field of architected materials. Their adoption continues to expand in areas demanding multifunctional performance—where stiffness, energy absorption, biological compatibility, and weight reduction must be simultaneously optimized (Al-Ketan et al., 2020).

The mechanical behavior of Triply Periodic Minimal Surface (TPMS) structures has been extensively studied due to their promising potential in lightweight and multifunctional engineering applications. Maskery et al. (2017) experimentally and numerically analyzed additively manufactured double Gyroid lattices, highlighting their energy absorption and failure modes. The foundational work by Ashby and Gibson (1997) on cellular solids provides a theoretical basis for understanding TPMS stiffness and strength characteristics. Additionally, Ngo et al. (2018) reviewed additive manufacturing technologies and discussed the advantages of TPMS architectures in aerospace and energy sectors due to their tunable mechanical properties and manufacturability. These studies collectively demonstrate the growing importance of TPMS lattices in advanced structural designs. A detailed comparison of key TPMS studies is shown in Table 2.

Table 2. Summary of Recent Numerical and Experimental Studies on TPMS-Based Lattices

Authors, Year	***Investigated Lattices***	***Aim of the Study***
Diab et al. (2016)	Primitive, Neovius, CLP foams	Elastic and conductive properties; anisotropy analysis via FEM
Fan & Wang (2025)	TPMS core in wood-plastic sandwich beams	Influence of graded TPMS cores on bending stiffness, strength, and toughness
Feng et al. (2023)	Adjustable TPMS structures	Curvature-wall method for isotropy and elastic control; validated numerically/experimentally
Fisher et al. (2023)	TPMS and related lattices	Created a TPMS design catalog with property data and CAD files
Fu et al. (2022)	Isotropic hollow TPMS lattices	Genetic algorithm optimization for load bearing and energy absorption
Silver-Thorn et al. (2013)	General porous structures	Review of modulus–porosity relationships for AM; accuracy emphasized for 20–50% porosity
Chatzigeorgiou et al. (2021)	TPMS vs. strut-based lattices	Biomedical implants; TPMS advantage in mimicking bone and reducing stress peaks
Ejeh et al. (2025)	Hybrid Primitive-based TPMS	Reinforced designs achieving isotropy and near-theoretical stiffness limits
COMSOL (2023)	Gyroid composites	Micromechanical modeling of Poisson's ratio and volume fraction effects
DeValk & Lakes (2021)	Gyroid lattice	Experimental validation of isotropic Poisson's ratio and modulus near H-S upper bound

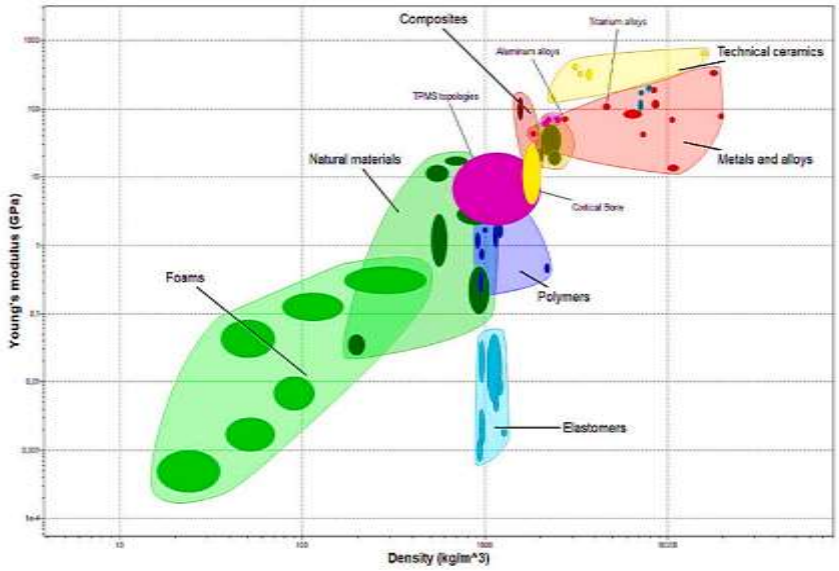


Figure 2. An Ashby chart showing the Young's modulus and density of TPMS topologies (Gyroid, Diamond, I-WP, and Primitive) compared to the target region of cortical bone (Chatzigeorgiou et al., 2021; Gibson & Ashby, 1997; Ashby, 1997).

MATERIAL AND METHOD

1.5. Design and Geometric Generation of Lattice Architectures

A lattice structure is characterized by a repeating unit cell, where the solid volume V_{solid} is compared to the total volume V_{total} to determine the volume fraction:

$$Volume\ Fraction = \frac{V_{solid}}{V_{total}}$$

The lattice geometry directly influences this volume fraction, as it dictates the distribution of solid material within the unit cell (Chatzigeorgiou et al., 2021).

In this study, three distinct TPMS geometries Gyroid, Primitive, and I-WP were selected due to their widely recognized mechanical behavior and fabrication feasibility. Each unit cell was modeled using implicit surface equations in MATLAB within a cubic design domain L×L×L, with periodic boundary conditions. The isosurface parameter t was varied within the range $0 \leq t \leq 1.5$ to investigate the influence of relative density on mechanical properties.

1.6. Input parameters

This study employs two primary input parameters: the relative density (ρ_{rd}) and the type of TPMS unit cell. These parameters play a critical role in determining the effective mechanical response of the lattice structures. Different TPMS geometries, due to their unique surface topologies, exhibit varied stiffness, energy absorption, and anisotropy characteristics, even under the same RD value.

1.6.1. Relative Density

The relative density (ρ_{rd}) is a dimensionless parameter that represents the ratio of the apparent density of a cellular structure to the density of its solid parent material. It is defined as follows:

$$\rho_{rd} = \frac{\rho^*}{\rho_s}$$

The ρ_{rd} governs many mechanical properties such as stiffness, strength, and energy absorption capacity. As the RD increases, stiffness and strength generally increase at the cost of weight savings and energy absorption efficiency. In this study, ρ_{rd} is controlled via the isosurface parameter (t) in the implicit mathematical formulations of TPMS geometries, allowing continuous gradation and fine-tuning of porosity across the structure.

1.6.2. Unit Cells

The unit cell represents the fundamental building block that is periodically repeated to form a complete lattice structure. Depending on their geometry, unit cells can be categorized into various types. In TPMS-based lattice architectures, the unit cell geometry is mathematically described using implicit equations that involve trigonometric functions.

In the current study, three distinct TPMS topologies Gyroid, Primitive, and I-WP were examined. These structures were selected due to their continuous curvature, high surface-to-volume ratio, and self-supporting capabilities, which make them suitable for additive manufacturing and mechanical performance optimization. The mathematical formulations used to define their implicit surfaces are detailed in the subsequent section.

Gyroid

$$t = sin(\frac{2\pi x}{L}) * cos(\frac{2\pi y}{L}) + sin(\frac{2\pi y}{L}) * cos(\frac{2\pi z}{L}) + sin(\frac{2\pi z}{L}) * cos(\frac{2\pi x}{L})$$

Primitive

$$t = cos(\frac{2\pi x}{L}) + cos(\frac{2\pi y}{L}) + cos(\frac{2\pi z}{L})$$

I-WP

$$t = cos(\frac{2\pi x}{L}) * sin(\frac{2\pi y}{L}) + cos(\frac{2\pi y}{L}) * sin(\frac{2\pi z}{L}) + cos(\frac{2\pi z}{L}) * sin(\frac{2\pi x}{L})$$

1.6.3. Porosity–Modulus Models

Various models have been developed to describe the mechanical behavior of porous materials, particularly focusing on how the elastic modulus decreases with increasing volume porosity. While some models assume a linear relationship, others incorporate more complex power-law or exponential functions. These expressions vary in accuracy depending on microstructural features such as pore shape, distribution, and connectivity.

The following table summarizes these approaches, presenting their general forms and main characteristics. It serves as a comparative reference for researchers working on porous structures such as TPMS lattices (Table 3; Silver-Thorn, 2013).

Table 3. Comparison of Models Describing the Relationship Between Porosity and Elastic Modulus. (Silver-Thorn, 2013)

General equation form General equation form Other Equations			
Linear equations	***Power equations***	***Exponential equations***	***Other equations***
$\mathbf{\frac{E_p}{E_0} = 1 - aP}$	$\frac{E_p}{E_0} = (1 - jP)^i$	$\frac{E_p}{E_0} = \exp(-mP)$	$\frac{E_p}{E_0} = (1 - P)/(1 + wP)$
	$\frac{E_p}{E_0} = 1 - uP^{2/3}$	$\frac{E_p}{E_0} = 1 - \exp(-r(1 - P))$	$\frac{E_p}{E_0} = 1 + [\frac{AP}{1-AP-P}]$
	$\frac{E_p}{E_0} = [1 - \left(\frac{P}{Q}\right)]^{KQ}$	$\frac{E_p}{E_0} = \exp[-(nP + gP^2)]$	$\frac{E_p}{E_0} = (1 - P^2)/(1 + yP)$
	$\frac{E_p}{E_0} = (1 - P^{2/3})^{1.21S}$		
	$\frac{E_p}{E_0} = (1 - P^{2/3})/(1 - P^{\frac{2}{3}} + P)$		
	$\frac{E_p}{E_0} = 1 - dP + gP^2$		
	$\frac{E_p}{E_0} = [1 - (\frac{P}{t})]^S$		

1.1.1. Gibson–Ashby Model

The Gibson–Ashby model is widely used to predict the equivalent mechanical properties of porous and cellular materials, including TPMS lattice structures. In this study, Young's modulus (E), shear modulus (G), and Poisson's ratio (ν) are expressed as functions of relative density (ρ_{rd}) for different TPMS geometries Maskery et al. (2018).

The general form of the Gibson–Ashby equations is expressed as:

$$E^* = C_E x E_s \times \rho_{rd}^{n_E}$$

$$G^* = C_G x E_S \times \rho_{rd}^{n_G}$$

$$v^* = f(\rho_{rd})$$

The following table presents the piecewise analytical expressions for the effective elastic modulus (E*), shear modulus (G*), and Poisson's ratio (ν*) of the Gyroid TPMS structure as a function of the relative density (ρ_{rd}), based on the study by Maskery et al. (2018).

Table 4. Mechanical properties of sheet-based TPMS structures

Mechanical Property		Condition	Expression
Gyroid (G)	Elastic Modulus (E*)	$\rho_{rd} \leq 0.45$	$\frac{E*}{Es} = 0.596 \times \rho_{rd}^{1.467}$
		$\rho_{rd} > 0.45$	$\frac{E*}{Es} = (0.962 \times \rho_{rd}^{2.351} + 0.038)$
	Shear Modulus (G*)	$\rho_{rd} \leq 0.45$	$\frac{G*}{Gs} = 0.777 \times \rho_{rd}^{1.544}$
		$\rho_{rd} > 0.45$	$\frac{G*}{Gs} = 0.973 \times \rho_{rd}^{1.982} + 0.027$
	Poisson's Ratio (ν*)	$\rho_{rd} \leq 0.5$	$\nu* = 0.192 \times e^{-1.349*\rho_{rd}} + 0.202$
		$\rho_{rd} > 0.5$	$\nu* = 0.402 \times \rho_{rd}^{2} - 0.603 \times \rho_{rd} + 0.501$
Primitive (P)	Elastic Modulus (E*)	$\rho_{rd} \leq 0.25$	$\frac{E*}{Es} = 0.317 \times \rho_{rd}^{1.264}$
		$\rho_{rd} > 0.25$	$\frac{E*}{Es} = 1.007 \times \rho_{rd}^{2.006} - 0.007$
	Shear Modulus (G*)	$\rho_{rd} \leq 0.25$	$\frac{G*}{Gs} = 0.705 \times \rho_{rd}^{1.189}$
		$\rho_{rd} > 0.25$	$\frac{G*}{Gs} = (0.953 \times \rho_{rd}^{1.715} + 0.047)$
	Poisson's Ratio (ν*)	$\rho_{rd} \leq 0.55$	$\nu* = 0.314 \times e^{-1.004*\rho_{rd}} + 0.119$
		$\rho_{rd} > 0.55$	$\nu* = 0.152 \times \rho_{rd}^{2} - 0.235 \times \rho_{rd} + 0.383$
I-graph and wrapped package-graph (IWP)	Elastic Modulus (E*)	$\rho_{rd} \leq 0.35$	$\frac{E*}{Es} = 0.597 \times \rho_{rd}^{1.225}$
		$\rho_{rd} > 0.35$	$\frac{E*}{Es} = (0.987 \times \rho_{rd}^{1.782} + 0.013)$
	Shear Modulus (G*)	$\rho_{rd} \leq 0.35$	$\frac{G*}{Gs} = 0.529 \times \rho_{rd}^{1.287}$
		$\rho_{rd} > 0.35$	$\frac{G*}{Gs} = (0.960 \times \rho_{rd}^{2.188} + 0.040)$
	Poisson's Ratio (ν*)	$\rho_{rd} \leq 0.13$	$\nu* = 2.597 \times e^{-1.004*\rho_{rd}} - 2.244$
		$\rho_{rd} > 0.13$	$\nu* = 0.201 \times \rho_{rd}^{2} - 0.227 \times \rho_{rd} + 0.326$

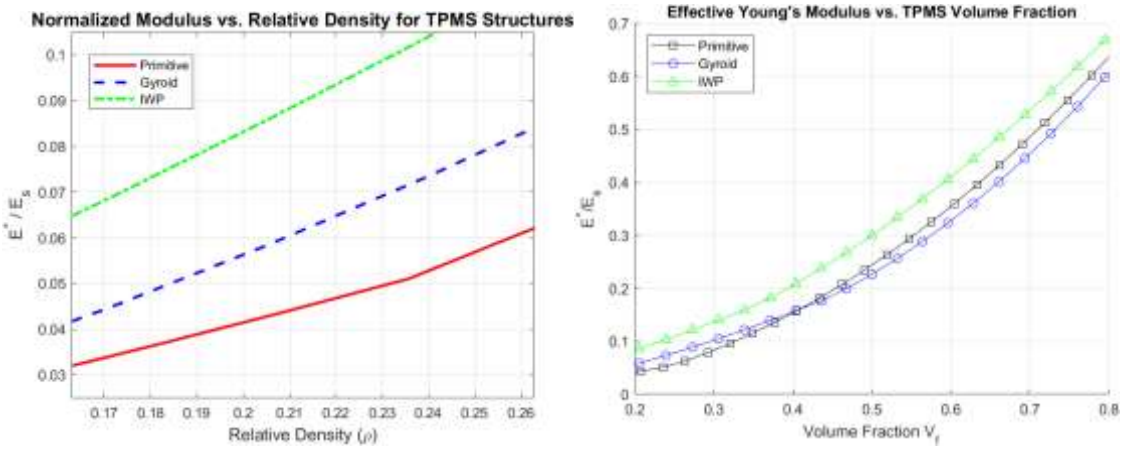
RESULTS AND DISCUSSION

In this study, three different types of TPMS (Triply Periodic Minimal Surface) structures were utilized: Gyroid, I-WP and Primitive. For each TPMS topology, the relative densities were computed as a function of the isosurface threshold parameter (*t*). Subsequently, the corresponding effective mechanical properties, namely Young's modulus (E*), shear modulus (G*), and Poisson's ratio (ν*), were determined. The variation of these mechanical properties with respect to *t* was investigated in detail within the range of $0 \leq t \leq +1.5$ for each TPMS type. These results provide a comprehensive comparison of the mechanical performance of the selected TPMS geometries across different porosity levels.

In order to evaluate the effective mechanical properties of TPMS-based lattice structures, a computational framework was implemented using MATLAB. Three types of TPMS topologies Gyroid, Primitive and I-WP were investigated over a predefined range of isosurface threshold values (t) between 0 and 1.5, divided into 60 equal intervals.

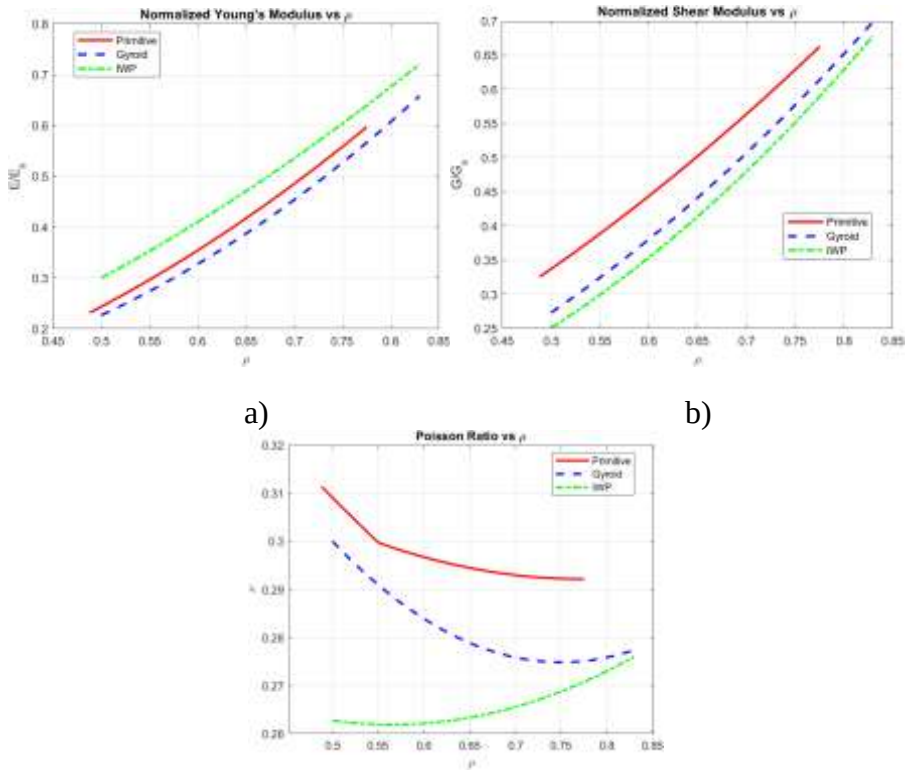
Once ρ_{rd} was determined for each t value and topology, the corresponding mechanical properties Young's modulus (E*), Shear modulus (G*), and Poisson's ratio (ν*) were calculated using empirical Gibson–Ashby-type models. These models relate normalized mechanical properties to relative density using power-law or piecewise analytical expressions derived from the literature.

Figure 3 illustrates the variation of normalized modulus and relative density for three different TPMS structures. Subfigure (a) shows how the normalized modulus changes with relative density, highlighting the differences in stiffness among Primitive, Gyroid and I-WP geometries. Subfigure (b) presents the effective Young's modulus as a function of TPMS volume fraction, emphasizing how each structure responds differently to increasing porosity.



a) b)

Figure 3. a) Variation of Normalized Young's Modulus with Relative Density for Different TPMS Structures b) Variation of the Effective Young's Modulus with volume fraction for different TPMS structures.



a) b)

c)

Figure 4. a) Variation of Normalized Young's Modulus with ρ for different TPMS structures b) Variation of the normalized shear modulus with ρ for different TPMS structures c) Variation of the Poisson ratio with ρ for different TPMS structures.

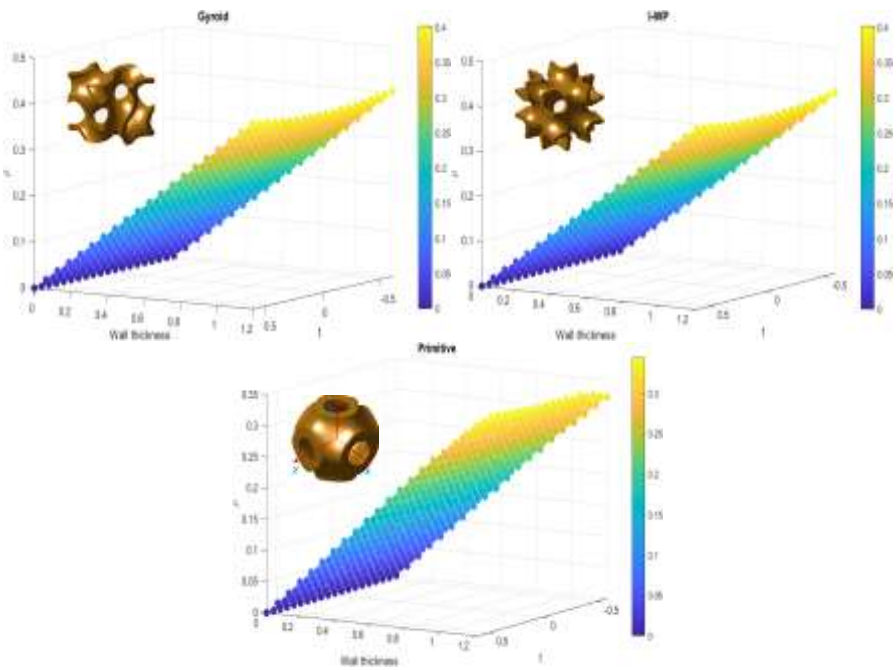


Figure 5. Three-dimensional surface plots of the relative density ρ for three different TPMS structures (Gyroid, I-WP, and Primitive) as a function of the isosurface parameter t and wall thickness.

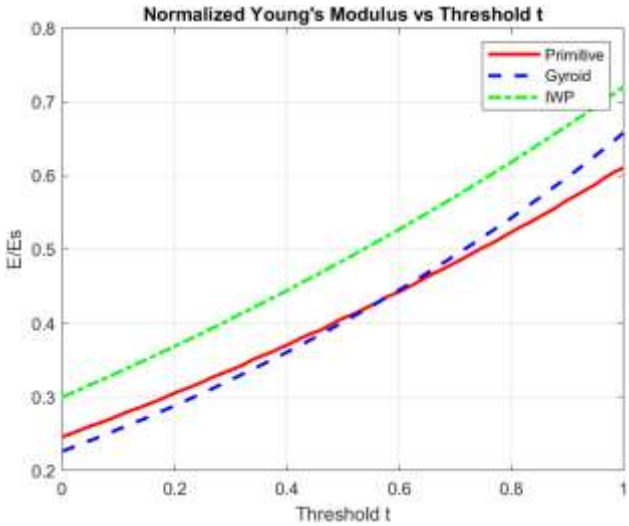


Figure 6. Variation of Normalized Young's Modulus with Isosurface Threshold (t) for Different TPMS Structures

This figure illustrates the variation of normalized Young's modulus (E/Es) as a function of the isosurface threshold parameter t for three distinct Triply Periodic Minimal Surface (TPMS) architectures: Primitive, Gyroid, and I-WP. The threshold t regulates the solid volume fraction within the unit cell, directly influencing the relative density and mechanical stiffness of each structure.

Comparison of Structures:

- The I-WP structure exhibits the highest normalized Young's modulus across the threshold range, indicating superior stiffness and load-bearing capability compared to the other two TPMS geometries.
- The Gyroid structure follows a moderately steep trend, showing intermediate stiffness values, reflecting its balance between flexibility and structural rigidity.
- The Primitive structure demonstrates the lowest normalized Young's modulus throughout, suggesting a more compliant and flexible lattice design.
- All three TPMS structures display nonlinear growth of stiffness with increasing t, consistent with the predictions of cellular solid models such as the Gibson–Ashby theory.

This analysis highlights that by adjusting the threshold t and selecting appropriate TPMS geometries, it is possible to tailor the elastic properties of architected and functionally graded materials for specific mechanical requirements.

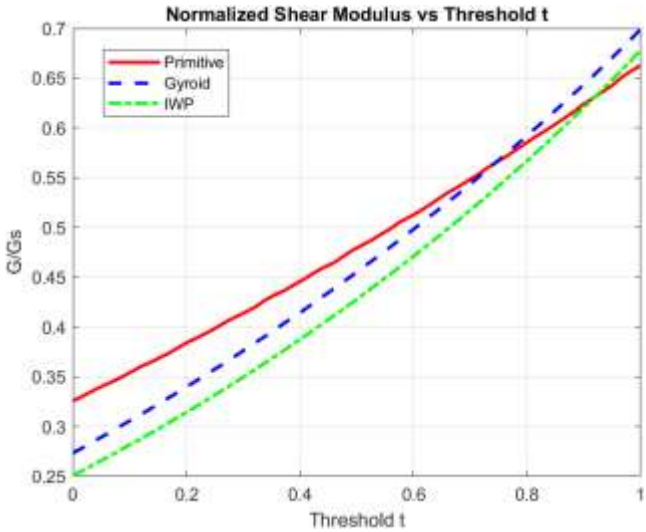


Figure 7. Variation of Normalized Shear Modulus with Isosurface Threshold (t) for Different TPMS Structures

This figure illustrates the variation of the normalized shear modulus G/Gs for three TPMS structures—Primitive, Gyroid, and I-WP as a function of the isosurface threshold parameter t, which controls the relative density (ρrd) of each structure.

Threshold Parameter (t): The t parameter ranges from 0 to 1 and regulates the solid volume fraction of the unit cell. As t increases, the relative density of the structure increases, leading to enhanced mechanical stiffness.

Normalized Shear Modulus (G/Gs): The vertical axis represents the shear modulus of the TPMS structures normalized by the shear modulus of the solid material (Gs). This provides a measure of the relative stiffness of the porous structures.

Comparison of Structures:

- Primitive (red solid line): Exhibits moderate shear modulus values throughout the range of t, indicating intermediate stiffness.
- Gyroid (blue dashed line): Shows slightly higher shear modulus than Primitive for most values of t, reflecting better shear resistance.
- I-WP (green dashed line): Demonstrates the lowest normalized shear modulus, making it the most flexible structure among the three.

Trend: For all structures, G/Gs increases monotonically with t, confirming that higher relative density enhances the shear stiffness. The difference between structures becomes more pronounced at intermediate t values, while they converge at high t near 1, indicating that denser configurations reduce structural performance differences.

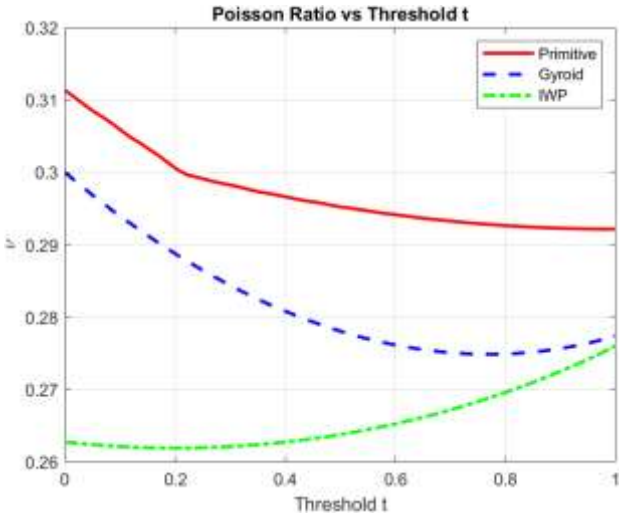


Figure 8. Variation of Poisson's Ratio with Isosurface Threshold (t) for Different TPMS Structures

This graph illustrates how the Poisson's ratio (ν) of three different TPMS (Triply Periodic Minimal Surface) structures—Primitive, Gyroid, and IWP—varies with the isosurface threshold parameter (t). The parameter 't' directly influences the relative density of the structure. Key observations from the graph:

Key Observations

- Overall Trend: For the Primitive and Gyroid structures, the Poisson's ratio generally decreases as the threshold 't' increases. This indicates that at higher densities, these materials exhibit reduced lateral expansion when subjected to axial strain. The IWP structure shows a slight decrease initially before starting to increase again after a 't' value of approximately 0.5.
- Primitive Structure: The Primitive structure consistently maintains the highest Poisson's ratio values across the entire range of 't'. This behavior suggests a more ductile and flexible response compared to the other two structures.
- Gyroid Structure: The Gyroid structure exhibits a lower Poisson's ratio than the Primitive structure, and it shows a noticeable decrease as 't' increases. This suggests the material becomes less deformable in the lateral direction.
- IWP Structure: The IWP structure shows the lowest Poisson's ratio up to a 't' value of around 0.2. This indicates a more rigid behavior in the lateral direction. After this point, the Poisson's ratio begins to increase.
- Non-linear Trends: The non-linear trends observed for each structure reflect the strong dependence of the Poisson's ratio on the specific micro-geometry and density distribution.

These results are crucial for designing materials where specific mechanical properties, such as lateral stiffness or flexibility, are desired. This is particularly relevant in fields like biomechanics, aerospace panels, or flexible protective layers, where precise material behavior is essential for performance.

CONCLUSION

In this study, the mechanical behavior of three TPMS architectures Primitive, Gyroid, and I-WP was investigated as a function of the isosurface threshold parameter *t*. The analyses revealed that increasing *t* enhances relative density and, consequently, both elastic and shear stiffness. Among the structures, the I-WP exhibited the highest stiffness, followed by the Gyroid, while the Primitive consistently showed the lowest values, indicating higher flexibility. Poisson's ratio results showed a general decrease with increasing density, with the Primitive maintaining greater lateral deformability and the I-WP displaying the most rigid response. These findings confirm that by tuning the threshold parameter and selecting suitable TPMS geometries, mechanical properties can be effectively tailored for advanced engineering applications.

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PRODUCTION AND CHARACTERIZATION OF MESOPOROUS ACTIVATED CARBON FROM CAROB TREE BIOMASS FOR ENVIRONMENTAL APPLICATIONS

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ABSTRACT

The valorization of non-edible carob pods (*Ceratonia siliqua L.*), an abundant resource in Algeria, represents a promising pathway for producing high value-added carbon materials. In this work, a mesoporous activated carbon was prepared from this biomass through phosphoric acid impregnation followed by controlled pyrolysis. The obtained material was characterized by thermogravimetric and differential scanning calorimetry analyses (TGA/DSC), X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), and specific surface area measurements (BET). The results revealed efficient carbonization between 300 and 450 °C, enhanced by the catalytic action of H_3PO_4, leading to an amorphous and highly porous structure. The activated carbon exhibited a high specific surface area (739.26 m^2/g), a mesoporous structure confirmed by an H4-type hysteresis loop, and an abundance of oxygenated functional groups favorable for adsorption. These properties provide the material with strong potential for environmental applications such as pollutant adsorption, catalysis, and filtration.

Keywords : Carob tree, activated carbon, chemical activation, porosity, environmental applications

INTRODUCTION

The carob tree (*Ceratonia siliqua L.*), belonging to the family Caesalpiniaceae, is a typical species of Mediterranean regions. Cultivated for ornamental, industrial, and agro-silvo-pastoral purposes, it has considerable socio-economic and ecological importance due to its strong ability to adapt to arid and semi-arid conditions (Rejeb, 1989; Girolamo et al., 2002). Its pods, mainly used in the pharmaceutical and food industries (Van Uden, 1981; Maria et al., 1997), represent an abundant and low-cost resource, yet still largely underexploited.

In North Africa, and particularly in northern Algeria, certain varieties of carob trees produce non-edible pods. The valorization of these residues represents a promising opportunity for the production of high value-added carbon materials. In particular, their transformation into biochar or activated carbon fits within the framework of a circular economy, combining sustainable management of agricultural waste, improvement of soil quality, and contribution to the fight against climate change. Owing to their lignocellulosic structure, these biomasses are

well suited for carbonization and chemical activation, leading to the formation of porous materials characterized by high specific surface area, good thermal stability, and a richness in oxygenated functional groups. These properties provide activated carbon derived from carob with remarkable potential for contaminant adsorption and various environmental applications.

Beyond its technical performance, the use of non-edible carob pods presents several advantages, including local availability, low cost, renewable nature, and non-toxicity. This valorization also contributes to local socio-economic development through the creation of income-generating activities, while reducing the environmental impact associated with the accumulation of such residues (Mahmoud et al., 2014).

In this context, the present study aims to explore the potential of non-edible carob pods as a raw material for the preparation of mesoporous activated carbon by chemical activation. The focus will be on the physicochemical characterization of the obtained material and the evaluation of its properties for environmental applications.

MATERIAL AND METHOD

Preparation of Activated Carbon from *Ceratonia siliqua* Pods

The pods of *Ceratonia siliqua*, collected in Constantine (Algeria) during the month of December, were carefully rinsed with tap water and then with distilled water to remove impurities. They were subsequently dried at 50 °C until a constant weight was obtained. The dried material was then ground and sieved to obtain particles with a diameter ranging between 160 and 500 µm.

Twenty grams of this powder were impregnated with 10 g of 85% phosphoric acid for 24 hours at room temperature. The mixture was then subjected to a two-step sonication process: a first phase of 30 minutes under ultrasound, followed by a one-hour rest period, and then a second phase of 30 minutes under ultrasound.

The resulting product was dried at 110 °C for 24 hours and then placed in a furnace at room temperature. Carbonization was carried out at a heating rate of 9 °C/min until reaching 450 °C, a temperature maintained for one hour. The carbonized residue was then purified by continuous washing with distilled water in a Soxhlet extractor until the effluent was free of phosphate traces.

Finally, the obtained activated carbon was dried at 100 °C until its mass stabilized, finely ground, and stored in a hermetically sealed glass container.

Characterization of Activated Carbon

Thermal Analysis (TGA/DSC)

The thermal analysis of the sample was carried out using a simultaneous thermal analyzer TGA/DSC 3+ (Mettler Toledo), capable of reaching temperatures up to 1600 °C. The measurements were performed under a controlled nitrogen atmosphere, with a constant heating

rate of 10 °C/min, from room temperature up to 1000 °C. This analysis allowed the evaluation of the thermal stability of the sample, the identification of decomposition stages, and the characterization of mass losses (TGA) as well as the associated thermal transitions (DSC).

Specific Surface Area Analysis (BET)

The textural characterization of the activated carbon was carried out using a specific surface area analyzer AutosorbiQ Station 2 (Quantachrome Instruments), with nitrogen as the adsorbate gas at liquid nitrogen temperature (77.35 K). The sample, with a mass of 0.317 g, was previously degassed under vacuum at 200 °C for 9 hours in order to remove moisture and surface-adsorbed gases. The Brunauer-Emmett-Teller (BET) model was applied to calculate the specific surface area from the obtained adsorption isotherm.

X-Ray Diffraction (XRD) Analysis

The crystalline structural analysis of the carbon material was carried out by X-ray diffraction using an Empyrean diffractometer (PANalytical) equipped with a copper anode, producing radiation characterized by wavelengths $K\alpha_1$ = 1.54060 Å, $K\alpha_2$ = 1.54443 Å, and $K\beta$ = 1.39225 Å, with a $K\alpha_2/K\alpha_1$ ratio of 0.5. The generator was set at 40 kV and 30 mA to ensure a stable intensity of the incident beam.

The scanning covered an angular range from 5.00° to 89.98° (2θ), with a step size of 0.013° in continuous mode, and was performed at room temperature.

Fourier Transform Infrared (FTIR) Spectroscopy Analysis

Fourier Transform Infrared (FTIR) spectroscopy was performed on our sample using a Bruker INVENIO-X spectrometer equipped with an RT-DLaTGS detector. Measurements were carried out in the spectral range of 4000 to 400 cm^{-1}, with a resolution of 4 cm^{-1} and 16 scans.

RESULTS AND DISCUSSION

Thermal Analysis (TGA/DSC)

The thermal behavior of the activated carbon as a function of temperature is shown in Fig. 1.

The TGA/DSC analysis shows that the sample activated at 450 °C with 10% phosphoric acid undergoes thermal transformation in several well-defined stages, typical of an efficient chemical activation process. The significant mass loss and intense exothermic effect observed between 300 and 450 °C indicate effective carbonization with the development of porosity. Phosphoric acid (H_3PO_4) plays a catalytic role in thermal degradation and contributes to the formation of porous structures stabilized at high temperature.

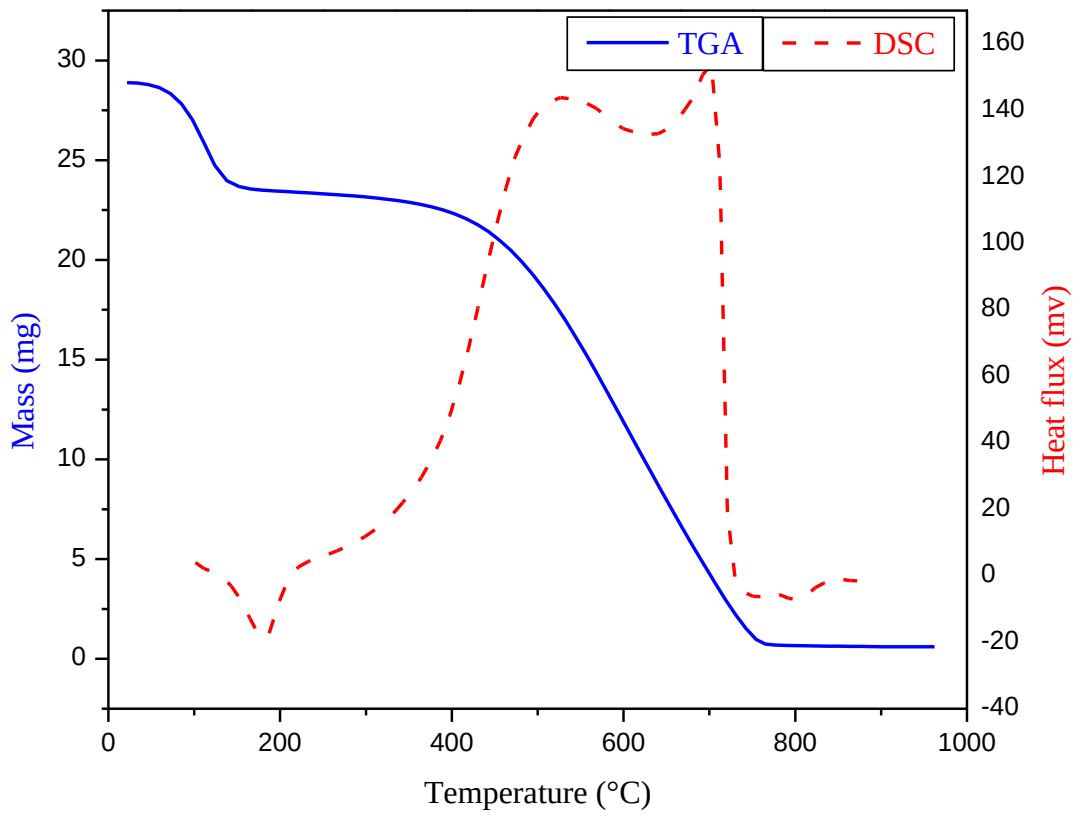


Fig. 1. Combined TGA and DSC curves of the analyzed activated carbon sample.

FTIR Analysis

The FTIR spectrum of the obtained activated carbon is presented in Fig. 2. It reveals the presence of various functional groups on the surface of the material, consistent with chemical activation using phosphoric acid. A broad band at 3410 cm^{-1} indicates the presence of hydroxyl (O–H) groups, potentially associated with phenolic or carboxylic groups, as well as adsorbed water (Zhang et al., 2020). A weak intensity at 2915 cm^{-1} suggests aliphatic (C–H) groups such as methyl or methylene groups (Ahuja et al., 2022). The band at 1710 cm^{-1} corresponds to carbonyl (C=O) groups, while the bands between 1580–1600 cm^{-1} and 1050–1250 cm^{-1} are attributed respectively to aromatic C=C bonds and C–O bonds (alcohols, acids, esters, ethers) (Mohamad et al., 2020). Finally, the peaks between 800–870 cm^{-1} confirm the presence of aromatic rings (=C–H).

This spectral profile, typical of chemically activated carbon, highlights the abundance of oxygenated functional groups, which significantly contribute to its adsorption properties (Esmail M et al., 2024).

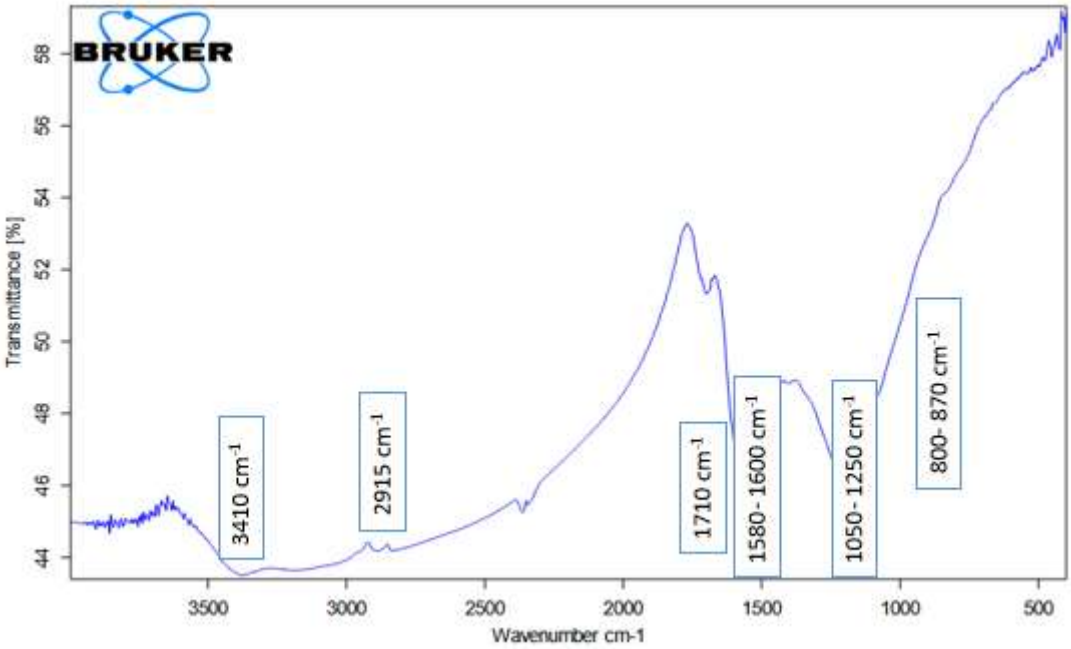


Fig. 2. FTIR spectra of the activated carbon sample.

XRD Analysis

The X-ray diffraction (XRD) pattern of the activated carbon obtained after activation is shown in Fig. 3. It highlights a predominantly amorphous structure. A broad and low-intensity halo centered around 2θ ≈ 22–26° is characteristic of amorphous or poorly crystallized carbon, which is typical of activated carbons derived from biomaterials treated at moderate temperatures (<600 °C) (Theeradit, Ph). A second broadening around 2θ ≈ 43° suggests local graphitic rearrangements without the complete formation of graphene or well-ordered graphite.

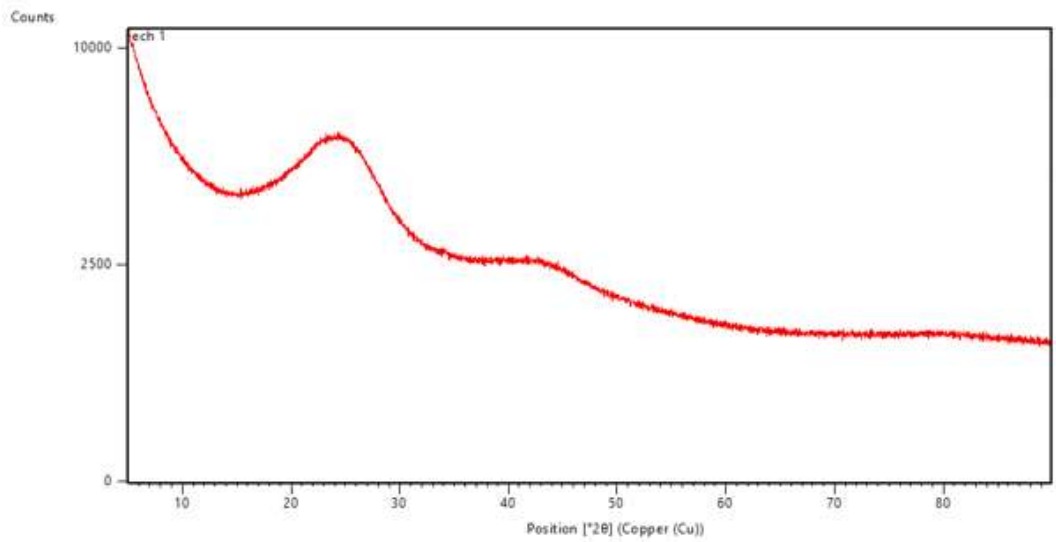
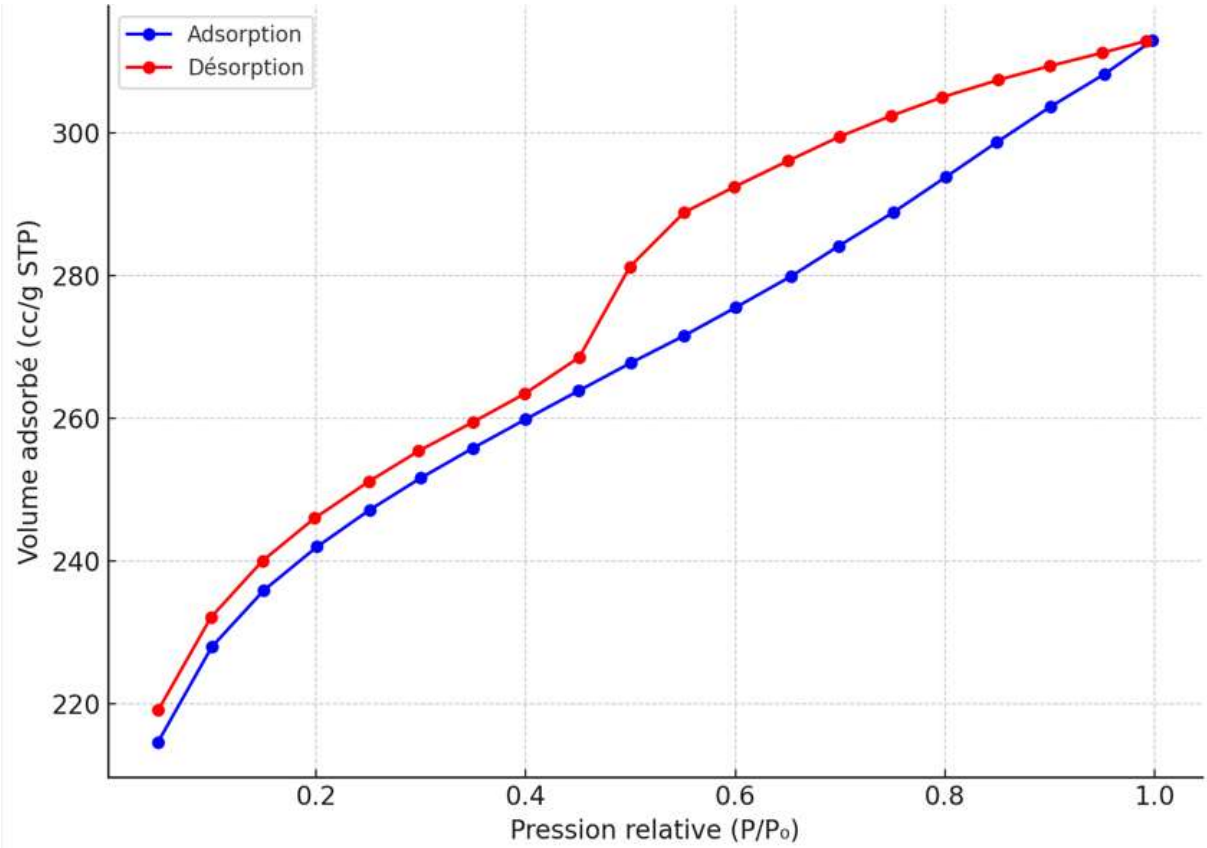


Fig. 3. XRD patterns of the activated carbon sample.

The absence of sharp and narrow peaks, as well as the lack of high-intensity secondary peaks, indicates a low degree of crystallinity and the absence of significant crystalline mineral phases (such as quartz or calcite) or metallic oxides. These observations confirm that the activated carbon obtained has a disordered structure, optimized for applications requiring high specific surface area and well-developed porosity.

BET Analysis

The nitrogen adsorption isotherm at 77 K for the activated carbon is shown in Fig. 4.



The BET equation with an infinite number of layers was used to determine the specific surface area:

$$\frac{V}{V_m} = \frac{Cx}{(1-x)(Cx+1-x)}$$

Where $x = \frac{P}{P_o}$ represents the ratio of the equilibrium adsorption pressure P to the saturation vapor pressure of the gas P_o. V is the volume of adsorbed gas under standard conditions (0 °C and 760 mmHg). V_m is the volume of gas under standard conditions required to cover the adsorbent with a monolayer. C is a constant characteristic of the isotherm.

The linear form of the BET equation can be written as:

$$\frac{x}{V(1-x)} = \frac{1}{V_m C} + \frac{C-1}{V_m C}x$$

By plotting $\frac{x}{V(1-x)}$ as a function of x, a straight line is obtained, with a slope of $\frac{C-1}{V_m C}$ and an intercept corresponding to $\frac{1}{V_m C}$. From these values, V_m can be calculated.

In our case, the following results were obtained:

$$\frac{1}{V_m C} = 8.76 \times 10^{-5} \text{cm}^{-3}$$

$$\frac{C-1}{V_m C} = 5.8 \times 10^{-3} \text{cm}^{-3}$$

$$\frac{1}{V_m} = \frac{C-1}{V_m C} + \frac{1}{V_m C} = 8.76 \times 10^{-5} + 5.8 \times 10^{-3} = 5.8876 \times 10^{-3} \text{cm}^{-3}$$

$$V_m = 169.848 \text{ cm}$$

The specific surface area was determined using the following relation:

$$\text{S} = \frac{\text{Vm} \cdot \text{NA} \cdot \sigma}{22414}$$

Where

$\text{NA} = 6.022 \times 10^{23}$ (Avogadro's number),
$\sigma = 0.162\, nm^2$ (area occupied by one nitrogen molecule),
22414 = molar volume in cm^3/mol (STP).
Thus, the specific surface area is:

$$S = 739.26\, m^2 g^{-1}$$

This result indicates a high specific surface area, characteristic of a highly porous material, suitable for applications requiring a large exchange surface, such as adsorption, catalysis, and filtration.

The nitrogen adsorption–desorption isotherm exhibits an H4-type hysteresis loop, typical of mesoporous materials with slit-shaped or lamellar pore structures. This behavior confirms the presence of non-uniform mesopores, possibly resulting from irregular particle stacking or specific chemical activation.

CONCLUSION

This work has demonstrated the feasibility of producing mesoporous activated carbon from non-edible carob pods, a locally available and low-cost biomass. Chemical activation with phosphoric acid and carbonization at 450 °C led to a material with a high specific surface area ($739.26\, m^2 g^{-1}$), an amorphous structure, and well-developed porosity, as confirmed by TGA/DSC, XRD, FTIR, and BET analyses.

These results highlight the potential of carob as an alternative resource for the production of sustainable activated carbons, suitable for adsorption and environmental treatment

applications. Beyond the scientific and technological interest, this valorization also contributes to agricultural waste management and the development of a circular economy at the regional level. Further studies will be necessary to evaluate the performance of this material in specific applications, particularly in the removal of organic and metallic pollutants from wastewater.

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BOLTING IN SPINACH; GENETIC AND ENVIRONMENTAL FACTORS

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ABSTRACT

Bolting in spinach is an undesirable physiological process in which the plant suddenly transitions from the leaf formation period to the flowering period. This process leads to economic losses by negatively affecting leaf yield and market quality. The bolting process is determined by genetic predisposition and environmental conditions. Genetically, some spinach varieties are more resistant to bolting than others. This highlights the role of genes regulating flowering in bolting-related genes such as *Flowering Locus T*, *Leafy (LFY)* and *Suppressor of overexpression of co1 (SOC1*) . These loci controlling bolting are directly or indirectly stimulated under specific environmental and endogenous conditions and initiate flowering by generating a series of internal signals. Environmental factors that accelerate bolting include long day length (photoperiod), high temperatures, and gibberellin, an endogenous hormones and key factor of spinach bolting-related genes. Additionally, environmental stresses, such as nutrient imbalance and water stress, can trigger this process. Understanding these factors is important for developing bolting-tolerant genotypes through breeding studies and for achieving sustainable production. In addition to cultural measures, such as selecting an appropriate sowing time, using late-bolting varieties, and managing temperature and light, controlling bolting in spinach requires a holistic approach that includes genetic selection and carefully managing environmental conditions. This review examines the mechanisms related to genetic and environmental factors that trigger bolting and provides a perspective on research and development trends and application prospects in this field.

Keywords: Bolting, Climate change, High temperature, Spinach

INTRODUCTION

Spinacia *oleracea* (spinach), a species of green leafy vegetable in the *Amaranthaceae* family, has been documented in the Plant List database and has a history dating back more than 2000 years to ancient Persia. Spinach's remarkable adaptability to diverse climates and its extensive versatility in various culinary preparations have contributed to its widespread cultivation around the globe (Koh et al., 2012; van Treuren et al., 2012; Xu et al., 2017). Spinach, a widely cultivated and consumed vegetable in different parts of the world, is a vital source of nutrients. It is rich in vitamins (group B, β-carotene (provitamin A), folates, and C, etc.), minerals, and antioxidants. Spinach is also a prominent vegetable with high iron content (van Treuren et al. 2012; Koh et al. 2012; Niu et al. 2023). Beyond its nutritional importance, spinach cultivation significantly supports global agriculture, contributing to the livelihoods of many farmers. Furthermore, depending on the variety type and environmental conditions,

spinach yields have been reported to range from 18.6 to 44.8 tons per hectare (Kuwahara et al. 2014; Safdar et al. 2022; Niu et al. 2023). According to data from 2024, the global production of spinach was approximately 34 million tons, while Turkey's production was approximately 237 thousand tons. Following China, which is the global leader, countries such as the USA, India, and Turkey are significant producers (Fao, 2024; Tüik, 2024). In this context, spinach is a pivotal vegetable type in terms of both enhancing production values and augmenting nutritional richness.

Climatic conditions represent a pivotal factor in the successful cultivation of spinach. As a cool climate vegetable, spinach exhibits a natural resistance to cold temperatures. However, this varies depending on the variety. With respect to soil, spinach can be cultivated in a variety of soil types; however, the interaction between soil characteristics and environmental conditions is a critical factor in determining successful cultivation outcomes. Therefore, in order to obtain a high-quality yield from a given unit area, it is essential to consider climate conditions, soil characteristics, and the selection of varieties resistant to environmental changes in spinach cultivation (Mutlu et al., 2016). In this context, the establishment of novel spinach breeding programs is imperative to cultivate new varieties that exhibit distinct morphological characteristics, including leaf texture, color, shape, posture, and petiole length. These characteristics are crucial for ensuring the resilience of the plants to fluctuating climatic and environmental conditions, in addition to meeting the evolving demands of the market. Indeed, extensive day exposure within breeding programs has been demonstrated to exert a substantial influence on numerous morphological characteristics, including but not limited to plant height and stem emergence. Consequently, it has been established that spring and summer spinach varieties exhibit a heightened propensity for flowering, resulting in a substantial decline in productivity (van Treuren et al. 2017; van Treuren et al. 2020; Cho et al. 2017).

The process of flowering and the early emergence of stems in spinach are among the most critical productivity-related traits, which are subject to the regulation of multiple signaling pathways and regulatory mechanisms (Arif et al., 2013; Chen et al., 2019). In recent years, significant advancements have been made in the field of flowering-related gene pathways and their molecular functions, particularly in model plants. However, there is a paucity of research focusing on the subject in spinach (Lee and An, 2015; Chitwood et al., 2016; Ma et al., 2016). This review will examine the molecular and environmental mechanisms that trigger flowering and emergence in spinach in the light of current studies. Additionally, it will offer insights into the current state of research, development trends, and potential applications in this domain.

WHAT IS BOLTING (FLOWERING AND EARLY EMERGENCE) IN SPINACH?

The yield of spinach cultivated in this manner is contingent upon numerous factors that must be meticulously evaluated during agronomic practices. As posited by van Treuren et al. (2017), the aforementioned factors encompass environmental variables, including temperature, light exposure, water, moisture, nutrition, and stress factors. Among the influential factors for spinach yield, early bolting/flowering is one of the most well-known important phenomena. Bolting, the premature onset of flowering and subsequent seed development in spinach plants, is a phenomenon that can lead to undesirable changes in the leaf flavor profile, including bitterness. Bolting has also been demonstrated to result in a reduction in plant leafing, thereby further reducing overall yield potential (Cho et al. 2017; Chen et al. 2019).

Spinach exhibits distinct bolting habits, categorized as follows: 'early bolting' (bolting 60 days after sowing), 'medium' (bolting 60-70 days after sowing), and 'late bolting' (bolting 70

days after sowing) (Chitwood et al. 2016). According to Kuwahara et al. (2014), spinach varieties commonly used in East Asia are known as early bolting varieties, while those used in Europe are usually late bolting varieties. The differences between Asian and Western spinach varieties are thought to be important for two reasons. The first section will address the origin and historical development of spinach, while the second will focus on the cultural and gustatory preferences of the populations inhabiting these two regions. Easterners have a marked preference for spinach varieties that exhibit smooth, narrow leaves and long petioles, while Westerners demonstrate a clear predilection for those varieties characterized by wrinkled leaves (Van der Vossen, 2004). Subsequent studies have reported the link between wrinkled leaves and late bolting in spinach (Sabaghnia et al., 2014; Kuwahara et al., 2014; Xu et al., 2017; Abolghasemi et al., 2021). This information can be used to improve the selection of varieties with late bolting as well as better economic performance under different environmental conditions (Abolghasemi et al. 2021).

Environmental factors, including temperature, photoperiod, and light quality, have been identified as crucial elements in the regulation of bolting. In contrast, stress factors such as water scarcity or nutrient deficiencies have been observed to enhance bolting tendency in plants. (Cho et al., 2017; Beharav & Hellier, 2020). The regulation of bolting in spinach involves multiple molecular pathways, each consisting of a distinct set of genes with specialized functions (Boss et al. 2004). In this context, the integration of spinach into the cultivation process constitutes a multifaceted undertaking, exerting an influence on the inherent trajectory of plant development. This process is influenced by a confluence of environmental and genetic factors. A thorough evaluation of the mechanisms underlying these factors is imperative. Consequently, the factors that precipitate bolting will be addressed under the headings of genetic and environmental factors.

GENETIC FACTORS AFFECTING BOLTING MECHANISMS IN SPINACH

In species such as radish, carrot, lettuce, and spinach, there is strong argument that the bolting mechanism is controlled by two important integrators; the first is the flower-inducing integrator, which is known as the “FLOWERING LOCUS T (*FT*)” and the second is flower-inducing integrator, which is known as the “CONSTANS (*CO*)”. Based on Kyoto Encyclopedia of Genes and Genomes, spinach holds these two key integrator in the circadian rhythm pathway. This also sheds light on the role of the perception of daily light duration in spinach in flowering. Therefore, photoperiod, vernalization, maturation, internal hormones, and environmental stimuli trigger early flowering or bolting by signaling along circadian rhythm pathway (Abolghasemi et al., 2021). The plant generally undergoes the flowering process via circadian rhythm pathway depending on the duration of light exposure when there are no environmental stress factors. On the other hand, changes in the length of daylight and exposure to different temperatures (especially high temperature) alter the internal hormonal balance, causing bolting in spinach. Changes in the duration of light exposure during the day affect flowering as follows:

The following proteins influence the control of flowering depending on long or short day conditions; FLAVIN-BINDING, KELCH REPEAT, F-BOX 1 (FKF1), GIGANTEA (GI), CONSTANS (*CO*), FLOWERING LOCUS T (*FT*), EARLY FLOWERING 4 (ELF4), CYCLING DOF FACTOR (CDF), SUPPRESSOR OF OVER-EXPRESSION OF CONSTANS 1 cryptochrome (CRY2) phytochrome A (PHYA) and phytochrome B (PHYB). In the early morning hours, *CO* and *FT* promoter regions interacts with CDF factor. In the afternoon of a long day (not in a short day), the FKF1 protein activated by blue light forms a complex with the GI protein. FKF1-GI protein complex increases CO transcription by

degrading the that CDF factor in the CO promoter region. By interacting with ELF4-GI, GI displaces from subnuclear compartmentalization in the nucleus during the day-night period and GI complex tends to bind CO promoter region resulting increased CO transcription (Amini et al., 2025; Kim et al., 2013). The accumulated CONSTANS-like proteins binds to the promoter region of the *FT* and *SOC1* integrators together with other transcription factors, thereby increasing transcription in long day candition (Campos-Rivero et al., 2017; Fatima et al., 2024).CO-protein stabilization also plays a role in controlling flowering after post-translational regulation. In post-translational regulation, the far led light-activated PHYA and blue light-activated FKF1 proteins stabilize the CO protein. Also, the CRY2 protein stabilizes CO by suppressing proteins that reduce CO stabilization (Figure 1 and 2) (Abolghasemi et al., 2021; Song et al., 2013). There are numerous findings indicating that the *LNK1* and *LNK2* genes play a critical role in controlling flowering via circadian rhythm pathway (De Leone et al., 2019). The proteins belonging to *LNK* family are not effective in controlling flowering alone, but interact with certain MYB-type transcription factors (in such case; LHY and circadion clock associated) to control the plant's circadian rhythms, photomorphogenic reaction, and photoperiod-dependent flowering. Due to the the lack of DNA binding domain of *LNK* type protein, *LNK1* and *LNK2* protein form morning complex with REVEILLE/LHY-CCA1-LIKE (RVE/LCL) REV4/REV8 like transcription factor for increasing transcription rate of *TOC1* and *PRR5* (Ma et al., 2018). It is stated that *TOC1* and *PRR5* coordinately regulate *CDF1* expression in a negative direction (Amini et al., 2025; Ito et al., 2008; Liu et al., 2021). In this case, it promotes the synthesis of the CO protein essential for flowering, and flowering occurs evetually. Changes in the photoperiod, which is one of the critical stimulants of the flowering mechanism, cause shifts in the circadian rhythm pathway in species such as spinach, and trigger early flowering/bolting (Chun et al., 2001; Hata et al., 2006; Vajjiravel et al., 2024).

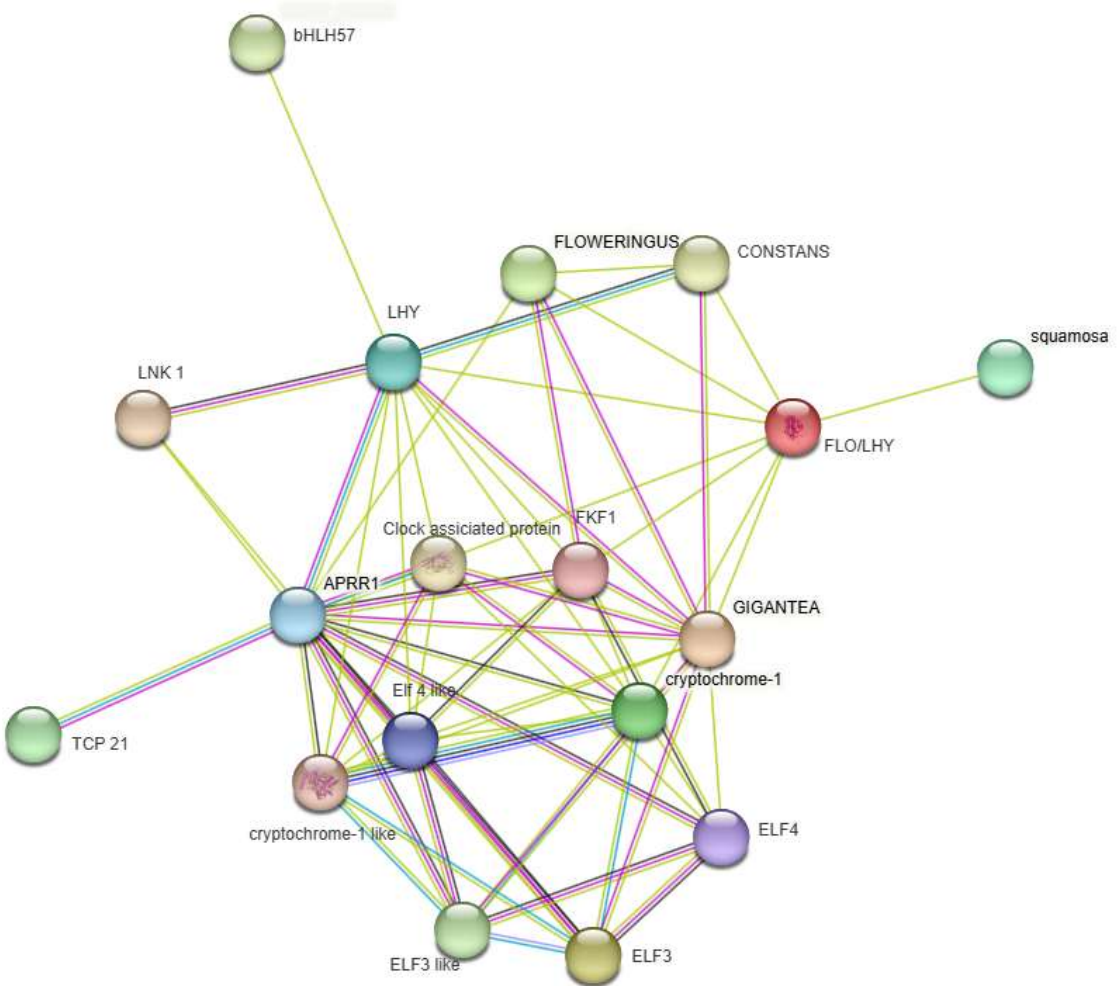

Figure 1. Protein-protein interaction of bolting related spinach proteins.

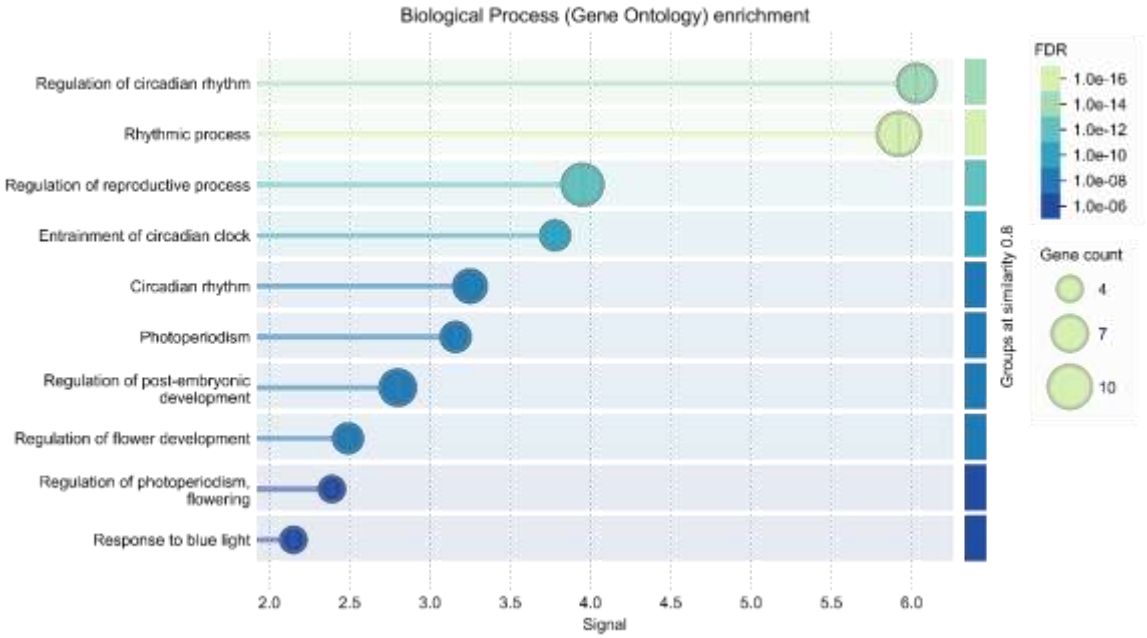

Figure 2. Gene Ontology enrichment of bolting related spinach proteins

Another important factor triggering bolting is complex relation between photoperiodic pathway and gibberellic acid hormone metabolism. The increase in ambient temperature also promotes bolting through gibberellic acid metabolism. The precondition for bolting is the stem elongation of the plant and its transition from the vegetative phase to the generative phase. Many researchers agree on gibberellic acid (GA) synthesis being responsible for stem elongation, cell division, and bolting (Binenbaum et al., 2018; Xie & Chen, 2020; Zeevaart et al., 1993). It has been demonstrated that there is a significant increase in the ratio of proteins belonging to the *GA20ox1* gene in spinach under long-day photoperiodic conditions (Lee & Zeevaart, 2007). *GA3ox* and *GA20ox1* genes are two important genes involved in gibberellic acid synthesis, and their high levels of transcription have triggered bolting in some species (García-Martinez & Gil, 2001). The light-activated product of the *PHYB* gene negatively regulates the *PHYTOCHROME-INTERACTING FACTOR 3-LIKE 5 (PIL5)* transcription factor (Ghorbani et al., 2021). Decreased PIL5 levels lead the active functioning of the *GA3ox* gene and cause ultimately the production of active GA. In shoot apex part, PHYB dependent GA biosynthesis is increased in model plant *Arabidopsis* (Hisamatsu & King, 2008). Moreover, there are many studies indicating that *FT* expression independently increases GA synthesis under long-day conditions (Bao et al., 2019). Thus, GA production increases both through light activation (*PHYB, PIL5*) and independently *FT* locus expression ((Bao et al., 2020). Two transcription factors, *TEMPRANILLO 1 (TEM1)* and *2*, which suppress the *FT* locus, also suppress the expression of the *GA3ox1,2* genes (Osnato et al., 2012). It also lowers the GA level in the plant. On the other hand, when there is sufficient light for a longer period of time, the effect of *TEM* genes, that suppress the *FT* locus and the *GA3ox1,2* genes, disappears, and the *FT* locus activates *AP1* via *SOC1* resulting GA synthesis/accumulation. In the presence of GA, *FT* and *SOC1* cause flowering of plants (Osnato et al., 2012; Srikanth & Schmid, 2011). Consequently, GA production triggered by day length or exogenous GA applications causes the plant to elongate first and then promote flowering through the circadian rhythm.

The final external stimulus that initiates bolting is high temperature stress. The effect of heat stress on flowering occurs via the autonomous pathway. It has been determined that the *FCA* and *FVE* genes located on the autonomous pathway are among the genes that function as thermosensors (Blázquez et al., 2003). It has been demonstrated that there is an increase in *FCA* expression with increasing temperature (Xu et al., 2025). SVP protein negatively regulates flowering integrator *FT* and *SOC1* locus. The increased *FCA*, *FVE* and *FLM-δ* activity at high temperatures compete SVP proteins, which prevent the binding of promoters necessary for flowering (Capovilla et al., 2014). The proteins and nucleic acids that perform this thermal sensing vary depending on the plant family (Zhu et al., 2025). The transcription of integrators that promote flowering begins with the detachment of SVP protein from the promoter region of these integrators. In addition, many studies have found evidence that PHYTOCHROME INTERACTING FACTOR4 (PIF4) directly activates the *FT* locus (Kumar et al., 2012). Besides this mechanism, mir156 another regulation mechanism that targets the *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL)* genes directly and *SPL* increases the expression of the *FT* and *SOC1* loci (Amini et al., 2025; Avşar & Esmaeili Aliabadi, 2017; Huijser & Schmid, 2011). In relation to all of this, the genetic mechanism that triggers bolting, especially in spinach, generally manifests itself through the circadian rhythm pathway. Light, high temperature, and endogenous/exogenous increased GA levels stimulate different sections of the circadian rhythm pathway, thereby affecting bolting in spinach. This situation has been observed in protein-protein interactions and gene ontology enrichment (Figure 1 and 2). It was created specifically for protein-protein interactions analysis by including proteins derived from various studies conducted on bolting in spinach.

4. MECHANISMS OF ENVIRONMENTAL FACTORS AFFECTING BOLTING

The nutritional and marketing value of vegetables is greatly affected by bolting. The economic value of spinach is significantly influenced by its yield performance, which is greatly affected by environmental factors such as light, temperature, water, humidity, and nutrition (van Treuren et al., 2017). The transition phase between vegetative growth and reproductive growth in the plant begins with bolting (stem elongation) (Mutasa-Göttgens et al., 2010). This is considered a critical event due to the coordinated effects of various environmental factors and developmental components (Melchinger, 1998; Chen et al., 2019), and is known to be one of the most important factors causing significant declines in quality, productivity, and serious economic losses in spring spinach production (Abe et al., 2014; Ma et al., 2016).

Spinach is easily influenced by photoperiod (Chun et al., 2000) and bolts in spring (Tang et al., 2018). New slow-bolting spinach varieties that can adapt to a wide range of photoperiods and climatic conditions are available (Bhattarai and Shi, 2021). Selecting appropriate varieties will increase breeding and production efficiency in spinach (Goreta and Leskovar, 2006).

Bolting and Flowering Factors

Vernalization, which is necessary for the formation of flower organs and the regulation of flowering, is the passage of the plant from the vegetative growth stage to the reproductive stage through a period of continuous low temperatures (Zhu, 2023).

Prerequisites for bolting include seed vernalization (Filek et al., 2010), plumule vernalization (Dai et al., 2023), devernalization (Yu et al., 2010), and vernalization (Akter et al., 2021). Amaranthaceae plants, including spinach, and *Brassicaceae* plants such as radish, Chinese cabbage, choy sum (*B. campestris* L. ssp. *chinensis* var. *utilis* Tsen et Lee), turnip, and mustard primarily undergo seed vernalization.

Bolting and Flowering Pathways

Changes in environmental conditions such as vernalization temperature and light can cause immature bolting in many vegetable crops, which reduces yield and commercial value (Ma and Dai, 2019; Moe et al., 1985). Many important bolting and flowering genes have been identified and functionally characterized in Arabidopsis. Arabidopsis is characterized by flowering axis elongation-type bolting (Chen et al., 2019), which provides a reference for studying bolting and flowering genes in spinach. Genetic studies in *Arabidopsis* have revealed that genes controlling bolting and flowering are involved in and can be assigned to different regulatory pathways, including photoperiod, vernalization, gibberellin, autonomous, environmental temperature, and age (Fornara et al., 2010). These pathways are related to plant development and environmental cues such as photoperiod and temperature (Cho et al., 2017).

Vernalization and Devernalization Pathway

Photoperiod pathway: In long-day plants such as spinach, early flowering can be triggered by long light periods. The length of this light period is particularly beneficial for flowering in plants such as spinach and garlic (Tan et al., 2024).

Gibberellin (GA) pathway: GA plays an important role in regulating flowering. In plants like lettuce, exogenous GA3 and low-temperature treatments significantly increase GA levels, leading to early flowering and flowering (Wang et al., 2022).

5. BOLTING MANAGEMENT

The effective management of bolting is of great importance to maintain both yield and product quality. To this end, agronomic and genetic strategies are evaluated in concert. Such strategies include, but are not limited to, appropriate planting time, temperature control, optimization of environmental conditions, minimization of stress factors, and variety selection. This approach is noteworthy as a successful management strategy within the production process. In this section, the subject of measures that can be taken for bolting management is addressed.

Optimum Planting Time

Bolting is a salient trait that must be considered in the context of cultivating spinach for year-round production, given its sensitivity to photoperiod (Chun et al. 2000). Exposure to prolonged daylight hours has been demonstrated to induce bolting in spinach, rendering the plant unmarketable (Goreta and Leskovar, 2006). Given that some commercially cultivated spinach is harvested multiple times, overwintered spinach, which is prone to spring bolting, can diminish the number of harvests that can be obtained, consequently reducing the total yield (Morelock and Correll, 2008). Chun et al. (2000) discovered that the process of bolting can be influenced by alterations in light conditions during the sowing stage. However, this method is not feasible for large-scale production due to the predominant practice of direct seeding in spinach cultivation (Koike et al. 2011). Indeed, bolting, defined as rapid stem elongation that eventually leads to flowering, can occur at any time of the year. However, spinach sown in spring or summer is more markedly prone to bolting. In this context, it has been reported that bolting can be prevented by controlling environmental conditions in the growth chamber, using spinach varieties with low photoperiod sensitivity, or planting 50-60 days before the onset of long summer days (Chitwood et al. 2016). Consequently, it is imperative to acknowledge that fall and early spring sowing should be conducted in temperate climate regions where spinach is cultivated, while summer sowing should be avoided.

Selection of Late Bolting Varieties

In the context of spinach cultivation, agronomic traits such as plant height, leaf erectness, and stem emergence are of significant importance. Favorable photoperiod insensitivity is a desirable characteristic for year-round production, and thus, the late or slow emergence of these traits is preferred (Chun et al. 2000). It has been demonstrated that slow or late emergence is a desirable trait in commercially cultivated spinach. This species is typically harvested multiple times during the growing season, thereby increasing the overall yield. Commercial spinach cultivation is highly mechanized (Morelock and Correll, 2008; Koike et al., 2011), and to adapt to high-density spinach production and mechanical harvesting, growers generally prefer spinach varieties with taller plant height and upright leaves. In this context, studies conducted over many years on the detection of genetic variation in spinach have revealed that there is a level of variation within the species that can be evaluated in terms of late emergence trait, suggesting that it may be possible to develop late emerging varieties through breeding programs (Goreta and Leskovar, 2006). In a study that classified bolting as early, medium, or late based on phenotypic data in spinach, it was reported that eight cultivars with late bolting, erect leaves, and a plant height of 9 cm (PI103063, PI169678, PI169684, PI171863, PI171865, PI174386, PI175929, and PI648963) could be used as parents in a spinach breeding program (Chitwood et al. 2016). In this regard, the late bolting of plants has been

identified as a primary focus of economic production and scientific research (Chitwood et al. 2016; Abolghasemi et al. 2021). Research has indicated that varieties such as 'Matador', 'Bloomsdale Long Standing', and 'Tyee' exhibit enhanced resistance to bolting (Kaşka, 2020).

Temperature and Light Control

Researchers have generally reported that the primary factors contributing to bolting are associated with day length and elevated temperatures (Shi et al., 2016). Ambient temperature is one of the factors that affect growth, development, and flowering time throughout the plant's life cycle. Research has demonstrated that the majority of plants exhibit early flowering in response to elevated temperatures (Jagadish et al., 2016). It has also been reported that many plant species have the ability to initiate flowering at the optimal time for their reproduction, which mainly depends on accurate measurement of seasonal changes in day length and temperature (Hayama et al. 2003; Shi et al. 2016). Photoperiod sensitivity is therefore a critical factor in measuring critical day length and determining flowering time in plants. A modification in this factor has been demonstrated to induce alterations in flowering time and can enhance adaptation to local environmental conditions in numerous plant species (Jung and Muller, 2009). In spinach, the optimal growth temperature range is between 10-20°C, while temperatures above 24°C can trigger bolting.

Fertilization

Excessive or deficient levels of certain elements in the soil have been demonstrated to alter flowering time (Bernier et al., 1993; Wada and Takeno, 2010). In general, malnutrition has been demonstrated to promote flowering. Pharbitis nil, a short-day plant, has been observed to induce flowering when cultivated under long-day conditions and when nutrients were scarce, but not when plants were supplied with standard nutrient solutions (Shinozaki et al., 1988; Wada and Takeno, 2010). These results suggest that the mechanism of induction due to nutrient deficiency is different between short and long day conditions. The induction of flowering is more pronounced under short-day conditions, and this effect is more significant in younger plants.

Nitrogen is one of the macroelements required by plants that regulate a wide range of biological processes (Vidal et al. 2014). In *Arabidopsis*, nitrate-limiting conditions have been reported to promote flowering under neutral or short-day conditions, and this response is independent of vernalization and autonomous pathways (Marín et al. 2011; Liu et al. 2013). However, when nitrate was limited, transcript levels of giberalic acid biosynthesis genes and GA3 concentrations were reported to increase (Liu et al. 2013). Few studies have focused on how other nutrients alter this timing. In alpine *Gnaphalium supinum*, flowering was increased due to a greater supply of phosphorus, while nitrogen was reported to have no effect (Petraglia et al. 2014). In *Arabidopsis*, flowering was delayed under low phosphorus conditions, while flowering was reported to be unchanged when high phosphorus was applied to plants (Kant et al. 2011). In this context, while elements such as phosphorus support root development and general plant health, excessive nitrogen fertilization can also trigger bolting by promoting rapid growth in the plant. Therefore, it should be kept in mind that balanced fertilization is important in cultivation.

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POSSIBLE MORPHOLOGICAL EVIDENCES FOR A NEW SPECIES OF *EUPALOPSELLUS* (ACARIFORMES: EUPALOPSELLIDAE): INSIGHTS FROM A FEMALE SPECIMEN COLLECTED FROM TÜRKİYE

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ABSTRACT

In this study, a single female mite specimen belonging to the genus *Eupalopsellus*, collected from Türkiye, was examined morphologically in detail. The specimen displays key morphological affinities with *Eupalopsellus kermaniensis* Mahdavi & Farzan, particularly in dorsal shield ornamentation and leg chaetotaxy. However, it also possesses several distinguishing features, such as markedly shorter dorsal body setae and notably longer setae ag_2 and ag_3. A pair of wing-like sclerotized structures adjacent to the anogenital shields, also present in *E. kermaniensis*, was observed in the examined specimen. Similar wing-like structures have been found in *E. oresbiosis* Smith Meyer & Ueckermann and *E. sellnicki* Smith Meyer & Ueckermann, indicating a degree of morphological resemblance to these species as well. Nevertheless, the absence of solenidion ω on tarsus IV of the specimen differentiates it from these taxa. The combination of these morphological characters, while closely resembling *Eupalopsellus* aff. *kermaniensis*, suggests that it may represent an as yet undescribed species of *Eupalopsellus*; however, further material is required to confirm this.

Keywords: Mite, taxonomy, diagnostic morphology, new species candidate

INTRODUCTION

Eupalopsellidae Willmann is a family belonging to the superfamily Raphignathoidea. Members of this family are active predators and they act as biocontrol agents of small arthropods (Fan, 2004; Khan et al., 2024). At present, Eupalopsellidae includes about 50 described species in five valid genera (Gerson and Walter, 1998; Beron, 2020; Khan et al., 2024). *Paraeupalopsellus* Smiley & Moser and *Acaciacarus* Gerson, Frost & Swift (syn. of *Mecognatha* Wood) are now members of the family Mecognathidae Gerson & Walter in the current classification (Gerson and Walter, 1998).

Currently, twenty species belonging to the genus *Eupalopsellus* Sellnick are known in the world (Beron, 2020; Khan et al., 2024), four of which have also been recorded in Türkiye: *E. olandicus* Sellnick, *E. rostridius* Summers, *E. prasadi* Bagheri & Khanjani and *E. deformatus* Fan (Doğan and Ayyıldız, 2004; Dönel and Doğan, 2010; Kasap et al., 2013; Doğan et al., 2017, Doğan, 2019).

In this study, a female specimen of the genus *Eupalopsellus* collected from Karasu Valley was herein described and photographed. Morphological comparisons with the related species in the genus revealed that, although the specimen exhibits certain characteristics similar to those of the related species, it also displays distinct differences in other observed morphological traits. The subject of this work is whether this specimen constitutes a new species, a question which was addressed by comparing it with other related species.

MATERIALS AND METHODS

The mite specimen was extracted using Berlese-Tullgren funnels, cleared in 60% lactic acid and mounted on microscopic slides in Hoyer's medium under a stereo microscope. Photographs were taken using a Leica DM 4000B phase-contrast light microscope. Body size and measurements of various structures of the body were taken in micrometers (µm) with the aid of the Leica Application Suite (LAS) Software Version 3.8. The terminology is based on Kethley (1990) and Grandjean (1944, 1946). Specimen examined was deposited in Acarology Laboratory of Erzincan Binali Yıldırım University (EBYU), Erzincan, Türkiye.

RESULTS

Family: Eupalopsellidae Willmann

Genus: *Eupalopsellus* Sellnick

***Eupalopsellus* aff. *kermaniensis* Mahdavi & Farzan**

Description

Female

Idiosoma oval. Length of body 298, width 191. Dorsum of idiosoma covered with four shields. Integument striated and punctuated except for shields. All dorsal shields punctuated. Length of propodosomal shield 73, width 99. Propodosomal shield with four pairs of setae (*vi*, *ve*, *sci*, *sce*), a pair of eyes and a pair post-ocular bodies; eyes 10, post-ocular bodies 20 in diameter. Length of metapodosomal shield 73, width 119. Setae c_1, d_1, d_2 located on metapodosomal shield. Setae c_2 located on striated integument, humeral shields absent. Length of opisthosomal shield 80, width 108, with three pairs of setae (e_1, e_2, f_1). Length of suranal shield 40, width 91, bearing two pairs of setae ($h_{1,2}$). Dorsal idiosomal setae faintly barbed; the barbs on the setae f_1, h_1, h_2 more prominent compared to the others. Lengths and distances of dorsal body setae as follows; *vi* 11, *ve* 12, *sci* 13, *sce* 15, c_1 13, c_2 20, d_1 14, d_2 15, e_1 15, e_2 15, f_1 35, h_1 31, h_2 31, *vi–vi* 24, *ve–ve* 54, *vi–ve* 20, *sci–sci* 10, *sce–sce* 84, *sci–sce* 38, *ve–sce* 27, *vi–sci* 51, *ve–sci* 44, c_1–c_1 82, c_2–c_2 137, d_1–d_1 79, c_1–d_1 59, d_2–d_2 107, c_1–d_2 51, d_1–d_2 17, e_1–e_1 66, e_2–e_2 96, e_1–e_2 28, f_1–f_1 72, e_1–f_1 25, e_2–f_1 49, h_1–h_1 43, h_2–h_2 79, h_1–h_2 18.

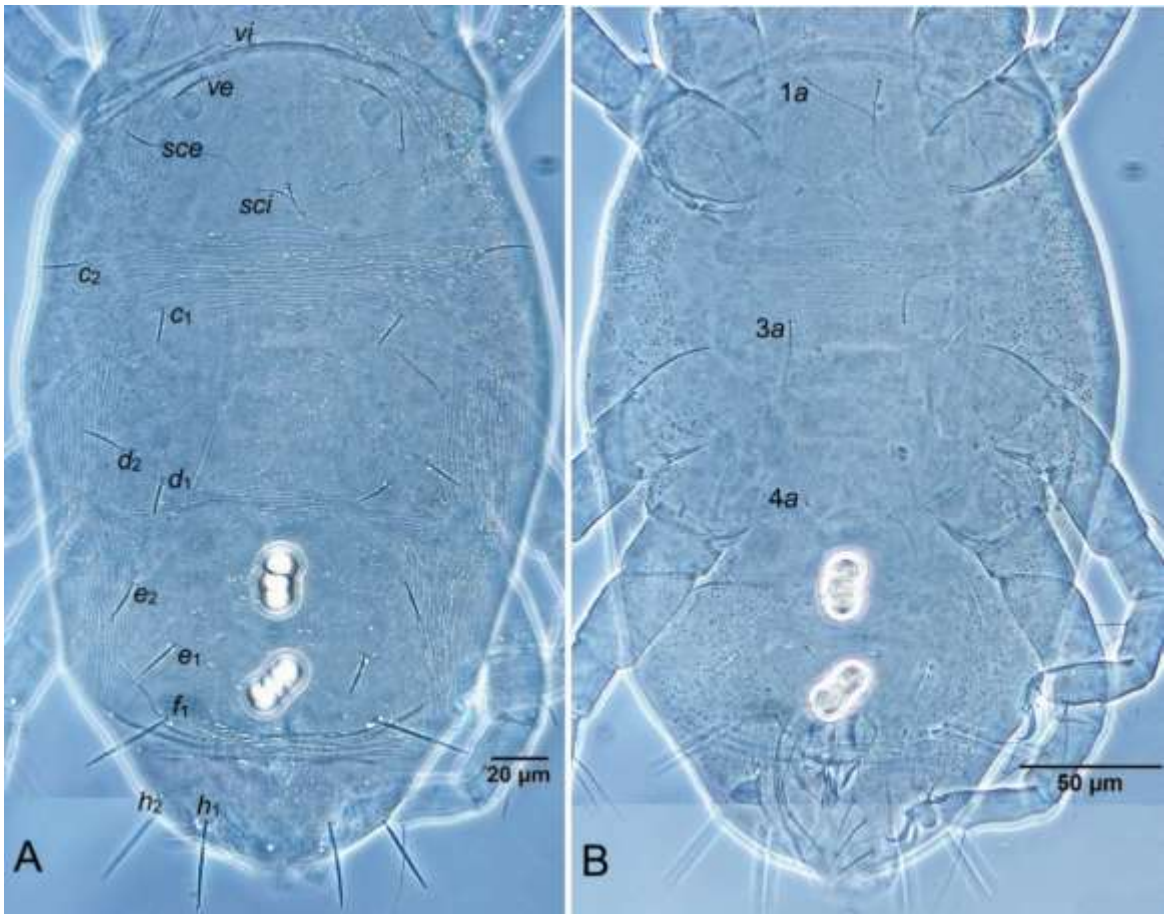

Figure 1. *Eupalopsellus* aff. *kermaniensis* (female). A) Dorsum of body, B) Venter of body.

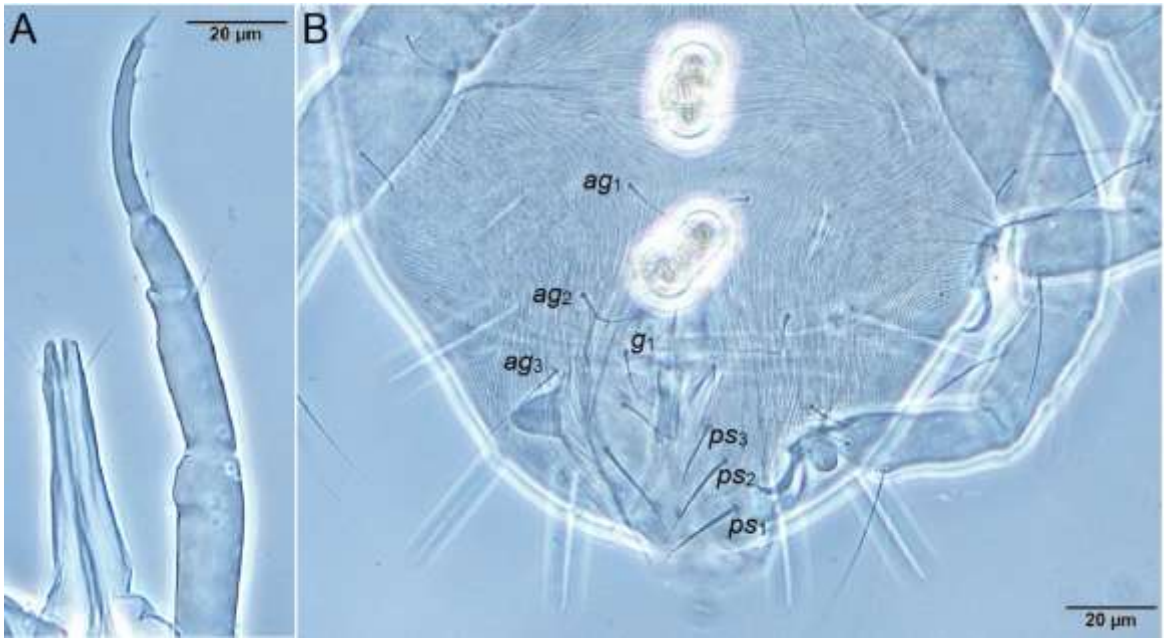

Figure 2. *Eupalopsellus* aff. *kermaniensis* (female). A) Elongated rostrum of subcapitulum and right palp, B) Anogenital region.

Coxisternal shields absent. Setae 1*a* situated between coxae I, setae 3*a* located between and anterior to coxae III and setae 4*a* on striated integument and located between coxae IV. Three pairs of aggenital setae ($ag_{1\text{-}3}$) present. A pair of wing-shaped sclerotized structures located below setae ag_3. A pair of genital setae (g_1) and three pairs of pseudanal setae ($ps_{1\text{-}3}$) present. Lengths and distances of the setae as follows; 1*a* 53, 3*a* 69, 4*a* 35, 1*a*–1*a* 25, 3*a*–3*a* 40, 4*a*–4*a* 28, ag_1 13, ag_2 25, ag_3 35, g_1 27, ps_1 21, ps_2 22, ps_3 18.

Lengths of legs; I: 198, II: 170, III: 168, IV: 174. Setal formulae of legs I-IV; coxae 2–1–2–2, trochanters 1–1–1–1 femora 4–4–3–1, genua 1(+1κ)–1–1–1, tibiae 5(+1φρ)–4(+1φρ)–4(+1φρ)–4(+1φρ), tarsi 10(+1ω)–9(+1ω)–6(+1ω)–6.

Gnathosoma 143, chelicerae 141 long. Palp tarsus 37, palp tibia 18 long and total length of palp 143. Number of setae on palp segments from palp trochanter to palp tarsus: 0-3-1-3-4/5?+1ω+1 terminal eupathidium. Tibial claw minute, and seta-like. Lengths and distance between subcapitular setae, *m* 32, *n* 50, *m*–*m* 8, *n*–*n* 15, *m*–*n* 30.

Material examined

1 ♀, from litter, 39°36'47.19"N 38°40'0.04"E, 1711 m a.s.l, the Karasu Valley, Türkiye, 27 September 2022.

DISCUSSION

In the examined specimen, a pair of wing-shaped sclerotized structures were observed just below the setae ag_3 (Figs 1B, 2B). In addition to this characteristic, the specimen closely resembles *Eupalopsellus kermaniensis* Mahdavi & Farzan in terms of leg setations and other morphological features (Mahdavi et al., 2013). However, the Turkish specimen differs in that the dorsal integument is striated and punctuated, the dorsal setae are significantly shorter compared to those of *E. kermaniensis*, and setae ag_2 and ag_3 are much longer (Figs 1A, 2B).

A pair of wing-shaped structures located on the sides of the anogenital shields are also present in *Eupalopsellus oresbiosis* Smith Meyer & Ueckermann and *E. sellnicki* Smith Meyer & Ueckermann (Smith Meyer & Ueckermann, 1984). Therefore, the Turkish specimen may also be closely related to these species. However, it can be easily distinguished from them by the absence of the solenidion ω on tarsus IV. In the mentioned species, solenidion ω is present on tarsus IV.

The combination of all these morphological characters indicates that the examined specimen is most closely related to *Eupalopsellus kermaniensis* among the previously mentioned species. So far, only a single specimen has been found, and no additional specimens of the same species have been encountered.

CONCLUSION

The findings indicate that the examined specimen can be identified as *Eupalopsellus* aff. *kermaniensis*, but it may represent a potential new species due to its morphological differences from the known species. However, to confirm this assumption and to make a description species-level identification, additional specimens from the same population or from similar habitats are required. Accordingly, detailed future sampling efforts and comparative morphological analyses will contribute significantly to clarifying the taxonomic status of this potential new species.

ACKNOWLEDGEMENT

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GIS MAPPING OF SOIL MACRONUTRIENTS FOR NUTRIENT MANAGEMENT IN HAZELNUT

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ABSTRACT

The purpose of this study was to determine and to map soil pH and macronutrient content in soils located in Ordu province. For that purpose, soil samples were collected from the study area to evaluate the soil fertility. The soil samples were analyzed to determine soil pH and total N, available P and available K, Ca, Mg. Then, soil nutrient maps were prepared by IDW method with this obtained data results. In the present study, the pH of the soils was between 4.91-6.25 indicating moderately acid to slightly acid. Maps of the soil macronutrients showed that total N content in soils was at sufficient level in the entire study site; available P, Ca and Mg content were found at the low and high level in the soils and available K content of the soils were at high level. In addition to, soil nutrients were significantly positively correlated with each other.

Maps showed that soil nutrients were at the high level in the study site. Soil nutrient maps indicated that macronutrients in soils were found at different levels in the study site and nutrient level was differ widely in the map. Soil nutrient maps revealed that study site should be managed differently according to the nutrient and it is important.

Keywords: Macronutrient, pH, acid soil, interpolation, GIS

INTRODUCTION

Soil nutrient mapping has a great importance as it provides essential information about the soil properties that affects plant growth (Tudi, 2022). Soil macronutrients includes nitrogen, phosphorus and potassium. Total nitrogen, available phosphorus and available potassium are basic parameters of soil fertility among soil nutrients. Mapping of the spatial distribution of soil macronutrient such as total nitrogen and available phosphorus, available potassium through mapping techniques is significant for agricultural management.

GIS methods have been widely used in soil science to detect spatial variability of nutrients in soils (Tiruneh et. al 2021). GIS application provides methods for identify the spatial distribution of soil properties and helps create a mapping model. Mapping of pH of the soil and nutrients are of considerable importance in hazelnut for nutrient management. It is essential to generate the maps of nutrients in soils in order to plan site specific fertilizer programme.

The purpose of this research was to determine soil pH and macronutrient content in soils of Hazelnut, located in Ordu and to generate a map of soil nutrients the areas that need a fertilization based on soil nutrient level for hazelnut.

MATERIAL AND METHODS

Study area

The study site is 12.5 da hazelnut orchards, located in Ordu province. It is situated at the longitude 402520-402685 East and latitude 4524840-4525015 North (WGS84 UTM Zone 37 North).

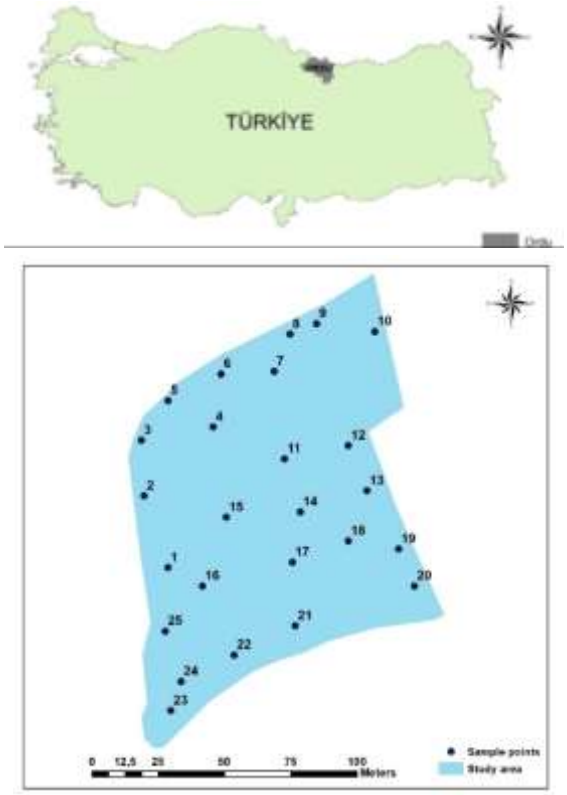

Figure 1. Location of study area and sampling point

Soil sampling and analysis

Twenty-five soil samples were collected at a depth 0–30 cm, in April 2025. The soil samples were taken randomly in study site (Fig. 1) and the sampling positions were geo-referenced and recorded using a GPS. This random soil sampling was taken before the application of fertilizer. This random sampling was taken prior to the soil fertilization.

The soil samples were dried in the shade and passed through a 2 mm sieve and analysed for soil pH, total N, available P, available Ca, available K, available Mg. Soil pH was detected using a pH meter at a 1:2.5 soil:water ratio (w:v) (Richards, 1954). Total N was determined by Kjeldahl method (Bremner 1965). Available P was determined by Bray Kurtz method through spectrophotometry (Kacar, 2009). Available Ca, Mg and K were extracted with neutral normal ammonium acetate solution (NH_4OAc–K) and were determined on an AAS (Kacar, 2009).

Mapping model

Maps of spatial distribution of soil pH and macronutrients were prepared in Arc GIS 10.5 software using the inverse distance weighted (IDW) interpolation method (Tiruneh et. al. 2021).

Statistical Analysis

Descriptive statistic of soil data (min, max., mean, standard deviation, coefficient of variation, skewness and kurtosis) were performed using Minitab 20. Pearson correlation was used to investigate relationships between soil pH and macronutrient. Significant differences were determined by Pearson least significant difference (LSD) test ($p < 0.05$).

RESULTS AND DISCUSSION

Descriptive statistics of soil fertility parameters

Descriptive statistic of soil data such as minimum, maximum. mean, standard deviation, coefficient of variation (CV), skewness and kurtosis are presented in Table 1. The soil fertility parameters were selected as soil pH and macronutrients; total N, available P, K, Ca and Mg. The coefficient of variation was calculated to investigate the variability of soil fertility parameters using the following order ≤25 low variations; >25 ≤50 moderate variations and >50 high variations (Khadka et al.2018). The results showed that the highest variability in coefficients of variation was in soil phosphorus while lowest variability in soil pH. In addition to this, CV was moderate variation for other soil macronutrients.

Table 1. Descriptive statistics of the soil fertility parameters in the study area

Properties	Mean	StDev	CV	Min	Max	Skewness	Kurtosis
pH	5.51	0.33	6.13	4.91	6.25	0.37	-0.01
N, %	0.193	0.051	26.4	0.13	0.35	1.48	2.47
P, mg kg^{-1}	18.30	18.64	101.8	0.01	60.75	0.91	0.03
K, mg kg^{-1}	475.7	208.4	43.8	204.4	1077	1.18	1.56
Ca, mg kg^{-1}	1143	544	47.5	419	2645	1.06	1.08
Mg, mg kg^{-1}	181.8	60.4	33.2	98.2	286.3	0.31	-1.30

Pearson correlation relations

Pearson correlation coefficients between data of soil fertility parameters are presented in Table 2. The soil macronutrients were positively correlated with each other in this study. Significant relationship was found between soil pH and available P ($r = 0.595$, $p<0.01$); available K ($r = 0.404$, $p<0.01$). In this study, there was no significant relationship between soil pH and total N, available Ca, available Mg. In addition to this, positive relationship was found between soil available Ca and Mg ($p< 0.01$); soil available P and soil available K ($p< 0.05$); soil available K and Mg ($p< 0.05$).

Table 2. Correlation relation between soil pH and macronutrient

	pH	N	P	K	Ca
N	-0.083				
P	0.595**	0.009			
K	0.404*	0.435*	0.487*		
Ca	0.073	0.258	0.181	0.221	
Mg	0.166	0.380	0.216	0.443*	0.840**

Classification of limit values for soil parameters

The classification of the limit values for soil pH and macronutrients are presented in Table 3. These limit values of the soil parameter were used for preparing soil maps.

Table 3. Classification of limit values for soil parameters

Parameter	Interpretation Classes						References
	Very low	**Low**	**Adequate**	**High**	**Very high**		
N %	<0.045	0.045-0.090	0.090-0.170	0.170-0.320	>0.320		Sillanpää, 1990
P, mg kg^{-1}	<2.5	2.5-8.0	8.0-25	25-80	>80		Sillanpää, 1990
K, mg kg^{-1}	<50	50-110	110-290	290-1000	>1000		Sillanpää, 1990
Ca, mg kg^{-1}	<238	238-1150	1150-3500	3500-10000	>10000		Sillanpää, 1990
Mg, mg kg^{-1}	<50	50-160	160-480	480-1500	>1500		Sillanpää, 1990
pH	Strongly acidic	Moderately acidic	Slightly acidic	Neutral	Slightly Alkaline	Strongly Alkaline	Richards, 1954
	<4.5	4.5-5.5	5.5-6.5	6.5-7.5	7.5-8.5	>8.5	

Map of soil pH and macronutrients

The maps of the soil pH and macronutrients are presented in Figure 2 and Figure 3. The soil pH of the study site varied from 4.91 to 6.25, indicating moderately acidic to slightly acidic. In addition to this, the map of soil pH showed that soils were more acidic in the west of the study area (Fig. 2).

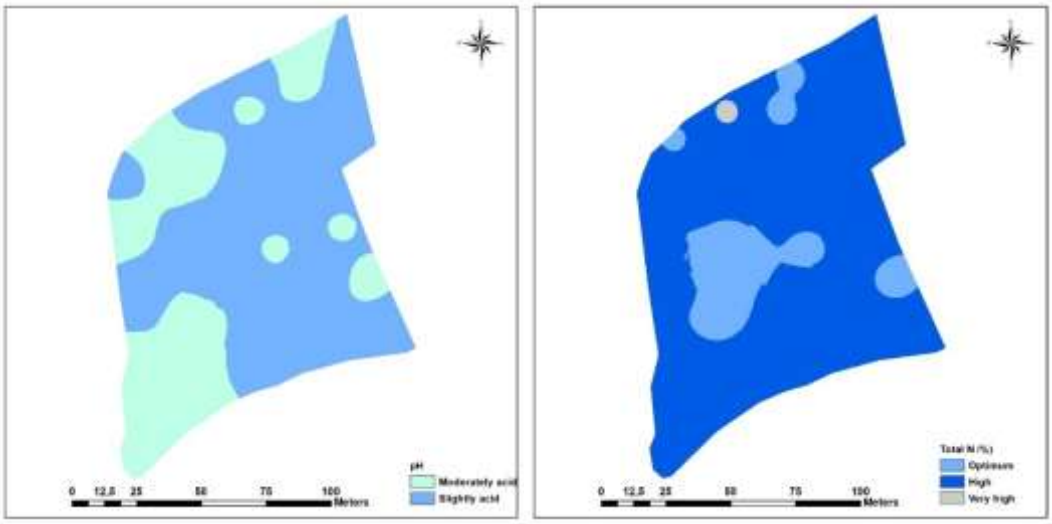

Figure 2. Map of soil pH and total nitrogen

The total nitrogen content of the soils was between 0.13 and 0.35 % with the mean of 0.19 %. (Table 1). The total nitrogen content was found at both adequate and high level in the study site. The map showed that most of study area was high level in total nitrogen content. (Fig. 2). In this study, available P contents of the soils varied from 0.01 to 60.75 mg kg^{-1} with the mean of 18.30 mg kg^{-1} (Table 1). This indicates that the available phosphorus contents in soils varied from very low to high level. The map in Fig. 3 showed that available P content in the soils was higher in the south eastern of the study site.

The map indicated that south part of the study area was insufficient in available phosphorus content (Figure 3). The available K content in the soils was between 204.4 and 1077 mg kg^{-1} with the mean of 475.7 mg kg^{-1}. The available potassium values were found at adequate, high and very high level in the soils. The map of soil available potassium showed that available K content was sufficient level in total study area. (Figure 3). Thus, available K content in the soils was high level in the study site.

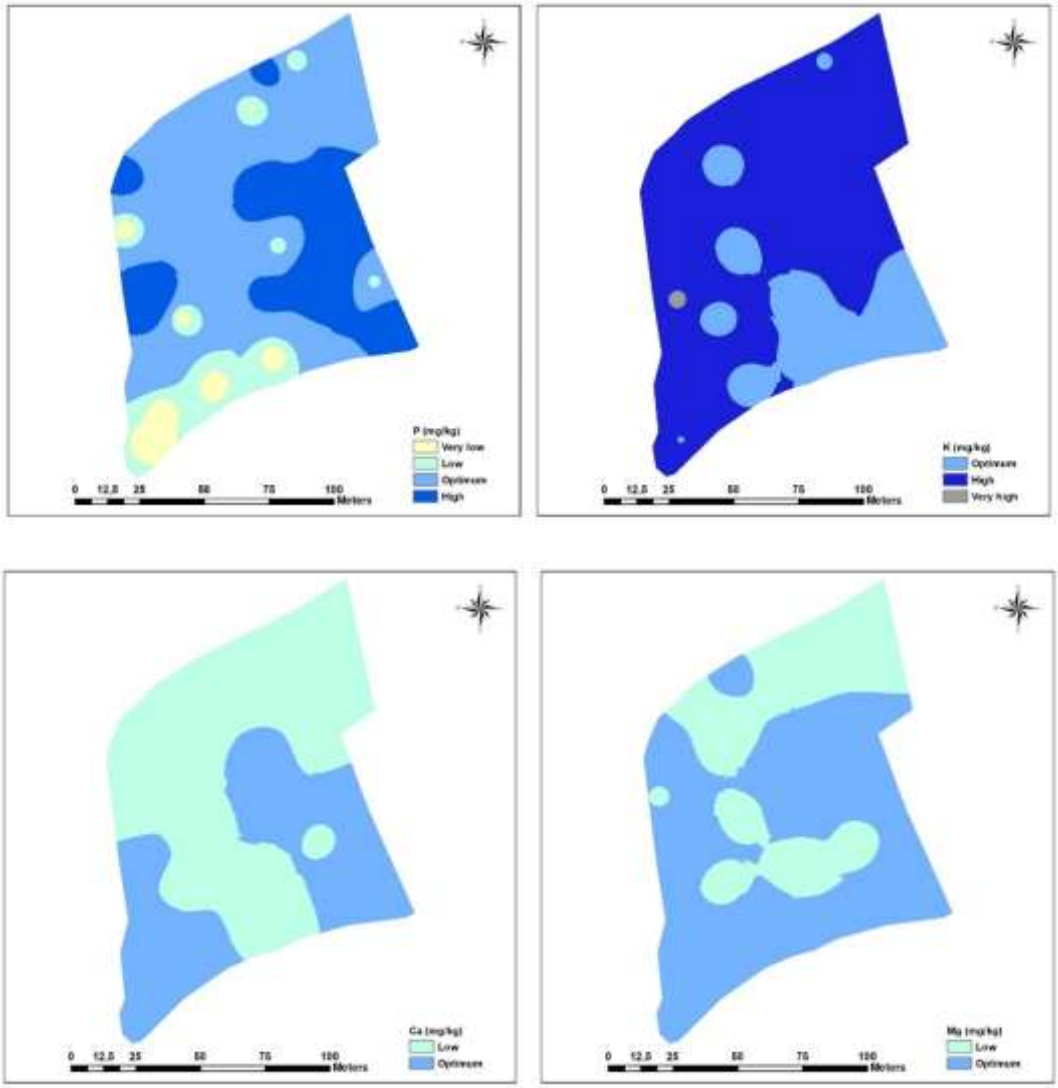

Figure 3. Map of soil available P, K, Ca and Mg

The available Ca content in the soils was between 419 mg kg^{-1} and 2645 mg kg^{-1} with the mean of 1143 mg kg^{-1} (Table 1). This map shows that available Ca in the soils was found at both low and adequate level (Fig. 3). In addition to, soil map shows that available Ca content in the soils was more present in south of the study site.

In this study, available Mg content ranged from 98.2 to 286.3 mg kg^{-1} with the mean of 181.8 mg kg^{-1} in soil. The map indicated that available magnesium contents in the soils were low and adequate level (Fig 3). The map showed that available Mg content in the soils was more present in the south of the study site. The majority of the study area was adequate in magnesium content and remaining area was low in magnesium content.

CONCLUSION

In this study, pH of the soils was generally slightly acidic and soil nutrients were found at different level. The spatial distributions map of the soil Ca and Mg showed both nutrients were adequate level in the south of the study site. However, soil available Ca and Mg contents were deficient in the majority of the study site. In addition to this, spatial distributions map of the soil available P indicated that soil P was deficient in the south western of the study site. There were differences in the amount of nutrients in the soils of the study site. P, Ca and Mg fertilization should be added to the fertilizer programme to increase nutrient content in the soils of hazelnut. The maps generated for the study may be useful for creating fertilizer programme and to determine the amount and kind of macronutrient to be applied based on the site specific nutrient management.

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CLASSIFICATION OF CURRENT ARTIFICIAL INTELLIGENCE APPLICATIONS ACCORDING TO THEIR FUNCTIONALITY

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ABSTRACT

With the advancement of technology in nature-related sciences, solutions in these fields are also shifting towards digitally focused approaches. Among these, fields such as agriculture, forestry, zootechnics, aquaculture, and biomedicine are leading breakthroughs in leveraging the benefits of artificial intelligence and Industry 5.0. Because Industry 5.0 is human-centered, the technology it seeks must be human-friendly and, therefore, close to nature. Therefore, the robot-nature relationship, including artificial intelligence, opens the door to a hybrid world. In this context, artificial intelligence provides us with the key to utilizing these two components. With machine learning and deep learning algorithms, artificial intelligence helps us leverage functions in the created environment and utilize them in the right direction. On the technical side, the correct use of data and algorithms allows us to manage technology effectively and achieve rapid results. This study compiles and discusses the applications of the technical aspects of Industry 5.0 and artificial intelligence used in the aforementioned fields of agriculture, forestry, zootechnics, aquaculture, and biomedicine. The article also discusses the impact of machine learning on modern and traditional methods in these fields. It also touches on assistive technologies such as 6G and IoT. Thus, the aim of this review is to inform and guide scientists on the most appropriate AI application(s) for their field of study.

Keywords: Artificial Intelligence, Bioscience, Machine Learning

INTRODUCTION

According to statistics on the world population, it is predicted that the world population will reach 10 billion by 2050 (Taha et al., 2025). Agriculture, forestry, zootechnics, aquaculture, and biomedicine are pioneering fields that will inevitably be affected by population growth. In this context, the use of technologies developed to make more effective use of nature's resources and to protect the world plays an important role. This role will be fulfilled by Industry 5.0 and artificial intelligence. In addition, interdisciplinary fields will also contribute to the process. Developments in robotics can be an example of this. Ultimately, it is essential for scientists to address these issues and develop innovative and sustainable systems to meet the needs of the growing population.

Machine learning, a subfield of artificial intelligence, has revolutionized resource management and decision-making by analyzing data and creating accurate prediction models. This technology facilitates informed and data-driven decision-making by enabling the identification and analysis of complex patterns in data from various sources using sensors and other equipment. Artificial intelligence applications, machine learning technologies, and applications working with the Internet of Things have shown remarkable growth in recent years.

The occurrence of several outbreaks and pandemics has further increased the global importance of these technologies (Ramachandran et al., 2022). Machine learning refers to the artificial intelligence method that enables computers to accomplish specified types of tasks and problem-solve issues without direct processing. This method enables a mechanism to be designed that can effectively execute a specific task by training a machine or computer on data related to that issue or mission (Bensoussan et al., 2020). Advances in computational capability and big data technologies are facilitating the evolution of deep learning, a highly sophisticated and practical form of machine learning. Deep learning has revolutionized fields such as object sensing, identification of powdered foods, crop nutrition sensing, leaf area metering, disease recognition, moisture stressed crops, plant health monitoring, fruit quality assessment, and other complex tasks involving large-scale data analysis (Fan, 2022). The performance of computer systems in these applications has reached extraordinary levels, enabling them to completely replace human intervention in certain operations or significantly support it. These technologies have permeated various sectors, including agriculture, where artificial intelligence, machine learning, deep learning, the Internet of Things (IoT), and other digital technologies are widely adopted, leading to the emergence of the concept of "smart agriculture" and the goal of achieving fifth-generation agriculture (Agriculture 5.0 (Ag5.0)) (Doshi & Varghese, 2022).

Agriculture 5.0 is an innovative, evolving version of agriculture that aims to address issues not covered by Agriculture 4.0. Agriculture 5.0 is a subcategory of Industry 5.0, a new concept that needs to be adopted, which involves integrating new technologies such as artificial intelligence, the Internet of Things (IoT), big data analytics, digital twins, blockchain, 6G networks, robotics, and human-machine interaction to manage various agricultural processes (Mourtzis et al., 2022).

Figure 1 shows the roadmap of the agricultural revolution from Agriculture 1.0 to Agriculture 5.0. The technologies offered in Agriculture 5.0, built upon the progress made from Agriculture 1.0 to Agriculture 4.0, will contribute to addressing some of the current global challenges of producing sufficient, affordable, and healthy food while protecting ecosystems (Taha et al., 2025). However, 6G technology, capable of providing transmission speeds of up to 1 Tbps, will expand IoT connectivity thanks to higher data speeds, minimal latency, and wider coverage. This will improve the management of agricultural resources by enabling advanced AI-based monitoring and precision farming techniques (Maddikunta et al., 2022).

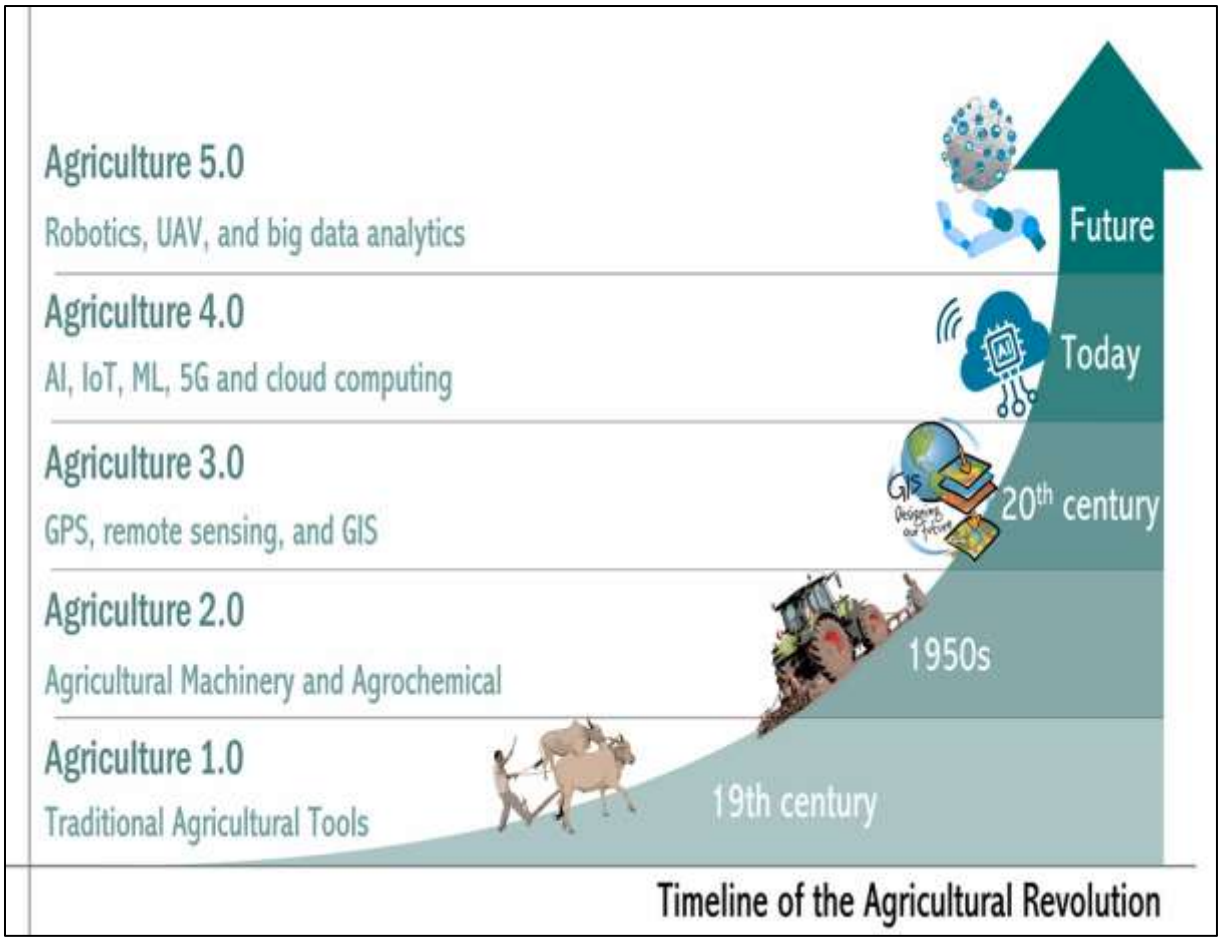

Figure 1. Timeline of the Agricultural Revolution (Taha et al., 2025)

Artificial intelligence, along with its core subfields such as machine learning and deep learning, is an integral part of Agriculture 5.0, and these areas play critical roles. Machine learning is a subclass of artificial intelligence that enables machines to learn automatically from data without the need for explicit programming. Machine learning is of critical importance for Agriculture 5.0 and assists in predicting crop yields, monitoring plant diseases, assessing nutrition and water status, detecting weeds, and forecasting adverse weather conditions such as rainfall, drought, and wind (Sarker, 2021). Deep learning, a subfield of artificial intelligence and machine learning, uses artificial neural networks (ANNs) inspired by human brain functions for complex computations. Deep learning methods such as convolutional neural networks (CNNs), recurrent neural networks (RNNs), autoencoders, and generative adversarial networks (GANs) are applied to specific agricultural challenges (Hong et al., 2020).

Plants are important components of ecosystems; they facilitate carbon exchange and provide food and habitat for both animals and humans. In terms of diversity and population, forest plants are at risk of extinction due to overuse and poor management (Fichtner & Härdtle, 2021).

Sustainable forest management refers to the conservative balancing of ecological, social, and economic objectives to ensure the permanence and resilience of natural ecosystems, particularly forest plants, while also enabling their utilization. The emergence of artificial intelligence is modernizing sustainable forest management by offering innovative solutions for monitoring, modeling, decision-making, and law enforcement (Fallah Madvari, 2022).

Forests are vital components of the environment and must be protected. For centuries, their management and protection have relied on traditional methods. However, the emergence of artificial intelligence, machine learning, and deep learning has transformed forest management (Shivaprakash et al., 2022).

Artificial intelligence primarily automates and enhances the analysis of complex datasets obtained from digital technologies such as satellites, drones, and aerial imagery, mimicking human intelligence. Artificial intelligence processes and interprets large amounts of data, thereby reducing dependence on manual labor (Buchelt et al., 2024).

Machine Learning, a subfield of artificial intelligence, works on algorithms that offer innovative solutions to compact environmental challenges through the efficient and accurate analysis of large amounts of data. Machine Learning facilitates better decision-making and precise forest resource management. Deep Learning, another subfield of Machine Learning, helps optimize forest resources more effectively by using techniques that are becoming increasingly important in sustainable forest management. Deep Learning systems utilize large-scale, high-dimensional data obtained from satellites, environmental sensors, and other components (Diez et al., 2021; Raihan, 2023).

Artificial intelligence is increasingly being incorporated into sustainable forest management to overcome challenges related to protection, resource management, and plant protection. This has enabled forest management and enhanced sustainability by assisting in decision-making processes. AI-powered tools mostly obtain data from remote sensors, while remote sensing involves the use of satellite imagery, aerial photography, and light detection and ranging (LiDAR) to provide a synoptic view (Börner et al., 2020).

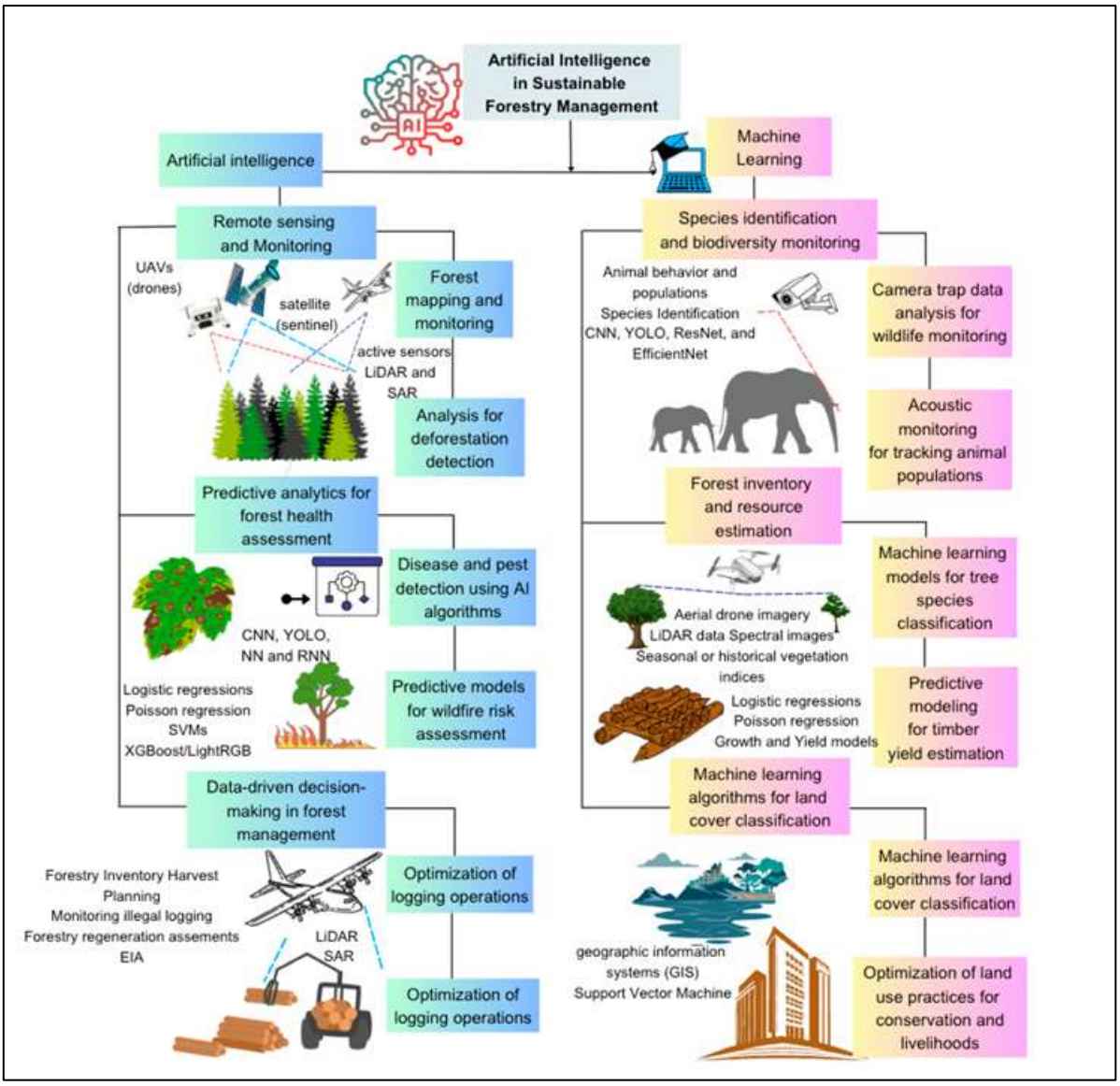

Figure 2. Overview of artificial intelligence and machine learning-based systems applied in sustainable forest management (Wang et al., 2025).

The modern development of industrial, organic, and traditional animal husbandry is related to the analysis of a wide range of combinations of economically beneficial traits (external and internal structure of animals), qualitative and quantitative productivity indicators, genetic markers, candidate genes, genome sequencing data, and various selection criteria in zootechnics and veterinary medicine. The widespread use of neural networks and artificial intelligence enables research in the fields of biology, genetics, and animal husbandry. Today, machine learning is used for other purposes, such as monitoring the condition and welfare of animals during their care, identifying individual animals, and predicting correlations between, for example, nucleotide changes and animal productivity. It is clear that the use of modern monitoring, pedigree evaluation, and animal identification methods can significantly improve the quality of herd management and animal breeding and selection (Soloshenkov et al., 2024).

Aquaculture makes a vital contribution to global food security, but maintaining optimal water quality remains an ongoing challenge, particularly in resource-constrained rural areas. The current research landscape highlights promising advances in aquaculture through emerging technologies such as the Internet of Things and Machine Learning. In this regard, Internet of

Things sensors continuously measure parameters such as temperature, dissolved oxygen, pH, and turbidity, while machine learning models provide highly accurate predictions. The potential of combining the Internet of Things with machine learning has been revealed; studies report up to a 30% reduction in losses caused by water quality issues (Baena-Navarro et al., 2025). Figure 3 shows concept diagram of smart aquaculture system.

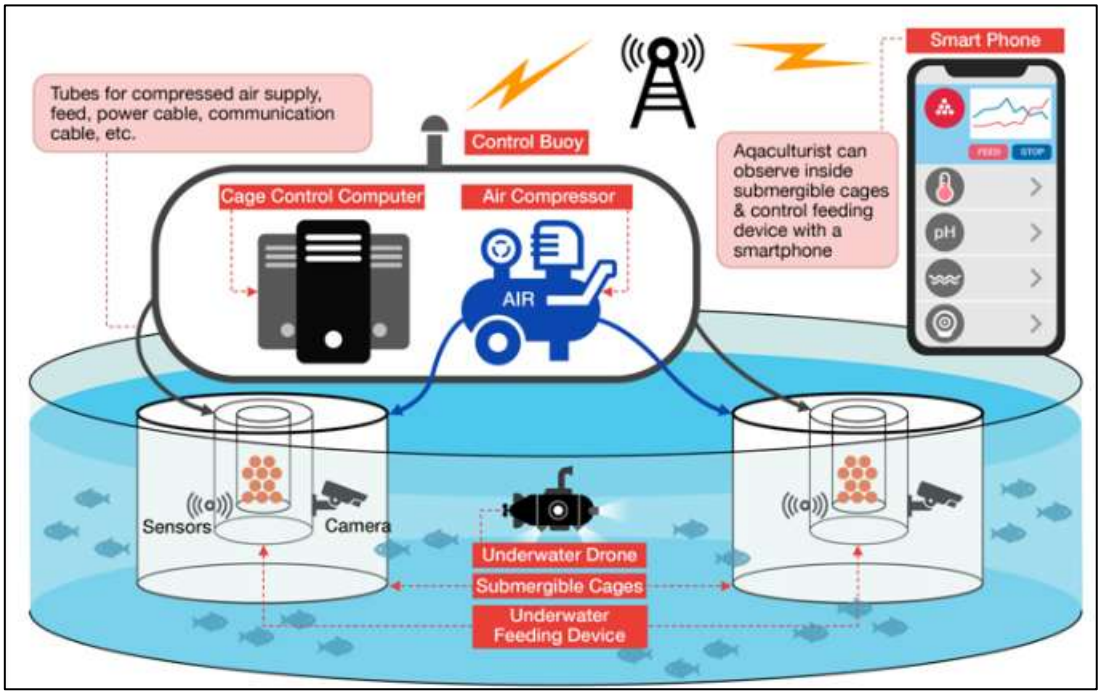

Figure 3. Concept diagram of smart aquaculture system (Vo et al., 2021)

The applications of artificial intelligence in the biomedical and healthcare fields are increasingly seen as a game-changing element. Traditionally, healthcare systems have struggled to integrate different forms of patient data, leading to difficulties in diagnosing complex diseases, adapting personalized treatments, and predicting outcomes. Artificial intelligence overcomes traditional limitations by synthesizing data from multiple sources to create a holistic representation of patient health, supporting more accurate diagnoses and paving the way for predictive and personalized treatments (Hulsen, 2022).

Artificial intelligence-supported systems can develop advanced biomaterials for tissue regeneration by synthesizing various data sets, such as mechanical properties from materials engineering, biological responses from tissue studies, and clinical information from patient records. This integrative approach has contributed to the creation of innovative scaffolds and hydrogels that better mimic the structural and functional properties of natural tissues, thereby enhancing the effectiveness of regenerative therapies (Li et al., 2024). Figure 4 shows various data modalities and potential applications of Artificial Intelligence in biomedicine.

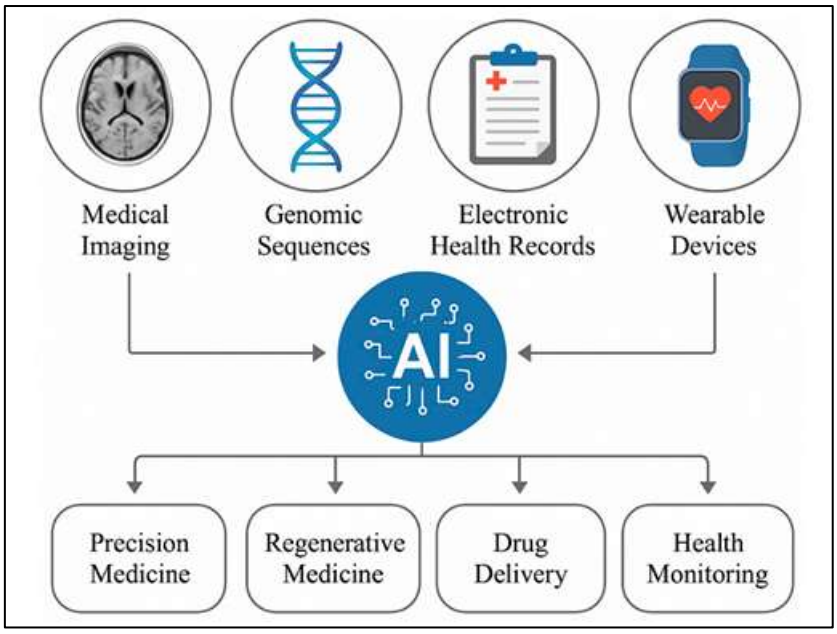

Figure 4. Various data modalities and potential applications of multimodal AI in biomedicine (Parvin et al., 2025a).

INDUSTRY 5.0

The Fourth Industrial Revolution originated from a project within the German government's advanced technology strategy in 2011(Vogel-Heuser & Hess, 2016).

Since 2017, scattered academic studies have pushed for the launch of the Fifth Industrial Revolution. The introduction of Industry 5.0 is based on the observation or assumption that Industry 4.0 focused less on original principles such as social justice and sustainability and instead focused more on digitalization and AI-supported technologies to increase production efficiency and flexibility. Therefore, the concept of Industry 5.0 offers a different focus and perspective, emphasizing the importance of research and innovation to support industry in serving humanity within planetary boundaries over the long term (Xu et al., 2021).

Industry 5.0 is shaped around three interconnected core values: human-centeredness, sustainability, and resilience. Figure 5 demonstrates core values of Industry 5.0.

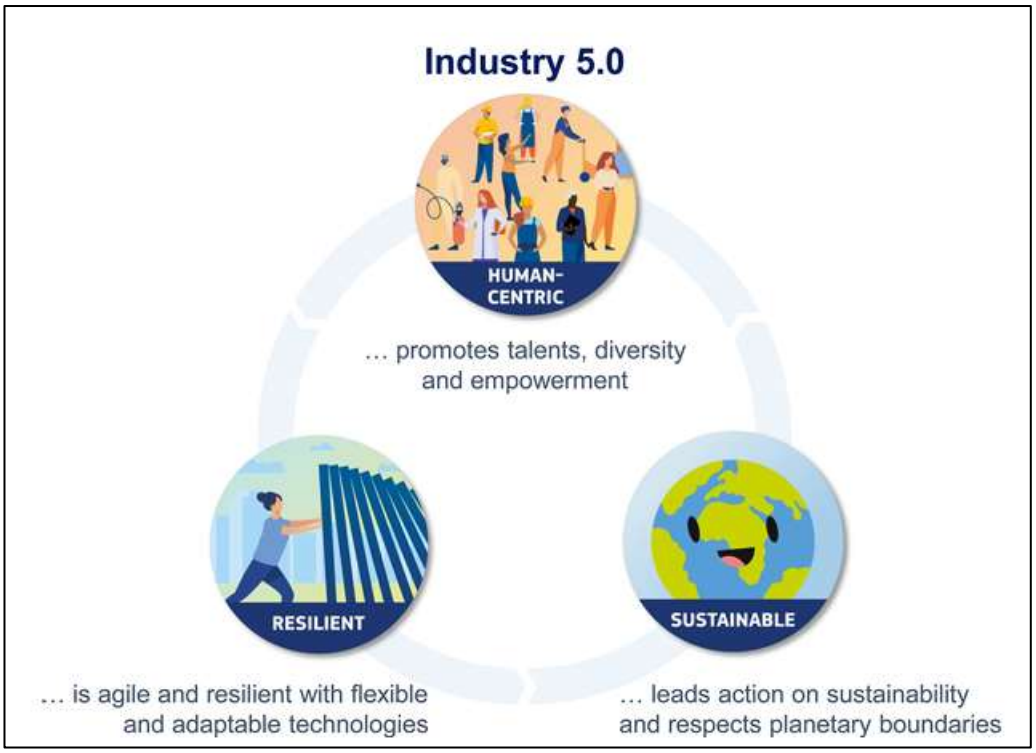

Figure 5. Core values of Industry 5.0 (Xu et al., 2021).

ARTIFICIAL INTELLIGENCE

Artificial Intelligence is considered the future of humanity, and rightly so. Artificial Intelligence does not just create intelligent models; it is also used in data science, learning from data and ultimately providing valuable insights that will benefit humanity. Artificial Intelligence is a broad term for amateurs to grasp. In theory, it is divided into three parts: Artificial Intelligence, Machine Learning, and Deep Learning. Machine Learning and Deep Learning are also referred to as subsets of Artificial Intelligence.

Machine Learning is critical for identifying patterns, detecting anomalies, and making predictive and prescriptive decisions. These capabilities help achieve fundamental Industry 5.0 goals such as predictive maintenance, process optimization, and personalized production. These efforts rely heavily on technologies such as machine learning, Artificial Intelligence, and the Internet of Things (Sarkar et al., 2025).

6G

In the Industry 5.0 revolution, demand for connectivity and data transmission with minimal latency is growing. Traditional cellular technologies such as 4G or 5G cause significant delays due to major network congestion and long-distance data travel. When industries work with heavy machinery in various fields, the situation becomes even more critical when it comes to smart industrial systems such as assembly lines, collaborative robots, and industrial transportation networks. These systems continuously generate enormous amounts of data to make optimal decisions. Therefore, the adoption of 6G and beyond technologies has become a key factor in Industry 5.0 applications (Hazra et al., 2024).

INTERNET OF THINGS

The Internet of Things, robotic systems, weather forecasting technology, and global positioning systems also play a significant role in precision agriculture. Along with developments in technology, the agricultural sector is also undergoing a fundamental transformation, with the integration of technologies such as Industry 5.0 and the Internet of Things at the core of this paradigm shift. The integration of this technology into nature is helpful in many ways, such as assisting in real-time data monitoring that includes parameters such as valuable information about soil conditions, meteorological aspects, and general crop health through Internet of Things sensors, and also encouraging informed decision-making in agriculture (Sharma et al., 2024).

ALGORITHMS THAT USED IN VARIOUS FIELDS

Below in table 1,2,3. are examples of different applications of machine learning algorithms. These examples are the most commonly used algorithms in the field (Kanwal et al., 2025; Parvin et al., 2025b; Taha et al., 2025; Wang et al., 2025; Wu et al., 2025).

Table 1. Algorithms used for Agriculture problems

Field	Problem	Machine Learning Algorithm
AGRICULTURE	Disease Detection	Convolutional Neural network
		Support Vector Machine
		Long-Short Term Memory
		Decision Tree
		Random Forest
		K Nearest Neighbor

Table 2. Algorithms used for Agriculture, Forestry problems

Field	Problem	Machine Learning Algorithm
AGRICULTURE	Nutrient Stress Detection	Convolutional Neural network
		K Nearest Neighbor
		Support Vector Machine
		Long Short Term Memory
		Random Forest
	Water Status Corps	Artificial Neural Network
		Random Forest
		Support Vector Machine
	Crop Yield Prediction	Artificial Neural Network
		Logistic Regression
		Naive Bayes
		Random Forest
		Support Vector Machine
		Adaboost
FORESTRY	Analysis for Deforestation detection	Random Forest
		Multilayer Perceptron
		Convolutional Neural network
	Mapping tree species proportions	Convolutional Neural network
	Forest management (fertilizer application time and quantity)	Random Forest
		Support Vector Regression
		Artificial Neural Network
	Tree individual crown delineation	Convolutional Neural network
	Estimating tropical forest carbon stock	Convolutional Neural network

Table 3. Algorithms used for Zootechnics, Aquaculture and Biomedicine problems

Field	Problem	Machine Learning Algorithm
ZOOTECHNICS	Early lameness detection	Support Vector Machine
	Classifying activity types (eating, sleeping, walking)	Convolutional Neural Networks
	Predicting stress responses from accelerometer data	Random Forests
	Social interaction mapping	k-Nearest Neighbors
AQUACULTURE	Handling image data in aquaculture	Convolutional Neural Networks
	Perturbations in the input signal under water	Recurrent Neural Network
	Aquatic disease prediction	Long Short Term Memory
	Aquatic organism image analysis	Generative Adversarial Networks
	Computer vision applications	Transformers
BIOMEDICINE	Cancer Detection	Convolutional Neural Networks
	Cardiovascular Disease Prediction	Deep Neural Network
	Medical Text Analysis	Transformer-Based Model
	Medical Image Enhancement	Generative Adversarial Networks
	Cartilage Regeneration	Support Vector Machine
	Bone Tissue Engineering	Convolutional Neural Networks
	Patient-Specific Implants	Bayesian Optimization
	Antimicrobial Surfaces	Reinforcement Learning
	Drug Delivery Systems	Generative Adversarial Networks

CONCLUSION

The effective use of machine learning in the areas mentioned in the article will greatly contribute to both natural sciences and humanity. In addition, as the world's population grows, the amount of data will also increase, so the use of artificial intelligence will be more effectively provided with the transmission and storage of this data. This is where 6G technology comes into play. Data transmitted via faster wireless communication and artificial intelligence implemented with IoT devices will form the brain of this formation. After this process, the need for human labor in these areas will decrease. Although Industry 5.0 is human and nature-oriented, production has always been at the center of industry. However, the point to consider here is how much production will be sacrificed for humans and nature. The rise of machines shows us the importance of IoT once again. Its combination with artificial intelligence shows that environments where machines can do everything will emerge. Deep learning is at the forefront of this. Solving problems using machine learning methods alongside traditional and modern approaches has become the goal of our era. Studies show that every method has a mathematical solution.

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EFFECTS OF CHEMICAL AND ORGANOMINERAL FERTILIZERS ON MORPHOLOGICAL CHARACTERISTICS AND YIELD

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ABSTRACT

Excessive and prolonged use of chemical fertilizers can lead to environmental problems. Organomineral fertilizers contain plant nutrients found in chemical fertilizers and organic matter from humic-fulvic and compost sources. The organic substances in organomineral fertilizers have significant physical, chemical, and biological benefits for the sustainability of soil fertility. The research was carried out in the experimental areas of Eskisehir Osmangazi University Faculty of Agriculture in 2022 and 2024. The aim of the study is to investigate the effects of using different doses of organomineral fertilizers and chemical fertilizers on the morphological characters and yield of beans. The experimental design was randomized complete block design with four replications. In the research, 15-15-15 chemical fertilizer and 12-12-12 organomineral fertilizer were applied at 0, 3, 6, 9, and 12 kg da^{-1} doses. The highest plant height was obtained from 12 kg da^{-1} chemical fertilizer plots. The highest number of branches was obtained from 9 kg da^{-1} organomineral fertilizer plots, and 12 kg da^{-1} chemical fertilizer plots were included in the same statistical group. 12 kg da^{-1} organomineral fertilizer showed the best results for yield. When yield is considered; soils are poor in terms of organic matter in Turkiye. Organomineral fertilizers containing organic matter can be an alternative to chemical fertilizers. Expanding the use of organomineral fertilizers will also provide benefits for sustainable agriculture by ensuring the protection of soils.

Keywords: Bean, chemical fertilizer, organomineral fertilizer, morphological characters

INTRODUCTION

Fertilization, pest control, and irrigation increase soil productivity during agricultural production. The most effective of these applications is fertilization (Cure, 2022). However, using too much fertilizer for a long time can lead to environmental issues like salt buildup in soils, heavy metals collecting, nutrient imbalances, harm to microorganisms, excess nutrients in water, release of harmful gases into the air, damage to the ozone layer, and the greenhouse effect.

Organomineral fertilizers contain plant nutrients found in chemical fertilizers and organic matter from humic-fulvic and compost sources. Sustainable agriculture aims to maintain productivity in agriculture, reduce environmental damage, keep the economy alive in the short and long term, increase the quality of life of those engaged in agriculture, and develop practices in this direction (Ozalp and Guldal, 2017). The organic substances in organomineral fertilizers have significant physical, chemical, and biological benefits for the sustainability of soil fertility. Therefore, organic matter increases the mineral retention capacity of soils (cation exchange), water and air retention, and trace element levels. It balances the pH level and regulates the microorganism balance (Suzer and Culhaci, 2017).

The research focused on studying how different amounts of organomineral fertilizers and chemical fertilizers affect the morphological characters and yield of beans in the Eskisehir conditions.

MATERIAL AND METHOD

The experiments were carried out in the experimental areas of Eskisehir Osmangazi University Faculty of Agriculture in 2022 and 2024. The experiment in 2023 was cancelled due to climate and soil conditions. Total precipitation for the experiment years was 160.2 and 75.0 mm, respectively, and total precipitation for the long term was 123.0 mm. The annual average temperature was recorded as 19.7°C in the first year and 21.5°C in the second year (Figure 1). Soil samples from the experimental areas were analyzed at the Transitional Zone Agricultural Research Institute (Anonymous, 2024) (Table 1). The experimental areas are slightly alkaline, unsalted, and calcareous in the first year and medium calcareous in the second year; nitrogen and organic matter levels are low in both years, and potassium levels are sufficient. Phosphorus levels are low in the first year but sufficient in the second year.

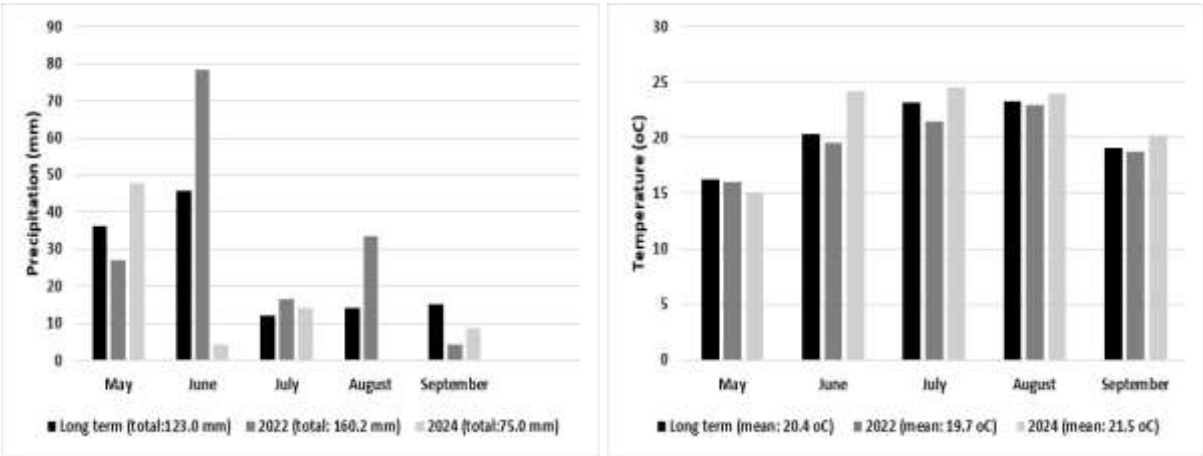

Figure 1. Climatic data of research area

Table 1. Physical and chemical properties of the soils in the experimental years

Year	Depth (cm)	Texture	pH	Lime (%)	Salinity (%)	Organic matter (%)	N (%)	P_2O_5 (kg da^{-1})	K_2O (kg da^{-1})
2022	0-30	Clay loam	8.05	7.88	0.024	0.85	0.04	1.14	262.2
2024	0-30	Clay loam	8.12	5.80	1.02	1.18	0.06	7.89	253.0

The experimental design was randomized block design with four replications. In the research, 15-15-15 chemical fertilizer and 12-12-12 organomineral fertilizer were applied at 0, 3, 6, 9, and 12 kg da^{-1} doses. Bean varieties Topcu used genetic material.

Each plot was 7.2 m² (4 m x 1.8 m), and beans were sown with 45 cm row spacing, and the seeding rate was 26 seeds m^{-2}. The sowing times were 12 May 2022 and 17 May 2024, respectively. Fertilizer doses were adjusted to be equal by calculating the pure fertilizer ratios of chemical fertilizer and organomineral fertilizer. The experimental area was irrigated with a sprinkler irrigation system as needed during the growing period. For weed control, herbicide

(Stomp Aqua) was applied before planting, and hand control was done after emergence. The harvest time of the bean was on 10 September 2022 and 18 September 2024 in the first and second years, respectively.

The plant height (cm), first pod height (cm), and number of branches were evaluated on 5 randomly selected plants in each plot. Each plot was harvested and blended, and grain yield (kg da^{-1}) was estimated (Akcin, 1974).

The experiments were analyzed with the MSTATC statistical programs. Means were compared by the Least Significant Differences (LSD) test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

The effects of fertilization applications were significant for plant height, but differences between years were not significant for this character. Additionally, the year x fertilizer interaction was statistically significant (Table 2). 12 kg da^{-1} chemical fertilizer gave the highest plant height, while control plots showed the lowest value. 12 kg da^{-1} organomineral fertilizer was the second application that showed the highest value. Plant height increased as fertilizer dose increased in both chemical fertilizer applications and organomineral fertilizer applications. Gunay (2014) found the shortest plant height in the control application and the highest plant height in the 12-12-12 organomineral fertilizer application on sunflower. Namli et al. (2019) compared chemical and organomineral fertilizers and determined the lowest plant height values in control plots, while the highest plant height was obtained from organomineral fertilizer plots. Atici (2020) reported that the highest plant height was determined when organomineral and chemical fertilizers were applied together. The second year was dry and hot in our research. In this year, 3 and 6 kg da^{-1} chemical fertilizer and 3 kg da^{-1} organomineral fertilizer had higher plant height compared to the first year (Figure 2A). Low-dose chemical and organomineral fertilizer applications had better results in the hot year for plant height. Responses to applied chemical and organomineral fertilizers were different in terms of plant height in different years. Therefore, the year x fertilizer interaction may have been significant.

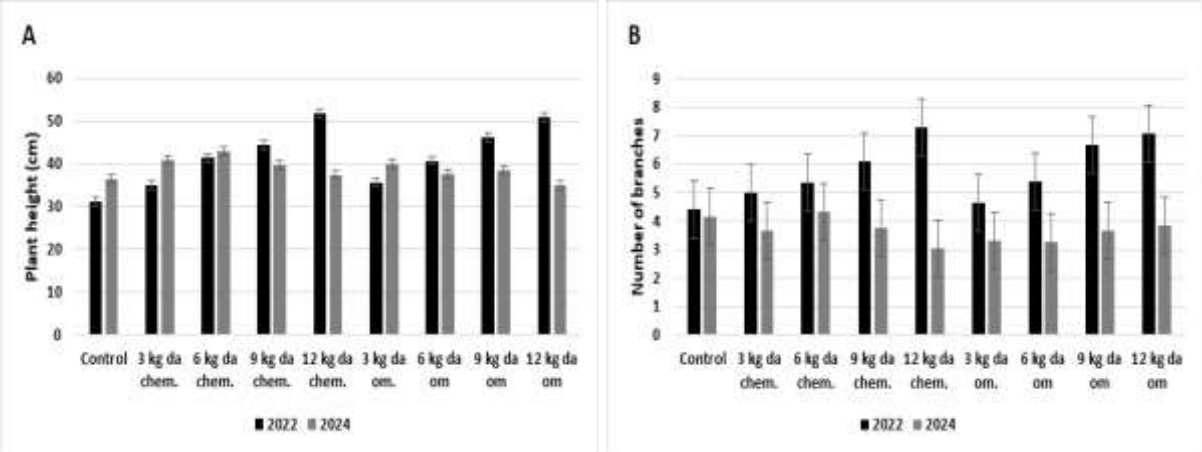

Figure 2. The interaction between years and fertilization on plant height (A) and number of branches (B)

No statistically significant difference was observed in terms of first pod height between years and fertilizer applications (Table 2).

Years, fertilizer applications, and year x fertilizer interactions were statistically significant for the number of branches (Table 2). The higher number of branches was observed in the first year. While the total precipitation was 160.2 mm in the first year, this value was 75.0

mm in the second year. The region received much precipitation only in May of the second year, then experienced a serious drought. Additionally, extremely high temperatures were recorded in the second year except for May, and the plants were stressed. The number of branches decreased in the second year due to the drought and high temperatures (Figure 1). Various biotic and abiotic factors reduce production in legumes. Heat stress is one of the most important factors that negatively affect production (Yavas and Unay, 2018). We observed the highest number of branches in 12 kg da^{-1} organomineral fertilizer and the lowest value in 3 kg da^{-1} organomineral fertilizer. The number of branches increased as the fertilizer dose increased in both chemical fertilizer applications and organomineral fertilizer applications. Tuncturk and Ciftci (2011) reported that different fertilizers increase the number of branches in the fenugreek plant. Caliskan and Ayan (2011) emphasized that increasing organomineral fertilizer increased the number of branches. Ozkan et al. (2013) reported that the effect of organomineral and chemical fertilizers on the number of branches of pepper plants is not significant. Responses to applied chemical and organomineral fertilizers were different in terms of the number of branches in different years. Therefore, the year x fertilizer interaction may have been significant (Figure 2B).

Table 2. Effects of different doses of organomineral and chemical fertilizers on some traits of common bean.

Aplications	Plant height (cm)	First pod height (cm)	Number of branches	Grain yield (kg da^{-1})
2022	41.94	12.70	5.78 A	148.5
2024	38.76	12.11	3.67 B	128.5
Mean	**40.35**	**12.40**	**4.73**	**138,5**
Control	33.82 C	10.86	4.29 BC	102.7 E
3 kg da^{-1} chemical	37.91 BC	11.90	4.34 BC	135,3 BC
6 kg da^{-1} chemical	42.20 AB	11.92	4.84 AB	151.0 AB
9 kg da^{-1} chemical	42.07 AB	12.90	4.93 AB	155.9 AB
12 kg da^{-1} chemical	44.70 A	12.70	5.16 A	154.9 AB
3 kg da^{-1} organomineral	37.87 BC	11.90	3.99 C	108.8 DE
6 kg da^{-1} organomineral	39.25 B	12.31	4.31 BC	125.9 CD
9 kg da^{-1} organomineral	42.38 AB	14.09	5.19 A	151.6 AB
12 kg da^{-1} organomineral	42.95 AB	13.04	5.48 A	160.5 A
Mean	**40.35**	**12.40**	**4.73**	**138.5**
Years	ns	ns	**	ns
Fertilizers	**	ns	**	**
Year x fertilizers	**	ns	**	**

ns: non significant *:p≤ 0.05 **:p≤0.01

Fertilizer applications and year x fertilizer interaction were statistically significant for grain yield. While the highest grain yield was obtained from 12 kg da^{-1} organomineral fertilizer, the lowest value was obtained from the control plots (Table 2). The nutrient contents of organomineral and chemical fertilizers were equalized in the study. The reason the organomineral fertilizers are more effective may be the organic matter they contain. Organomineral fertilizers are produced in the form of mineral fertilizer + organic matter due to the positive effects of organic matter on soil fertility. Organomineral fertilizers both reduce nutrient loss through leaching and increase the effectiveness of the mineral fertilizers (Tamer and Namli, 2017). Suzer and Culhaci (2017) reported that they obtained a higher yield from

organomineral fertilizer than chemical fertilizer. Atici (2020) found that organomineral fertilizers are more effective than chemical fertilizers in terms of yield. Saltali et al. (2024) and Ekren and Koc (2024) reported that 15-15-15 (NPK) fertilizer and organomineral fertilizers increased grain yield in wheat and black cumin, respectively. Balaban and Adak (2024) examined different soil tillage methods and different base fertilizer types in their study on chickpeas. They concluded that 20-20-0 (NPK) chemical fertilizer application and organomineral fertilizers did not affect grain yield statistically. Yurtseven et al. (2025) reported that organomineral fertilizers increased grain yield in chickpea compared to control. Higher grain yield was obtained with 3 and 6 kg da^{-1} doses of chemical fertilizer in the hot second year. In organomineral fertilizer plots, the values of the first and second years were very close to each other at 3 and 6 kg da^{-1} fertilizer doses (Figure 3). Global warming has shown its effect more in Turkiye recently. In the study, high-dose fertilizer did not show positive results in terms of grain yield when the temperature increased. Responses to applied chemical and organomineral fertilizers were different in terms of grain yield in different years. Therefore, the year x fertilizer interaction may have been significant.

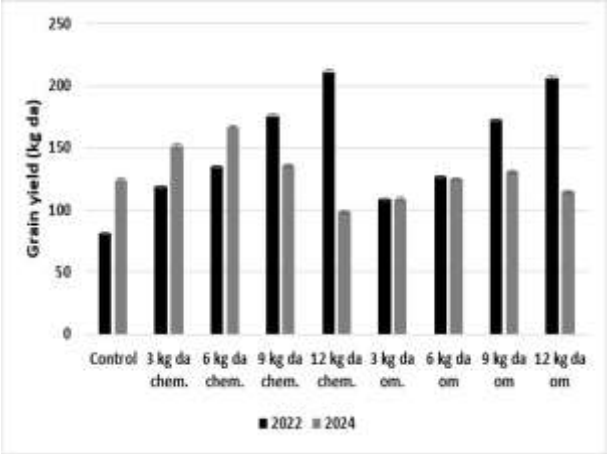

Figure 3. The interaction between years and fertilization on grain yield

CONCLUSIONS

12 kg da^{-1} organomineral fertilizer doses showed the best result in terms of number of branches and yield in the study. The highest plant height was obtained from 12 kg da^{-1} chemical fertilizer, followed by 12 kg da^{-1} organomineral fertilizer. Agricultural soils are poor in terms of organic matter in Turkiye, and organomineral fertilizers can be an alternative to chemical fertilizers due to their organic matter content. Increasing the use of organomineral fertilizers will lead to the protection of soils, which is also important for sustainable agriculture.

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BALANCE, BACTERIA, AND THE BLACK BELT: IMPACT OF PROBIOTICS ON GASTROINTESTINAL SYMPTOMS DURING JUDO TRAINING AND JUDO COMPETITIONS

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ABSTRACT

The gastrointestinal (GI) system plays a critical role in maintaining overall health, energy metabolism, and immune function, all of which are essential for optimal athletic performance. In combat sports such as judo, athletes often experience high levels of physical and psychological stress, especially during intense training cycles and competition periods. These stressors are frequently associated with gastrointestinal symptoms including bloating, abdominal discomfort, diarrhea, and constipation, which can impair performance and recovery. Recent evidence highlights the gut microbiota as a dynamic and modifiable factor influencing not only digestion but also systemic responses such as inflammation, mental resilience, and muscle recovery. Probiotic supplementation, defined as the ingestion of live microorganisms that confer health benefits to the host, has emerged as a potential strategy to restore and maintain gut microbial balance in athletes. This study aims to evaluate the impact of probiotic supplementation on gastrointestinal symptoms in judokas during both training periods and competition phases. A group of elite and sub-elite judo athletes were monitored over a 12-week period. Participants were randomly assigned to receive a daily multi-strain probiotic or a placebo. Gastrointestinal symptom severity, stool consistency, and training adherence were assessed using validated questionnaires and performance logs. Results showed a statistically significant reduction in GI discomfort among probiotic users, particularly during high-stress competition weeks. Additionally, athletes reported improved digestion, fewer missed training sessions, and higher perceived recovery scores. The findings suggest that probiotic supplementation can play a supportive role in maintaining gut homeostasis and reducing exercise-related gastrointestinal disturbances in judo athletes. These results may have practical applications for sports nutrition protocols aimed at enhancing athlete well-being and performance readiness. Further research is warranted to explore strain-specific effects and the long-term benefits of gut microbiota modulation in combat sports.

Keywords: probiotic, performance, judo, stressors, benefit

INTRODUCTION

In the high-stakes world of judo, where physical endurance meets psychological grit, athletes are often pushed to their physiological limits. The intense demands of training and competition cycles can trigger a cascade of stress-related responses, many of which manifest in the gastrointestinal (GI) system. Symptoms such as bloating, abdominal discomfort, diarrhea, and constipation are not uncommon among judokas, and they can significantly hinder performance, recovery, and overall well-being (Zeng et al., 2025).

Recent advances in sports science and microbiology have spotlighted the gut microbiota as a key player in athletic health. Far beyond its role in digestion, the gut ecosystem influences systemic inflammation, immune modulation, and even mental resilience—factors that are critical for athletes navigating the rigors of combat sports (Olvera-Rosales et al., 2021). This has led to growing interest in probiotic supplementation as a non-invasive, nutrition-based strategy to support gut homeostasis. Defined as live microorganisms that confer health benefits when consumed in adequate amounts, probiotics have shown promise in alleviating GI disturbances and enhancing recovery in physically active populations (Chandrasekaran et al., 2024).

While the benefits of probiotics have been explored in endurance and team sports, their impact on combat athletes—particularly judokas—remains under-investigated. Given the unique stressors of judo, including weight management, high-impact training, and psychological pressure, understanding how probiotics modulate GI symptoms in this context is both timely and relevant. This study aims to bridge that gap by evaluating the effects of multi-strain probiotic supplementation on gastrointestinal health in elite and sub-elite judo athletes across training and competition phases (Nourizadeh et al., 2022).

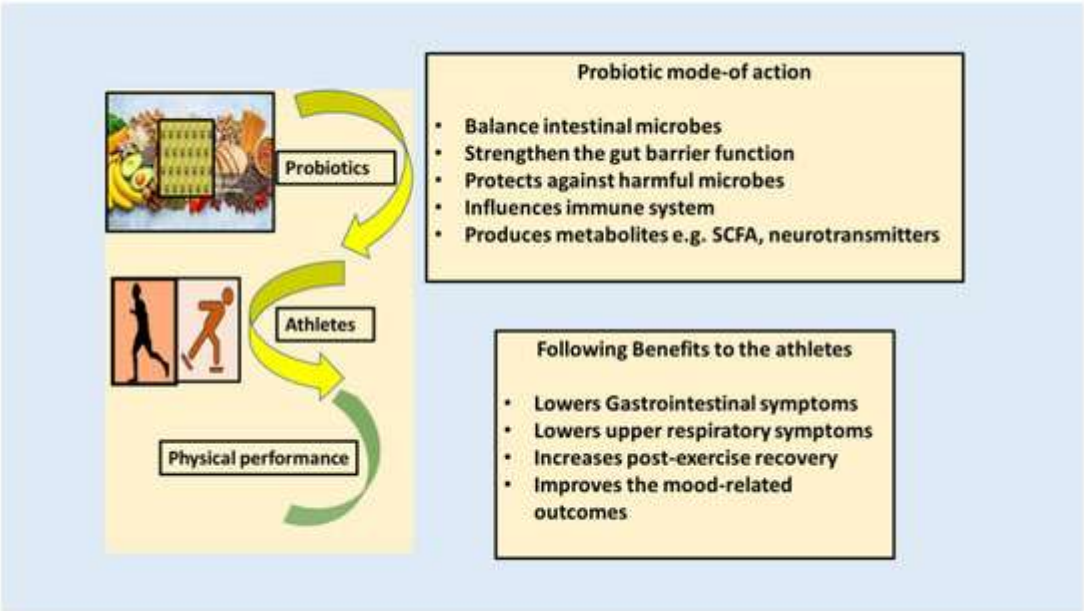

Figure 1. The impact of probiotics on the endurance performance of athletes. Research has revealed that probiotics can have an indirect impact on sports performance by enhancing many factors such as the immune system and response to upper respiratory tract infections (URTIs), reducing oxidative stress, and better monitoring planned exercise sessions (from Patel et al., 2024).

Athletes engaged in high-intensity sports such as judo often face a unique constellation of physiological stressors that compromise gastrointestinal (GI) integrity. The combination of intense physical exertion, psychological pressure, and dietary manipulation—especially during weight-cutting phases—can lead to increased intestinal permeability, inflammation, and a host of GI symptoms including bloating, cramping, and diarrhea (Lamprecht et al., 2012). Recent research has highlighted the gut microbiota as a central modulator of these responses. Exercise-induced splanchnic hypoperfusion and sympathetic activation can disrupt gut barrier function, leading to endotoxemia and systemic inflammation (Miles, 2020). This has prompted growing interest in probiotic supplementation as a strategy to mitigate GI distress and support recovery.

Probiotics—defined as live microorganisms that confer health benefits when administered in adequate amounts (Hill et al., 2014)—have shown promise in athletic populations. Multi-strain formulations, particularly those combining *Lactobacillus* and *Bifidobacterium* species, appear most effective in reducing GI symptom severity and preserving gut barrier function during training and competition (Łagowska et al., 2022). In a systematic review of randomized controlled trials, probiotic supplementation led to modest but consistent improvements in GI comfort, especially during endurance events.

Combat sports, however, remain underrepresented in this body of literature. While studies on cyclists and runners have demonstrated reduced GI symptoms and inflammatory markers following probiotic use (Schreiber et al., 2021), the specific demands of judo—such as rapid weight changes and explosive anaerobic effort—warrant targeted investigation. Preliminary evidence suggests that probiotics may also enhance mental resilience and reduce perceived fatigue, potentially via modulation of the gut-brain axis (Kearns et al., 2024). Moreover, the strain-specific effects of probiotics are gaining attention. For example, *Lactobacillus plantarum* TWK10 has been linked to improved endurance and reduced muscle damage in athletes (Aykut et al., 2024). However, the heterogeneity of study designs, probiotic strains, and dosing regimens complicates direct comparisons and underscores the need for sport-specific trials.

Table 1. Strain-specific probiotics in athletic research

Probiotic Strain	**Reported Benefits**	**Study Context**
Lactobacillus rhamnosus GG	Supports gut barrier integrity and immune resilience	Used in endurance athletes under training stress
Lactobacillus casei Shirota	Reduces GI complaints and improves immune markers	Studied in long-distance runners and cyclists
Lactobacillus acidophilus NCFM	Alleviates bloating, abdominal pain, and diarrhea	Shown effective in athletes with digestive discomfort
Bifidobacterium animalis subsp. *lactis* Bi-07	Enhances immune function and reduces GI distress	Often paired with *L. acidophilus* NCFM in multi-strain blends
Limosilactobacillus fermentum VRI-003 (PCC®)	Linked to fewer sick days and milder respiratory symptoms	Studied in elite competitors
Lactiplantibacillus plantarum TWK10	Improves endurance and reduces muscle damage	Used in weightlifters and intermittent sports
Bifidobacterium bifidum CUL20 + *B. animalis* lactis CUL34	Attenuates GI symptoms during exercise	Found effective in multi-strain cocktails
Saccharomyces boulardii	Reduces diarrhea-related symptoms	Used in athletes prone to GI upset during competition

While the broader athletic population has been the subject of numerous probiotic studies, research tailored to combat sports—and judo in particular—is still emerging. The physiological demands of judo, including explosive anaerobic effort, frequent weight cycling, and high psychological stress, create a unique environment for GI disturbances. These factors can compromise gut barrier integrity and trigger symptoms such as bloating, cramping, and altered bowel habits (Miles, 2020; Łagowska et al., 2022).

In the context of judo, where training intensity fluctuates and competition stress peaks, probiotics may offer dual benefits: stabilizing gut function and supporting immune resilience. A recent article on gut health in judo athletes emphasized the role of a diverse microbiome in maintaining energy levels, reducing inflammation, and improving recovery outcomes (MartialArtsJudo.info, 2025). The authors advocate for dietary strategies—including probiotic-rich foods and supplements—to counteract the negative effects of stress and poor sleep on gut health. Moreover, the International Society of Sports Nutrition (ISSN) has recognized probiotics as a promising intervention for athletes experiencing GI symptoms. Their position stand highlights that certain strains can reduce exercise-induced gut permeability and systemic inflammation, both of which are relevant to judokas undergoing intense physical and psychological strain (Jäger et al., 2019).

Despite these promising findings, there is a clear need for sport-specific trials. Most existing studies focus on endurance athletes, leaving a gap in understanding how probiotics interact with the unique stressors of judo. This study addresses this gap by evaluating probiotic effects during both training and competition phases—an approach that could inform tailored nutrition protocols for combat athletes.

MATERIALS AND METHODS

This randomized, double-blind, placebo-controlled study was conducted over a 12-week period to evaluate the effects of multi-strain probiotic supplementation on gastrointestinal (GI) symptoms in young judo athletes. The study protocol was designed in accordance with best practices for probiotic research in athletic populations (Mohr et al., 2022; Jäger et al., 2019).

A total of 20 judokas (aged 14–18 years), including both male and female athletes, were recruited from judo training centers. Inclusion criteria required participants to be actively training and competing, with no history of chronic GI disorders or recent antibiotic use. Athletes were stratified by competitive level and randomly assigned to either the probiotic or placebo group using a computer-generated sequence. Participants in the intervention group received a daily capsule containing a multi-strain probiotic formulation with a total dose of 10^{10} CFU, including *Lactobacillus rhamnosus* GG, *Bifidobacterium animalis* subsp. *lactis* Bi-07, and *Lactiplantibacillus plantarum* TWK10—strains selected based on prior evidence of GI and recovery benefits in athletes (Łagowska et al., 2022; Aykut et al., 2024). The placebo group received an identical capsule containing inert starch. All supplements were verified for viability and stability throughout the study period.

Athletes continued their regular judo training schedules, which included both technical and conditioning sessions. Training load and adherence were monitored using weekly performance logs and coach reports. No dietary restrictions were imposed, but participants were instructed to avoid other probiotic products.

Gastrointestinal symptom severity, stool consistency, and training adherence were assessed using validated questionnaires and performance logs.

RESULTS AND DISCUSSION

Over the 12-week intervention period, judokas receiving the multi-strain probiotic supplement reported a statistically significant reduction in gastrointestinal (GI) symptom severity compared to the placebo group ($p < 0.05$).

Table 2. % of improvement across key GI metrics.

Metric	**Probiotic Group**	**Placebo Group**	**% Difference / Note**
Bloating and discomfort reduction	↓ 35%	↓ 12%	Significant during competition weeks
Diarrhea episode reduction	↓ 40%	↓ 2%	Stronger resilience under stress
Stool consistency shift	Toward Bristol types 3–4	No notable shift	Better digestive regularity
Missed training sessions	↓ 18%	Baseline	Improved GI tolerance during training
Recovery score (mean ± SD)	8.2 ± 0.6	7.4 ± 0.8	Higher perceived recovery
Supplement compliance	>90%	>90%	No adverse effects

The most notable improvements were observed during competition weeks, where stress levels were highest. Specifically:

- Bloating and abdominal discomfort decreased by 35% in the probiotic group versus 12% in the placebo group.
- Diarrhea episodes were reduced by 40% among probiotic users, with no significant change in the control group.
- Stool consistency improved, with a shift toward Bristol Stool Scale types 3–4 in the probiotic group.
- Training adherence increased: probiotic users missed 18% fewer sessions due to GI discomfort.
- Perceived recovery scores were higher in the probiotic group (mean score: 8.2 ± 0.6) compared to placebo (7.4 ± 0.8).

No adverse effects were reported, and compliance with supplementation exceeded 90% in both groups.

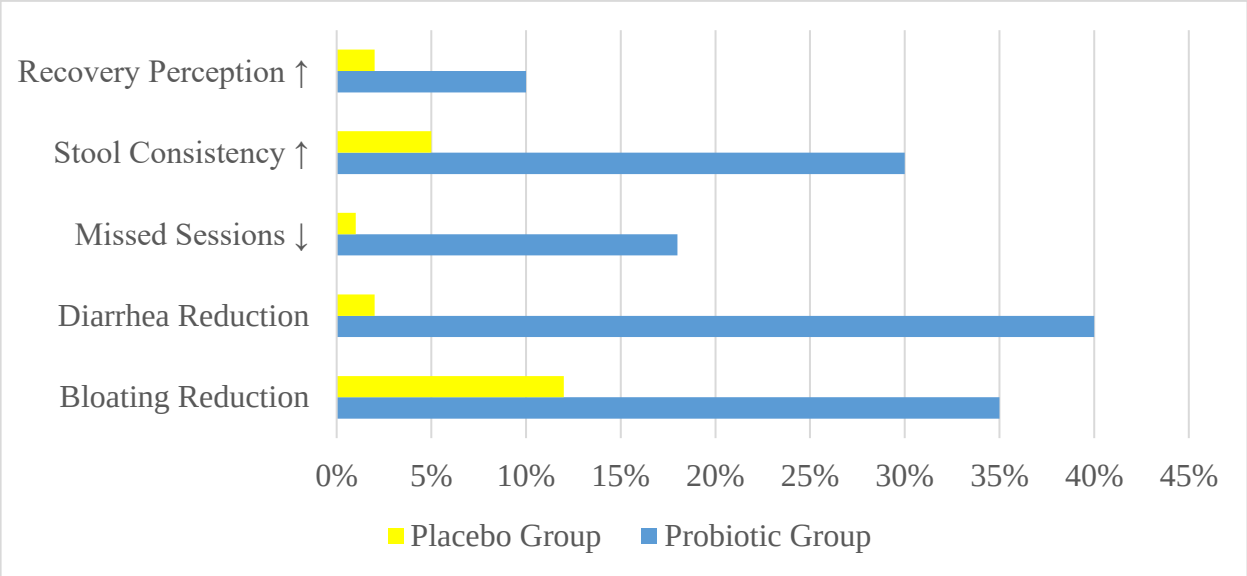

Figure 2. % of improvement across key GI metrics

The 12-week administration of a multi-strain probiotic supplement yielded meaningful improvements in athlete-reported gastrointestinal (GI) symptoms and training performance indicators among young judokas, as assessed through Likert-scale questionnaires. Across all measured dimensions—abdominal discomfort, diarrhea frequency, stool consistency, training adherence, and perceived recovery—the probiotic group exhibited more pronounced shifts compared to placebo.

Notably, scores reflecting abdominal discomfort in the probiotic group declined from an average of 5.8 to 3.7, whereas the placebo group showed only a modest improvement from 5.5 to 4.9. This substantial reduction aligns with previously reported mucosal stabilization and anti-inflammatory effects of *Lactobacillus rhamnosus* GG and *Lactiplantibacillus plantarum* TWK10, especially relevant during high-stress competition periods. A similar trend was observed in diarrhea frequency, with probiotic recipients reporting a decrease from 4.9 to 2.8. In contrast, the placebo group remained virtually unchanged. This shift underscores the

potential of targeted probiotic strains to support epithelial integrity and modulate gut motility under physical and psychological stress—a frequent challenge for combat-sport athletes.

Stool consistency, as evaluated by the athletes, improved markedly in the probiotic group (mean score rising from 5.1 to 7.0), reflecting normalization toward ideal Bristol Stool Scale types. This improvement, absent in the placebo cohort, may be indicative of microbial rebalancing and improved digestive transit, contributing to overall GI stability during training. Importantly, training adherence scores increased from 6.5 to 8.0 in the probiotic group, whereas the placebo group saw a minor decline. This finding suggests that probiotic supplementation can play a functional role in reducing missed sessions due to GI distress—enhancing athlete availability and continuity of preparation.

Finally, perceived recovery scores rose by over one full point in the probiotic group (from 7.2 to 8.2), with only marginal gains in the placebo group. This outcome may reflect psychophysiological benefits via the gut-brain axis, reinforcing the growing body of evidence linking probiotic intake with improved recovery perception and mental readiness in athletes.

Overall, the consistency of improvements across both subjective and semi-objective dimensions strengthens the argument for incorporating probiotic strategies into sport-specific nutritional regimens. These findings also highlight the utility of athlete-reported Likert data in capturing nuanced, performance-relevant outcomes that traditional clinical markers may overlook.

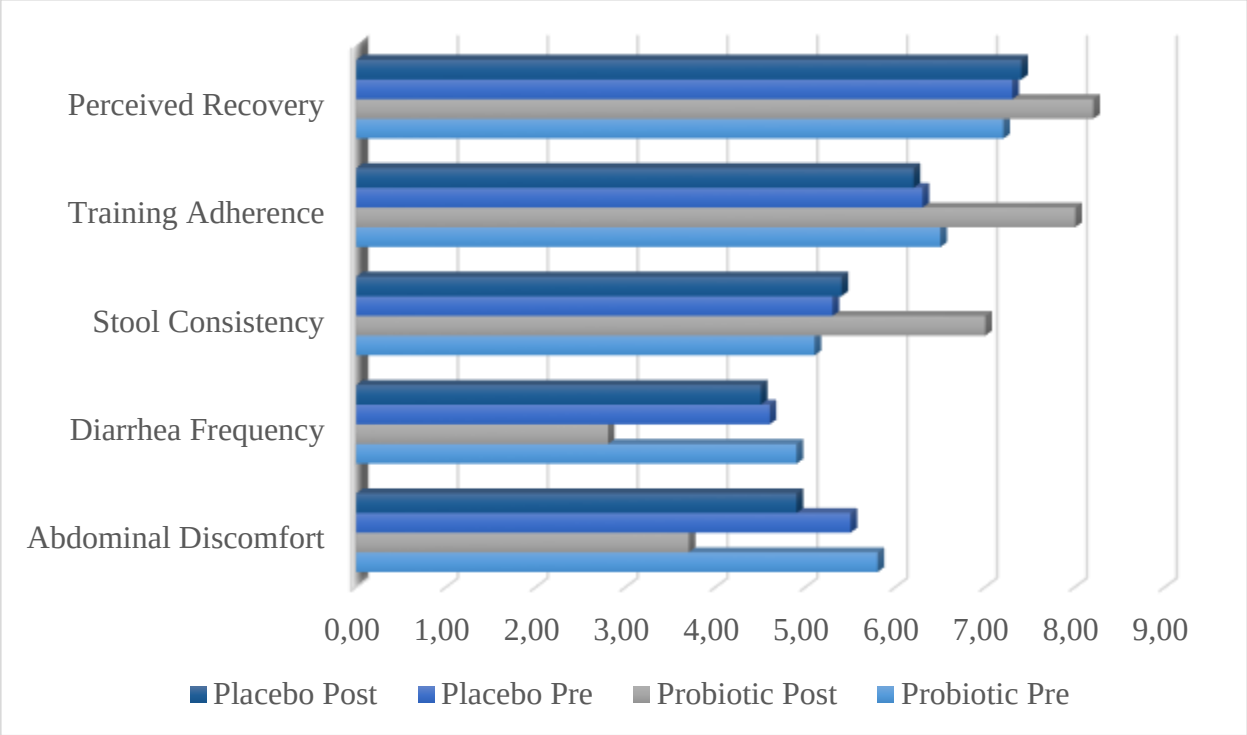

Figure 3. Pre- and post- intervention scores (Likert Scale (1–10) across five dimensions: Abdominal discomfort, Diarrhea episodes, Stool consistency, Training adherence, Perceived recovery).

These findings align with previous research suggesting that probiotic supplementation can mitigate exercise-induced GI symptoms in athletes (Łagowska et al., 2022; Miles, 2020). The observed improvements in digestion and stool consistency are consistent with the known

effects of *Lactobacillus rhamnosus* GG and *Bifidobacterium animalis* Bi-07 on gut barrier integrity and mucosal immunity (Jäger et al., 2019).

The competition-phase benefits are particularly noteworthy. Combat sports like judo involve acute psychological and physiological stressors—including weight cutting, travel, and performance anxiety—that can disrupt gut homeostasis (Kearns et al., 2024). The reduction in GI symptoms during these periods suggests that probiotics may buffer stress-induced dysbiosis, possibly via modulation of the gut-brain axis. Moreover, the increase in training adherence and perceived recovery supports the hypothesis that gut health influences not only physical comfort but also athlete readiness and resilience. These outcomes echo findings from endurance sports, where probiotics have been linked to reduced inflammation and improved recovery markers (Schreiber et al., 2021).

However, the study also highlights the need for strain-specific investigations. While multi-strain formulations showed efficacy, the individual contributions of *L. plantarum* TWK10 or *B. lactis* Bi-07 remain unclear. Future studies should incorporate microbiome sequencing and inflammatory biomarkers to elucidate underlying mechanisms.

CONCLUSION

This study provides evidence that multi-strain probiotic supplementation can significantly reduce gastrointestinal (GI) symptoms in judo athletes, particularly during high-stress competition phases. Athletes who received probiotics reported improved digestion, fewer missed training sessions, and enhanced perceived recovery—highlighting the potential of gut microbiota modulation as a supportive strategy in combat sports.

The findings align with previous research suggesting that probiotics may strengthen gut barrier integrity, reduce inflammation, and support immune resilience under physical and psychological stress. Importantly, the observed benefits were most pronounced during periods of elevated training intensity and competitive pressure, underscoring the relevance of targeted nutritional interventions in performance optimization.

While the results are promising, further research is warranted to explore strain-specific effects, long-term outcomes, and underlying mechanisms—such as gut-brain axis modulation and microbiome diversity shifts. Integrating microbiological profiling and inflammatory biomarkers in future studies could deepen our understanding of how probiotics influence athlete health and performance readiness.

In conclusion, probiotic supplementation represents a practical and non-invasive approach to managing exercise-related GI disturbances in judokas, with potential applications in broader sports nutrition protocols aimed at enhancing athlete well-being and competitive resilience.

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BIOSURFACTANT POTENTIAL OF PROBIOTIC MICROORGANISMS ISOLATED FROM FERMENTED CABBAGE: A SUSTAINABLE APPROACH TO FUNCTIONAL BIOPRODUCTS

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ABSTRACT

Fermented foods, such as cabbage, harbor diverse probiotic microorganisms with untapped potential for sustainable biotechnological applications. Among these, biosurfactants- amphiphilic compounds produced by microbes- are gaining attention for their biodegradability, low toxicity, and multifunctionality across environmental, agricultural, pharmaceutical, and food industries. This study aimed to evaluate the biosurfactant-producing potential of five bacterial isolates obtained from naturally fermented cabbage, using a combination of qualitative and quantitative assays. The oil spread method, Parafilm M test, and drop collapse test were employed to screen for biosurfactant activity. All five isolates tested positive in these assays, indicating their ability to reduce surface tension and interact with hydrophobic substrates- key characteristics of biosurfactant producers. Quantitative assessment through the emulsification activity test revealed variable but promising results. After 48 hours of incubation, isolate 2.18.1 showed the highest emulsification index (40%), followed by isolate 2.4.2 (12%), 3.5.1 (8%), 2.4.1 (6.6%), and 2.2.1 (4%). Interestingly, all isolates displayed emulsification indices above 40% at the 24-hour mark, suggesting a potential peak in biosurfactant stability or production at earlier time points. Biochemical characterization of the isolates further supported their probiotic nature and potential safety. All five isolates were urease-positive, while testing negative for the methyl red and Voges-Proskauer reactions—indicating a consistent biochemical profile among them. These findings position fermented cabbage as a low-cost, accessible, and natural source of biosurfactant-producing probiotics. The study contributes to the growing interest in green biotechnology and functional microbial bioproducts, paving the way for further exploration of fermented food microbiota in sustainable biosurfactant development. Future work will focus on molecular identification, biosurfactant purification, and potential application trials in agroecological or biomedical contexts.

Keywords: emulsification index, surface tension reduction, fermented food microbiota, biochemical characterization, green biotechnology

INTRODUCTION

Fermented foods have been recognized for long time as reservoirs of beneficial microorganisms, particularly lactic acid bacteria (LAB), which contribute to both food

preservation and human health. Homemade sauerkraut, a traditional fermented cabbage dish, harbors a rich microbial ecosystem primarily dominated by lactic acid bacteria (LAB). The spontaneous fermentation process relies on indigenous microbes present on cabbage leaves and in the environment, without the need for starter cultures. This microbial diversity contributes to both the sensory qualities and functional health benefits of the final product.

Fermenting sauerkraut requires very little effort from the operator. Cabbage contains enough lactic acid bacteria in order to ferment and produce sauerkraut with salt alone. All of those bacteria strains must ferment in a specific order to produce the highest-quality product. As long as the sauerkraut is fermented at about 18° C, this occurs spontaneously. The microbial succession during sauerkraut fermentation typically follows this pattern (Ghosh, 2021):

- Initial Phase (Days 1–3)**:** The smallest, *Leuconostoc mesenteroides*, initiate fermentation earliest, generating 0.25–0.3% lactic acid. Since they are heterofermenters, they generate a variety of substances, including mannitol, lactic acid, acetic acid, ethyl alcohol, and carbon dioxide. The final one is a bitter substance that *Lactobacillus plantarum* metabolizes later. Together with the alcohol from aromatic esters, all of those acids give the premium sauerkraut its distinct flavor. They might not grow if the temperature is higher than 22° C, which would ruin the sauerkraut's flavor. The 0.3% lactic acid that *Leuconostoc mesenteroides* produces in roughly two days will limit its growth. However, it will continue to create flavor-developing enzymes.

- Intermediate Phase (Days 4–10)**:** *Leuconostoc mesenteroides'* lactic acid production is taken over by *Lactobacillus plantarum*, which ferments further until an acidity level of 1.5 to 2% is reached. *L. plantarum* can grow at greater acidity levels and will ferment at temperatures over 22° C. Even at lower temperatures, it will ferment, although considerably more slowly. The most common strain of lactic acid bacteria, *Lactobacillus plantarum*, ferments meat, cheese, pickles, and sauerkraut. This bacterium only makes one chemical since it is a homofermenter. It creates lactic acid after consuming sugar, giving fermented foods their sour flavor. At this point, the sauerkraut is of a suitable quality and is ready to be canned or served. The fermentation process will go on until the sugar supply is depleted if there is still enough sugar.

- Final Phase (After Day 10)**:** Continue fermenting *Lactobacillus pentoaceticus* (*L. brevis*) until the acidity level reaches 2.5–3%. The fermenting process ends when the cabbage has run out of sugar.

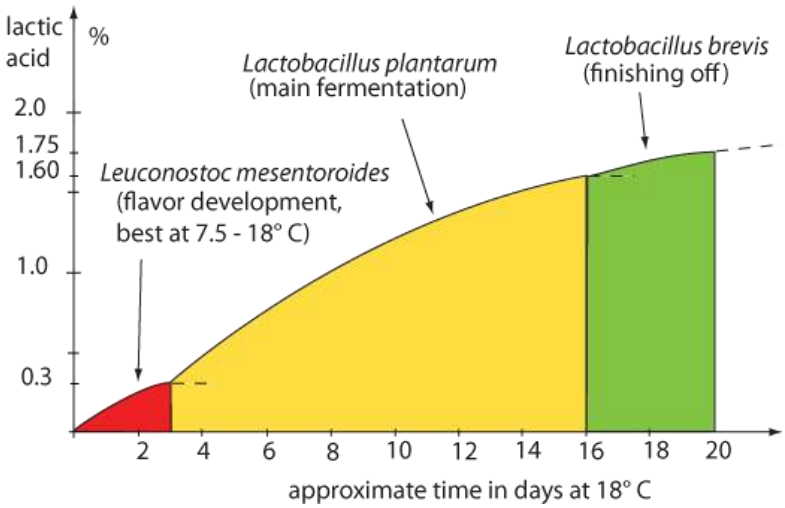

Figure 1. The microbial succession during sauerkraut fermentation (from https://www.meatsandsausages.com/fermenting-pickling/sauerkraut/fermentation).

Homemade sauerkraut undergoes natural lacto-fermentation that transforms shredded cabbage into a living probiotic food. As the head-space oxygen is consumed, beneficial lactic acid bacteria flourish, producing a tangy environment that preserves the vegetables and delivers a spectrum of viable microorganisms straight to the gut. These live cultures help maintain microbiota balance, supporting digestion and immune health in ways that pasteurized commercial sauerkraut simply cannot match. By avoiding heat treatment, home-fermented sauerkraut also retains a wealth of bioactive compounds. The fermenting bacteria break down cabbage cell walls, boosting the bioavailability of vitamins like B12 and K2 and concentrating antioxidants that combat oxidative stress. Finally, it stands out as an affordable, sustainable functional food, rooted in tradition yet perfectly aligned with modern needs for gut-friendly, culturally meaningful nutrition.

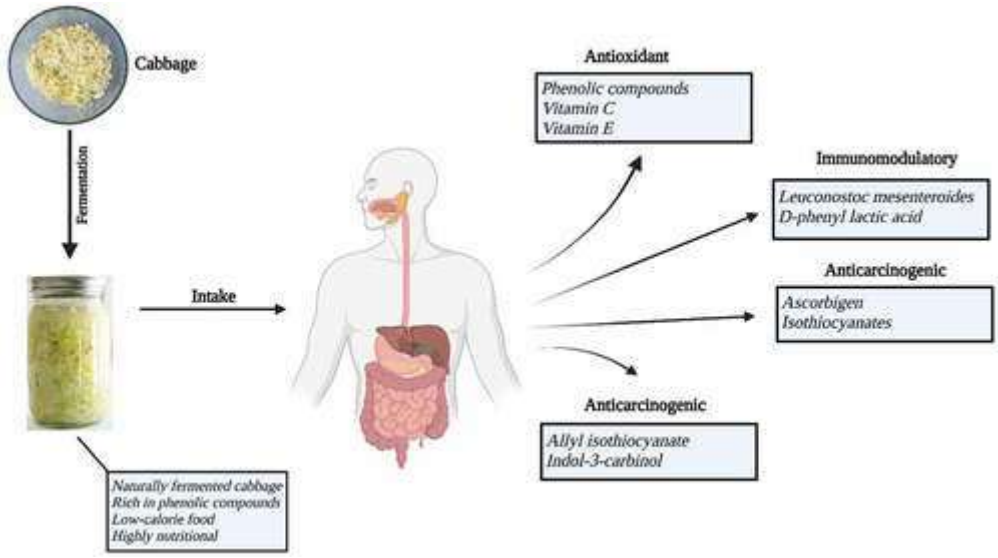

Figure 2. Health benefits of consuming sauerkraut (from Siddeeg et al., 2022).

Research has highlighted the ability of bacteria to produce biosurfactants, particularly glycolipids and lipopeptides, which exhibit emulsifying, antimicrobial, and antiadhesive

properties. These biosurfactants are biodegradable, non-toxic, and stable under a wide range of environmental conditions, making them attractive alternatives to synthetic surfactants in food, pharmaceutical, and environmental sectors (Sarubbo et al., 2015). The multifunctionality of biosurfactants extends to food emulsification, pathogen inhibition, and biofilm disruption, with implications for food safety and shelf-life enhancement. In agriculture, biosurfactants facilitate nutrient solubilization and biocontrol, while in pharmaceuticals, they serve as drug delivery agents and antimicrobial coatings. The organisms most frequently identified as biosurfactant makers are *Pseudomonas* sp., *Acinetobacter* sp., *Bacillus* sp., and *Arthrobacter* sp. However, the usage of these substances is limited and unsuitable for use in the food industry because of the infectious nature of the generating organisms (Hajfarajollah et al., 2014).

Recently, scientists have been trying to introduce an additional classification of biosurfactants, namely probiotic biosurfactants, which are produced by probiotic bacteria, so probiotic bacteria that produce biosurfactants are gradually moving towards the center of attention in the scientific community.

Table 1. Microorganisms considered as probiotics (from Hajfarajollah et al., 2018).

Lactobacillus Species		**Bifidobacterium Species**	**Other lactic acid bacteria**	**Non lactic acid bacteria**
L. acidophilus	*L. paracasei**	*B. adolescentis*	*Enterococcus faecalis*	*Propionibacterium freudenreichii**
L. amylovorus	*L. plantarum**	*B. animalis*	*Enterococcus faecium*	*Bacillus cereus var. toyoi*
L. crispatus	*L. rhamnosus*	*B. bifidum*	*Leuconstoc mesenteroies*	*Escherichia coli strain nissle*
L. delbrueckii	*L. bulgaricus**	*B. infantis*	*Pediococcus acidilactici*	*Saccharomyces cerevisiae*
L. gallinarum	*L. fermentum**	*B. longum*	*Sporolactobacillus inulinu*	*Saccharomyces boulardii*
L. johnsonii	*L. helveticus*	*B. essencis*	*Streptococcus thermophilus**	*Bacillus lactis*
L. gasseri	*L. reuteri*	*B. breve*	*Lactococcus lactis**	
L. casei		*B. lactis*		

* Reported as biosurfactant producer

Their production from probiotic strains isolated from fermented cabbage aligns with sustainable development goals by utilizing low-cost substrates and reducing reliance on chemical surfactants. This study aims to examine the biosurfactant-producing ability of five bacterial isolates isolated from naturally fermented cabbage, using a combination of qualitative and quantitative assays.

MATERIAL AND METHODS

Sample Collection and Bacterial Isolation

Naturally fermented cabbage samples were collected under sterile conditions from homemade preparations. Approximately 10 g of each sample was homogenized in 90 mL of sterile saline solution (0.85% NaCl) and serially diluted. Aliquots (100 µL) from appropriate dilutions were plated on de Man, Rogosa and Sharpe (MRS) agar and incubated anaerobically at 30°C for 48–72 hours. Distinct colonies were selected based on morphology and purified by repeated streaking.

Preliminary Screening for Biosurfactant Production

Five bacterial isolates were selected for biosurfactant screening using a combination of qualitative assays:

1. Oil Spread Method

This assay was performed to assess oil displacement activity. A Petri dish was filled with 10 mL of distilled water, and 10 µL of motor oil was added to form a thin layer. Then, 10 µL of cell-free supernatant (obtained by centrifuging cultures at 6000 rpm for 10 min) was gently placed at the center of the oil layer. The diameter of the clear zone formed was measured after 1 minute. A larger zone indicated higher biosurfactant activity (Nayarisseri et al. 2018).

2. Parafilm M Test

To evaluate surface activity on hydrophobic surfaces, 100 µL of cell-free supernatant was dropped onto a strip of Parafilm M. The shape and spread of the droplet were observed after 1 minute. Flattening or spreading of the droplet indicated the presence of surface-active compounds. Distilled water and uninoculated medium served as negative controls (El-Sahed et al., 2020).

3. Drop Collapse Test

A modified drop collapse assay was conducted using a 96-well microtiter plate. Wells were coated with 5 µL of motor oil and allowed to equilibrate for 24 hours. Then, 2 µL of cell-free supernatant was added to the center of the oil-coated wells. Drop shape was observed after 1 minute. Collapse or spreading of the drop indicated positive biosurfactant activity. Controls included SDS (positive) and distilled water (negative) (Nayarisseri et al. 2018).

Quantitative Assessment

Isolates showing positive results in all three qualitative assays were subjected to further quantitative analysis with determination of emulsification index (E24) (Eldin et al., 2019). These results were used to rank biosurfactant-producing potential.

Sensory profile of homemade sauerkraut

Sensory profiling of fermented vegetables is essential for linking fermentation dynamics to consumer perception. Homemade sauerkraut presents a complex interplay of taste, aroma and texture attributes that shift as lactic acid bacteria convert cabbage sugars into organic acids and volatile compounds. Capturing these organoleptic changes through structured evaluation helps to elucidate how fermentation time, salt concentration and cabbage variety influence overall quality. A thorough sensory assessment lays the groundwork for refining home-fermentation protocols and enhancing product consistency.

RESULTS AND DISSCUSION

Biosurfactant Activity Screening

Fermented cabbage, commonly consumed as sauerkraut, has emerged as a promising source of probiotic strains with biosurfactant-producing capabilities. Biosurfactants are amphiphilic compounds synthesized by microbes that reduce surface and interfacial tension, offering diverse applications in biotechnology, agriculture, and food systems. Fermented cabbage harbors a rich microbial ecosystem dominated by LAB such as *Lactobacillus plantarum*, *Lactobacillus brevis*, and *Leuconostoc mesenteroides*. These strains have demonstrated resilience under gastrointestinal conditions and possess probiotic traits including acid and bile tolerance, antimicrobial activity, and adhesion to intestinal epithelium. Studies by Zielińska et al. (2014) and Touret et al. (2018) confirmed the isolation of LAB strains from sauerkraut with high survival rates under low pH and bile salt conditions, suggesting their potential for probiotic applications.

All five bacterial isolates obtained from naturally fermented cabbage tested positive in qualitative biosurfactant assays, including the oil spread, Parafilm M, and drop collapse tests. These results confirm their ability to reduce surface tension and interact with hydrophobic substrates—key traits of biosurfactant producers (Gurkok and Ozdal, 2023; Vandana and Singh, 2018). Ghume et al. (2021) demonstrated biosurfactant activity in bacteria isolated from Indian fermented foods using drop collapse and oil spreading assays, identifying strains with bile salt hydrolase (BSH) activity and non-hemolytic profiles.

Biochemical Characterization and Probiotic Potential

All isolates were urease-positive**,** and negative for methyl red and Voges-Proskauer tests**,** indicating a consistent biochemical profile. These traits align with non-pathogenic, acid-tolerant LAB typically found in fermented vegetables (Khagwal et al., 2019; Marco and Wei, 2025). The absence of mixed acid fermentation and acetoin production suggests metabolic stability and safety, reinforcing their probiotic potential.

Emulsification Index (E24) Assessment

Quantitative evaluation using the emulsification activity test revealed variable but promising biosurfactant production across isolates. After 48 hours of incubation, isolate 2.18.1 exhibited the highest emulsification index (40%), followed by 2.4.2 (12%), 3.5.1 (8%), 2.4.1 (6.6%), and 2.2.1 (4%). Interestingly, all isolates showed E24 values above 40% at the 24-hour mark**,** suggesting a potential peak in biosurfactant production or stability at earlier time points—a phenomenon consistent with previous findings in LAB-derived biosurfactants (Kayath et al., 2016; Amaro da Silva et al., 2020).

Table 2. Emulsification Index (E24%) of Isolates at 24 h and 48 h.

Isolate ID	E24 (%) at 24 h	E24 (%) at 48 h
2.18.1	42.5	40.0
2.4.2	41.2	12.0
3.5.1	40.8	8.0
2.4.1	40.3	6.6
2.2.1	40.1	4.0

Note: Values represent mean of triplicate experiments. Standard deviation < ±2%.

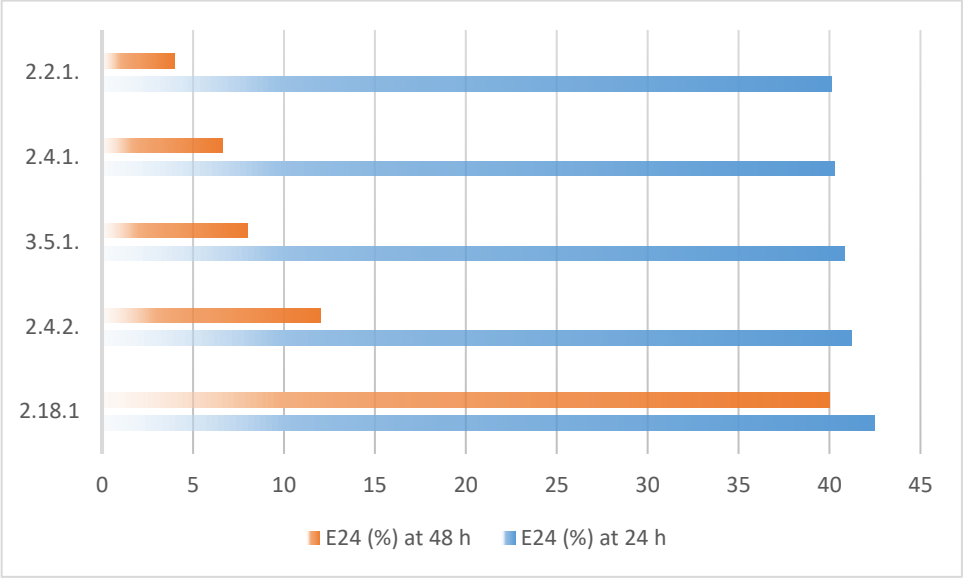

Figure 3. Temporal dynamics of emulsification index: isolate performance and stability at 24 vs. 48 hours.

A bar graph on Figure 2 illustrating E24 values at 24 h and 48 h for each isolate would clearly depict the decline in emulsification activity over time, highlighting isolate 2.18.1's sustained performance. This trend may reflect biosurfactant degradation, reabsorption, or metabolic shifts in stationary phase (Ozdal et al., 2021).

The rate of decline in emulsification index between 24h and 48h for each isolate shows the biosurfactant stability. Decline Rate is the absolute loss in emulsification index over the 24 h period. Isolate 2.18.1 shows the smallest decline (2.5 % points/day), while 2.2.1 loses activity most rapidly (36.1 % points/day).

Sensory profile of homemade sauerkraut

In this section, descriptive analysis and a nine-point hedonic scale were applied to key attributes—sourness, saltiness, umami, aroma intensity and crispness—at three fermentation milestones (early, mid and late). Tracking these sensory shifts not only identifies the optimal consumption window but also informs the design of consumer-driven quality benchmarks. Ultimately, this approach bridges traditional practices with scientific rigor to optimize both flavor development and consumer appeal.

Table 3. Nine-point hedonic scale at three fermentation milestones (early, mid and late) of homemade sauerkraut and sensory change rates in homemade sauerkraut.

Attribute	**Early (3 days)**	**Mid (7 days)**	**Late (14 days)**	**Δ Score**	**Change rate (units/day) = (score at 14th day–score at 3rd day) / 11 days**
Sourness	5	7	8	+3	+0.27
Saltiness	6	7	8	+2	+0.18
Aroma Intensity	5	7	8	+3	+0.27
Umami	4	6	7	+3	+0.27
Crispness	7	6	5	–2	–0.18
Overall Liking	6	7	6	0	0.00

The sensory trajectory of homemade sauerkraut reveals marked shifts in organoleptic attributes as fermentation advances. Sourness rose from a middling score of 5 at day 3 to a pronounced 8 by day 14, mirroring the cumulative production of lactic acid by *Lactobacillus* spp. and decline in pH (Johanningsmeier and McFeeters, 2006). Saltiness followed a parallel upward trend (6 → 8), reflecting progressive diffusion and concentration of brine, while aroma intensity climbed from 5 to 8, indicating escalating levels of volatile esters and organic acids that enrich the fragrance profile (Ghosh, 2021; Major et al., 2022).

Umami perception increased steadily (4 → 7), suggesting gradual breakdown of cabbage proteins into free amino acids and small peptides, a phenomenon previously linked to enhanced nutritional and flavor quality in fermented vegetables (Major et al., 2022). Conversely, crispness declined from 7 to 5, as cell wall pectins and hemicelluloses degrade under enzymatic and acidic conditions (Ciska et al., 2000). This textural softening underscores the trade-off between microbial activity and structural integrity over extended fermentation. Overall liking peaked at day 7 with a score of 7 before dipping slightly to 6 by day 14. This mid-fermentation zenith aligns with consumer studies showing optimal acceptance when sourness, saltiness, and aroma achieve balance without excessive acidity or loss of crunch (Fadhil et al., 2020). The plateau in liking despite continued acidification suggests an ideal consumption window around one week, beyond which textural losses may outweigh flavor gains.

Collectively, these sensory dynamics affirm that controlling fermentation duration is key to tailoring homemade sauerkraut quality. Early stages favor crisp texture and mild tang, mid stages maximize consumer appeal through flavor–texture equilibrium, and late stages intensify sour notes at the expense of bite. Future work should correlate these sensory patterns with microbial succession and metabolic profiles to refine guidelines for both home fermenters and artisanal producers.

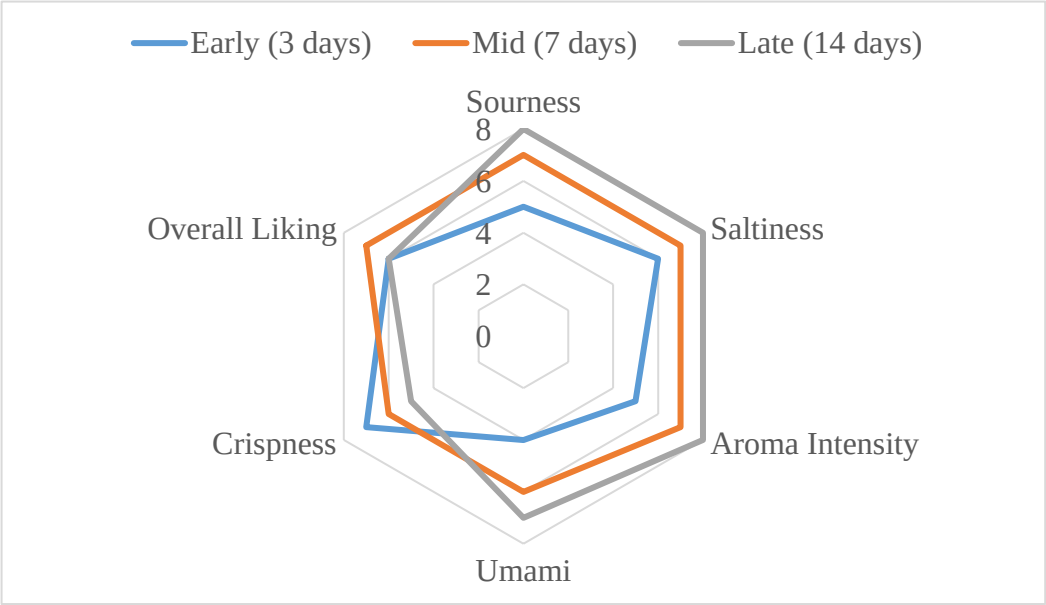

Figure 4. Nine-point hedonic scale at three fermentation milestones (early, mid and late) of homemade sauerkraut.

The calculated change rates across fermentation time offer insight into the dynamic sensory evolution of homemade sauerkraut and help pinpoint which attributes develop rapidly versus those that remain static or decline (Table 3). Sourness, Aroma Intensity, and Umami each show the highest change rate of +0.27 units/day, highlighting their rapid development during fermentation. These increases mirror microbial activity, especially by *Lactobacillus plantarum* and *Leuconostoc mesenteroides*, which drive lactic acid production and amino acid release—key contributors to both sourness and umami intensity. Rising aroma also reflects growing concentrations of volatile organic compounds generated by heterofermentative pathways in mid-late fermentation (Johanningsmeier and McFeeters, 2006; Major et al., 2022). Saltiness, with a change rate of +0.18 units/day, increases moderately. This likely reflects osmotic redistribution as salt diffuses deeper into cabbage tissues and the brine becomes more concentrated due to water release and microbial metabolism. This slower rate suggests saltiness stabilizes earlier than aroma or acidity. Crispness stands out with a negative change rate of –0.18 units/day, indicating steady textural softening. The decline stems from pectin breakdown and increased microbial enzymatic activity, especially by acidophilic lactobacilli, which compromise plant cell wall integrity (Ciska et al., 2000). This confirms the common sensory trade-off in fermented vegetables: flavor improves while texture diminishes. Overall Liking shows no net change (0.00 units/day)**,** despite fluctuations at individual time points. Liking peaked mid-fermentation (day 7), when flavor maturity and residual crispness coexisted. However, by day 14, the loss of texture may have offset further aroma and sourness gains, plateauing consumer satisfaction (Fadhil et al., 2020).

These change rates offer practical insight: flavor attributes such as sourness and umami progress swiftly and predictably, while texture degradation lags behind but steadily erodes acceptability beyond a certain threshold. The 7-day mark may represent an optimal sensory balance for homemade sauerkraut—where the benefits of active fermentation align with peak palatability.

CONCLUSION

This study demonstrates that naturally fermented cabbage is a valuable source of probiotic lactic acid bacteria capable of producing health-promoting biosurfactants. Five isolates—predominantly *Lactobacillus plantarum*, *L. brevis*, and *Leuconostoc mesenteroides*—not only exhibited strong acid and bile tolerance but also passed multiple qualitative biosurfactant assays, confirming their ability to reduce surface tension and interact with hydrophobic substrates. Biochemical profiling further reinforced their safety and metabolic stability, aligning with traits expected of food-grade probiotics.

Quantitative emulsification tests revealed that all isolates achieve peak activity within the first 24 hours, with isolate 2.18.1 maintaining the highest E24 value (40%) and the slowest decline rate (2.5 percentage points/day) over the subsequent 24 hours. These findings highlight both the potency and relative stability of LAB-derived biosurfactants from fermented cabbage, suggesting their suitability for applications where sustained emulsification is critical.

By integrating sensory profiling of the sauerkraut matrix—tracking rapid increases in sourness, aroma, and umami alongside texture softening—this work bridges microbial functionality with consumer perception. The mid-fermentation stage emerged as the optimal balance point, offering insights for timing bioproduct harvests to maximize both microbial yield and product quality.

Collectively, these results underscore the dual potential of probiotic LAB from fermented cabbage—as gut-health agents and as sustainable producers of food-grade biosurfactants. Future efforts should focus on optimizing fermentation parameters, scaling up biosurfactant recovery, and characterizing molecular structures to unlock targeted applications in food stabilization, agriculture, and biopharmaceutical formulations.

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HEAT AND COLD STRESS IN WATER BUFFALOES (BUBALUS BUBALIS): A REVIEW

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ABSTRACT

The direct effect of global warming is climate change, and increases in temperature and drought severity are observed due to climate change. This situation increases the negative effects of heat stress on buffaloes rather than cold stress. Although buffaloes have developed a series of adaptation mechanisms against heat and cold stress, their thick skin and low density of hair, as well as their low number of sweat glands, reduce their tolerance to heat stress. This may have a detrimental effect on the welfare and comfort of buffalos, as well as their milk yield, reproductive efficiency and health status, and may also have negative consequences for sustainable production. Therefore, it is important to understand the anatomical, physiological and behavioral responses of buffaloes to heat and cold stress and to discuss production losses in order to reduce the negative effects of global warming and to maintain sustainability. This review aims to understand the responses of buffalos to heat and cold stress and to discuss care, feeding and management strategies that can reduce the effects of heat stress. Due to the effects of global warming, water buffalo grazing grounds, wetlands, and ponds are being negatively impacted by drought. Therefore, systems such as shade, fans and showers should be integrated into the shelters to meet the cooling needs of the buffaloes. Furthermore, in hot regions, a focus on nutritional management should be placed on reducing the severity of heat stress. In the long term, water buffalo resistant to heat stress should be selected.

Keywords: Water buffaloes, Heat stress, Productivity, Health, Physiology, Behavior

INTRODUCTION

It is expected that global warming will increase temperatures by 3.5–5.5 °C in the 21st century (Napolitano et al., 2023), resulting in higher temperatures and more severe droughts (Nardone et al., 2010). This situation poses a significant challenge for water buffaloes (Bubalus bubalis) and their farms, as it does for other species of animal (Napolitano et al., 2023). For this reason, many researchers agree that the effects of heat stress on animals are more controversial than those of cold stress (Cheng et al., 2022; Okuyucu et al., 2023). Climate change resulting from global warming can negatively affect the welfare, productivity, and health of water buffaloes (hereafter buffalo, unless otherwise stated).

Buffaloes are a resilient farm animal that plays a vital role in agricultural economies worldwide, primarily raised for milk, meat, and skin production (Hussain et al., 2023). These animals, which evolved through natural selection, are able to adapt to a variety of challenging environmental conditions (Yáñez-Pizaña, et al., 2020). However, their method of dissipating heat is anatomically and physiologically different from cattle (Bos taurus and indicus). Briefly, buffaloes have i) fewer sweat glands, ii) thicker and darker skin, iii) lower hair density and iv) lower respiratory rate compared to cattle. These physiological and anatomical differences cause

buffalo to exhibit relatively less effective respiratory evaporative cooling compared to cattle (Marai and Haeeb 2010a). Therefore, buffalo naturally exhibit cooling behaviors such as wallowing in wet environments to alleviate heat stress. However, under intensive farming conditions, buffaloes are constantly confined to barns. This can increase indoor temperatures during warmer seasons and result in heat stress. Furthermore, the decline in lakes, rivers, and ponds in rural areas caused by drought also limits opportunities for wallowing for pasture-raised buffalo. These developments may make buffalo increasingly vulnerable to future increases in temperature and drought, exposing them to more severe heat stress. This could result in significant welfare issues, milk yield losses, and reduced reproductive performance for buffaloes (Hussain et al., 2023). Therefore, i) understanding the physical, behavioral and physiological responses of buffaloes to heat and cold stress, and ii) discussing the impact levels of these stress sources on animal welfare and productivity will contribute to the development of applicable care, nutrition and herd management strategies.

To the best of our knowledge, while there is a wealth of information on heat and cold stress in cattle, information on this topic in buffalos is limited. Addressing this knowledge gap is expected to significantly contribute to the existing scientific literature. The present review aimed to i) investigate adaptive traits of buffalo to heat, ii) investigate the physiological, physical and behavioral responses of buffalos to heat and cold stress, iii) discuss the effects of heat and cold stress on the welfare, milk yield, reproductive performance and health of buffaloes and iv) evaluate applicable care, feeding, structural and management strategies.

SEASONAL CONDITIONS AND HEAT/COLD STRESS

The effects of environmental conditions on buffaloes are complex. Climatic factors such as temperature, humidity, air movement (wind), and solar radiation are the most important factors determining heat exchange between the animals' bodies and the environment (Okuyucu et al., 2023). Buffaloes are most productive in a range of temperatures considered the thermal neutral zone. Therefore, buffalo should be at optimum temperature, humidity, and wind speed (temperature 13-18°C, humidity 55-65%, wind speed 5-8 km/h, and moderate solar radiation; Marai and Haeeb 2010a). When buffaloes are exposed to conditions outside the thermal neutral zone or if the ambient temperature reaches more extreme limits, it creates a significant stress load on them. Because climatic data such as solar radiation, wind speed, and precipitation are not always readily available when assessing heat stress in buffalos, the temperature-humidity index (THI) is commonly used. This index combines air temperature and humidity to provide information about animal comfort (Petrocchi Jasinski et al., 2023). Information on this topic is limited because few studies have documented optimal THI values for buffalos. However, a few studies have reported optimal and extreme values for buffaloes (Table 1; Choudhary and Sirohi, 2019; Petrocchi Jasinski et al., 2023). In cases where the THI value exceeds 72 in buffaloes, a partial decrease in milk yield may be observed (Table 1).

Table 1. THI values and stress levels

THI[1]	**Description**
< 72	Optimum
72-79	Mild heat stress
80-89	Moderate heat stress
90	Severe heat stress

[1]THI: temperature-humidity index

ADAPTIVE TRAITS OF BUFFALOES TO HEAT/COLD

The primary function of thermoregulation is to maintain body temperature within a specific range where cellular functionality can be maintained (Marai and Haeeb 2010a). Therefore, buffaloes have evolved several physiological, endocrine, and behavioral adaptations that support thermoneutrality. Several morphological and physiological traits that control thermoregulatory mechanisms differ between buffaloes and cattle (*Bos taurus* and i*ndicus*). Anatomical features such as epidermis colour and thickness, hair density and number of sweat glands differ between buffaloes and cattle.

It is well known that buffalo skin and hair have a high concentration of melanin in their basal cells (Garcia et al., 2023). This gives them an advantage in protecting themselves from ultraviolet radiation. However, dark skin and hair absorb higher concentrations of radiation, which makes them vulnerable to heat stress (Napolitano et al., 2023). Another anatomical adaptation of buffalo is their thicker skin compared to cattle (Marai and Haeeb 2010b). This serves as a thermal insulator against hot weather. Furthermore, their skin is covered with numerous capillaries that facilitate heat dissipation through pathways such as conduction, radiation and convection. Also, buffaloes have less sweat glands than cattle (Marai and Haeeb 2010a). Numerous authors have reported that the morphological and anatomical differences mentioned above cause buffalos to be adversely affected by solar radiation when exposed to direct sunlight (Marai and Haeeb 2010b; Napolitano et al., 2023).

Buffaloes have lower physiological adaptations to extreme heat or cold than cattle. Buffaloes have a lower body temperature and respiratory rate than cattle (Marai and Haeeb 2010a). The physiological and behavioral responses of buffalos to heat and cold stress are summarized in Figure 1.

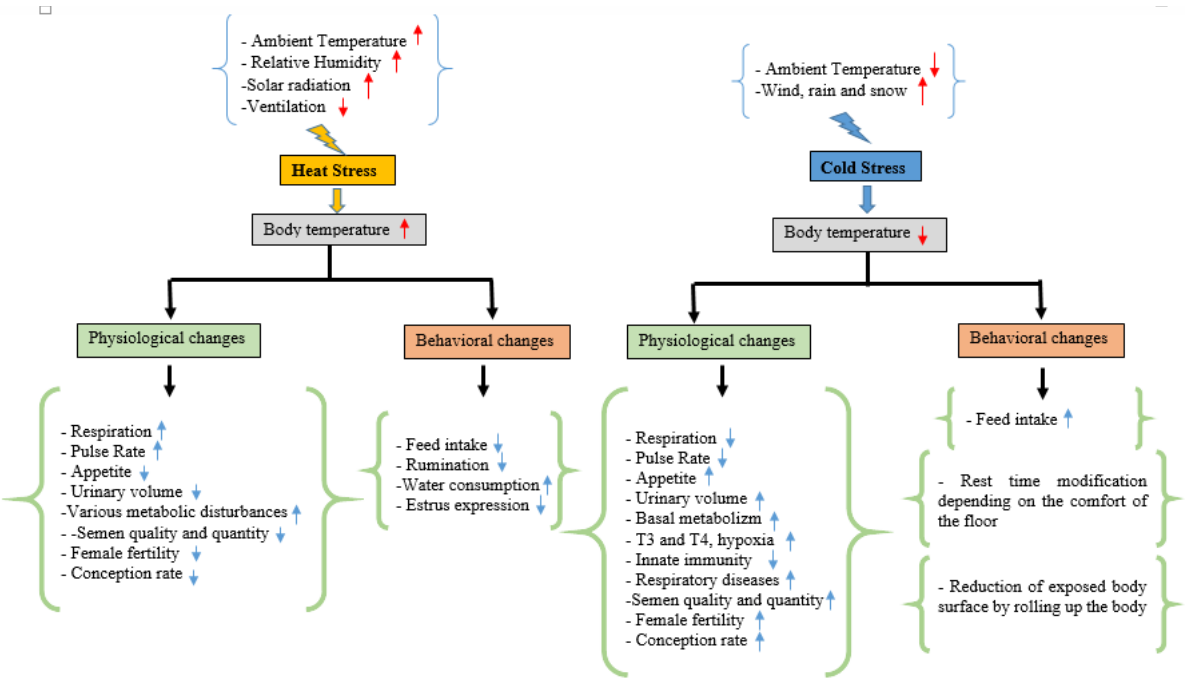

Figure 1. Physiological and behavioral responses of buffaloes to heat and cold stress. Adapted from Marai and Haeeb (2010b), Napolitano et al. (2023) and Belsare and Pandey (2008)

EFFECTS OF HEAT/COLD STRESS ON BUFFALOES

Since the effects of heat stress on buffaloes are more severe than cold stress, this section focuses mostly on the results of studies examining the relationships between heat stress and milk production, milk composition, reproductive performance and animal welfare in buffaloes.

In the study conducted on Mediterranean Italian (de Rosa et al., 2009) and Murrah (Upadhyay et al., 2007; Pawar, et al., 2012; Pawar, et al., 2013) buffaloes, it was reported that heat stress negatively affected the milk yield of buffaloes. Also, some studies reported that heat stress reduced milk fat content of Mediterranean Italian (Pasquini et al., 2018; Salari et al., 2013; Matera et al., 2022) and Murrah buffaloes (Pawar, et al., 2012). Similarly, previous studies on Mediterranean Italian (Salari et al., 2013; de Rosa et al., 2009; Matera et al., 2022) emphasized that heat stress reduces milk protein content in buffaloes. In a previous study on Mediterranean Italian (Matera et al., 2022), SCC and EC values increased as heat stress increased.

Heat stress can also affect the reproductive efficiency of buffaloes. Studies on Murrah buffaloes have shown that heat stress reduces pregnancy rates (Upadhyay et al., 2007) and estrus expression (Dash et al., 2015a; Dash et al., 2015b). Additionally, Khairy et al. (2007) reported that heat stress reduced oocyte quality in Egyptian buffalo cows.

STRATEGIES FOR REDUCING HEAT STRESS

Heat stress places a significant burden on the metabolism of buffalos (carbohydrate and protein metabolisms). Therefore, steps to mitigate the effects of heat stress should be addressed in two stages: improving environmental conditions and improving genotypic structure. While strategies to improve environmental conditions yield quicker results, improving genotypic structure is a time-consuming process. Therefore, priority should be given to improving environmental conditions and feeding management, while also prioritizing marker-assisted selection practices for heat- and humidity-tolerant animals to improve genotypic structure.
The impact of global warming on the thermoregulation mechanisms of buffalo means that wallowing areas, shaded areas, sprinklers, fans and ponds are required for those raised in extensive or intensive farming conditions during the hot seasons. Several researchers have highlighted the positive effects of cooling on various welfare traits of buffaloes in hot regions (Hoque et al., 2018; Petrocchi Jasinski et al., 2023). A study conducted on Nili-Ravi buffaloes found that dry matter intake, milk production and the contents of fat, protein and lactose were higher in cows cooled with shade, fans and showers than in cows cooled with shade alone. (Ahmad et al., 2018). Several authors found that in Mediterranean Italian buffaloes, cows with a swimming pool had a shorter calving-to-conception interval than those without a swimming pool (Neglia et al., 2009). The results of previous studies show that shade, shower and sprinklers can improve milk production and reproductive performance of cows in buffaloes. Also several authors have reported that wallowing in buffaloes can reduce body heat load and prevent increases in clinical parameters (Petrocchi Jasinski et al., 2023). They also emphasized that wallowing can improve the feeding and social behavior of buffaloes.

With some feeding manipulations, negative effects can be reduced during periods when buffaloes are exposed to heat stress. In a study in Mediterranean Italian buffaloes (Evangelista, et al., 2022), it was reported that Zn and Se supplementation improved milk yield. In addition, another study reported that an improvement in pregnancy rates was observed in buffaloes given vitamin E + sodium selenite supplements during the summer season (Megahed et al., 2008). In summary, combining dietary antioxidants may reduce the negative effects of heat stress on buffaloes (Petrocchi Jasinski et al., 2023). However, further studies on this topic are needed.

CONCLUSIONS

Buffaloes have lower resistance to heat stress compared to cattle due to differences in their anatomical (thicker, darker skin and lower hair density) and physiological (fewer sweat glands) traits. Therefore, it is clear that they will be more affected by heat stress than cold stress as temperature increases and drought severity increases, which are the main effects of global warming. Also, the increase in drought severity, a result of global warming, also negatively affects wallowing areas and wetlands. As a result, management strategies such as cooling, shading and air conditioning should be emphasized to improve the environmental factors of buffaloes. Strategies to reduce heat stress in buffalo during hot periods through nutritional manipulation should be identified. However, studies evaluating the relationship between heat stress and nutritional manipulation in buffaloes are limited. Further research on this topic is needed. In addition, long-term strategies should primarily focus on identifying heat stress-resistant buffalos at the genomic level and developing heat stress-resistant genotypes of animals through selection and controlled crossbreeding practices.

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AN OBSERVATION ON AN ANOMALY IN THE SCE SETA DISRUPTING BILATERAL SYMMETRY IN *TYCHEROBIUS IZMIRENSIS* AKYOL & KOÇ (ACARIFORMES: CAMEROBIIDAE)

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ABSTRACT

This study reports the first morphological variation observed in the *sce* seta on the idiosoma of *Tycherobius izmirensis* (Acariformes: Camerobiidae), a mite species known from Türkiye. Among the 90 examined female specimens of the species, the anomaly was detected in only one specimen, in which the right *sce* was found to be shorter and structurally simpler than that of the one on the left side. This variation is considered as a rare structural anomaly that disrupts the species' bilateral symmetry. It is evaluated that this observed morphological deviation may have resulted from a random developmental disturbance and could be included within the category of *fluctuating asymmetry*.

Keywords: Acari, anomaly, asymmetry, dorsal seta, morphology, Raphignathoidea

INTRODUCTION

Members of the family Camerobiidae Southcott are typically found in very low numbers within their habitats (Bolland, 1986; Paredes-León et al., 2016; Zmudzinski, 2020; Mirza et al., 2022). These mites can be readily distinguished from other members of the superfamily Raphignathoidea by their slender and elongated legs. Idiosoma of these mites is oval or nearly circular, gnathosoma is typically located beneath prodorsum, the basal segments of chelicerae are fused to form stylophore, peritremes are positioned either medially or laterally on stylophore, palps are short, and palp tibial claw is sword-shaped (Southcott, 1957; Fan and Zhang, 2005; Mirza et al., 2022).

Camerobiidae, in terms of species richness, represents the second largest family within Raphignathoidea (Akyol and Koç, 2006; Fan and Walter, 2011; Khanjani et al., 2011, 2014; Ulucay et al., 2016; Akyol, 2020; Doğan and Fan, 2025). To date, the family comprises over 175 valid species within seven genera (Fan and Walter, 2006a,b, 2011; Beron, 2020, 2022; Mirza et al., 2022; Escobar-Garcia et al., 2023, 2024; Doğan and Fan, 2025). The genus *Tycherobius* Bolland currently includes 26 described species worldwide (Beron, 2020; Mirza et al., 2022). Of these, *Tycherobius izmirensis* Akyol & Koç is known only from Türkiye (Akyol and Koç, 2017; Doğan, 2019; Beron, 2020). This species is distinguished by the following characteristics: fourteen pairs of serrated and lanceolate setae arranged in a typically symmetrical pattern on the dorsal idiosoma, the absence of setae *pdx*, setae c_1 being the longest of the dorsal body setae, *sci*/*sce* ratio 2.27 and d_1/d_2 ratio 3.85, bifurcated peritreme, and the presence of four setae on femur I and two setae on femur IV (Akyol and Koç, 2017, 2020).

This study reports the first documented morphological variation in the *sce* seta on idiosoma of *Tycherobius izmirensis*. In addition to contributing new insights into the morphological diversity of *T. izmirensis*, the study serves as an important case report for identifying developmental variations in mites.

MATERIALS AND METHODS

The mite specimens were extracted using Berlese-Tullgren funnels from litter, soil and moss samples collected within the scope of a research project (121Z986) conducted in the Karasu Valley of the Munzur Mountains region. The specimens were cleared using a 60% lactic acid and permanent slides were prepared in Hoyer's medium for microscopic examination. The images were captured using a Leica DM 4000B phase contrast microscope equipped with an integrated digital camera (DFC450 C). The nomenclature of body setae follows the terminology proposed by Kethley (1990). Species identification was carried out based on morphological characteristics using relevant literature (Fan and Walter, 2006b; Akyol and Koç, 2017, 2020; Mirza et al., 2022).

RESULTS

During the examination of 90 female specimens of *Tycherobius izmirensis*, it was observed that the external scapular seta (*sce*) on the right side of one specimen was significantly shorter (15 µm) and structurally simpler than its counterpart on the left side (Fig. 1). The abnormal specimen was collected from litter under an oak tree, 39°12'13.4"N 38°34'28.7"E, the Karasu Valley, Türkiye, 1053 m a.s.l., 10 March 2021. The idiosomal measurements of the species ranged: length 295–328, width 230–270 µm. Setae *sci* 55–72, *sce* 30–47, c_1 189–225, c_2 56–65, d_1 166–197 and d_2 38–55 µm.

The present study constitutes the first report of a morphological variation in the *sce* seta of *T. izmirensis*.

DISCUSSION

Mites are morphologically bilaterally symmetrical organisms. This means that their bodies can be divided vertically into two similar halves (right and left) along the sagittal plane, giving them mirror image symmetry. This symmetry is regarded as a fundamental morphological characteristic of mites and many other animal groups, reflecting their evolutionary adaptations. Although mites exhibit this symmetry, some individuals within a population may deviate from this pattern and display various asymmetrical features. Such asymmetrical variations typically manifest as disruptions in the symmetrical arrangement of paired structures (Doğan and Ayyıldız, 2023).

In the present work, among the 90 examined female specimens of *Tycherobius izmirensis*, only one individual was found to exhibit a right-side *sce* seta that was shorter and structurally simpler than that left-side one (Fig. 1). Such structural asymmetrical variations, which disrupt bilateral symmetry, are considered morphological anomalies (Bingül et al., 2017; Doğan and Fan, 2025).

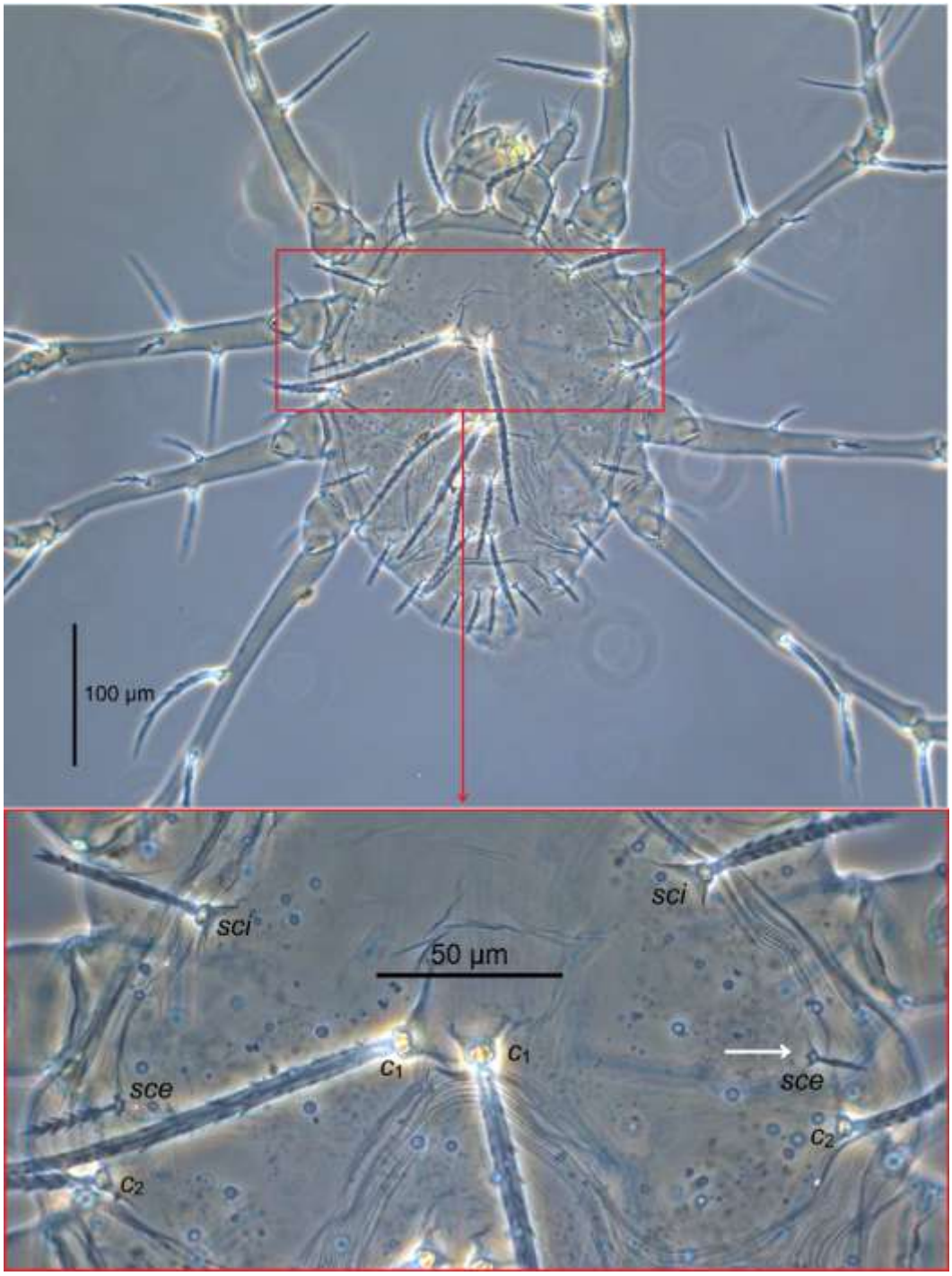

Figure 1. *Tycherobius izmirensis* (female). Variation of the right seta *sce* (indicated by an arrow).

Mites may exhibit variation in both the number and structural characteristics of their dorsal body setae (see Figure 2 for some illustrative examples). McGregor (1950), Bolland (1986), Fan and Walter (2006a), Khanjani et al. (2013, 2014), and Doğan and Fan (2025) have documented various variations in the number of dorsal setae in some species of the genera *Neophyllobius* and *Tycherobius* in Camerobiidae. Within these studies, it has been reported that *Neophyllobius ostovani* Khanjani & Ahmad Hoseini has a single (unpaired) *pdx* seta (Khanjani et al., 2014). In *N. yunusi* Akyol & Koç, one female was observed to have a duplex setae *pdx* on the left side, while a protonymph bore a single duplex setae *pdx* (Doğan and Fan, 2025). Only one *pdx* seta has been reported on the dorsal idiosoma in three species of *Tycherobius*: *T. virginiensis* (McGregor), *T. acicula* Fan & Walter and *T. emadi* Khanjani, Hajizadeh, Ahmad Hoseini & Jalili (McGregor, 1950; Bolland, 1986; Fan and Walter, 2006a; Khanjani et al., 2013).

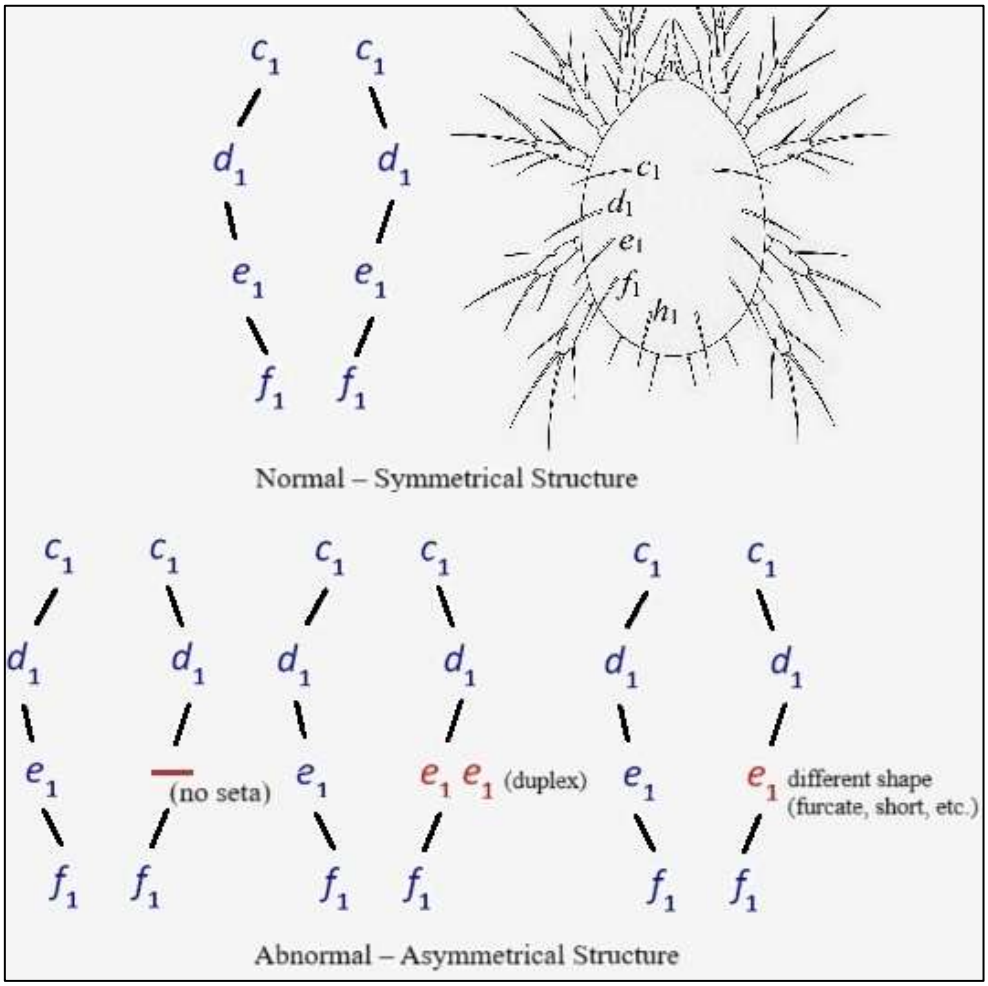

Figure 2. Representative illustration of several types of variation in dorsal body setae in mites.

To date, no morphological variation has been reported for *Tycherobius izmirensis*. However, a review of the literature reveals that similar asymmetrical structural abnormalities in dorsal setae have also been observed in several species belonging to the family Stigmaeidae. Such anomalies have been recorded in *Eustigmaeus absens* Doğan, *E. jiangxiensis* Hu, Chen & Huang, *E. segnis* (Koch), *Ledermuelleriopsis ayyildizi* Doğan, and *Stigmaeus erzincanus* Doğan, Bingül, Dilkaraoğlu & Fan (Bingül et al., 2017; Doğan and Doğan, 2024; Doğan, 2025a,b). In one male specimen of *E. absens*, the e_1 seta on the left side was structurally weaker

compared to the one on the right (Doğan, 2025b), while in a male of *E. jiangxiensis*, the c_1 seta on the right side was observed to be weaker than that of its left-side counterpart (Doğan, 2025a). In a female specimen of *E. segnis*, the e_1 seta on the right side was markedly shorter and structurally simpler than that of one on the left (Bingül et al., 2017). Similarly, in a female of *Ledermuelleriopsis ayyildizi*, the d_1 seta on the right side was considerably thinner than its counterpart on the left (Doğan and Doğan, 2024). In *Stigmaeus erzincanus*, the h_3 seta on the right side of a female was shorter and thinner than that of the left-side seta, while in a male, the e_1 seta on the left side was shorter than that of the corresponding right-side seta (Bingül et al., 2017).

The fact that the observation was limited to a single specimen that the variation does not represent a systematic trend at the population level such as *directional asymmetry* or *antisymmetry* characterized by a random distribution between left and right sides (Graham et al., 1993; Palmer, 2005, 2009; Bingül et al., 2017). Therefore, the observed morphological deviation is most likely a random fluctuation that arose during development due to genetic or environmental factors, and in this context, it may be classified as *fluctuating asymmetry*. Fluctuating asymmetry refers to minor, non-directional deviations from expected symmetry in morphological traits, resulting from developmental instability (Van Valen, 1962; Palmer and Strobeck, 1986; Møller and Swaddle, 1997; Tomkins and Kotiaho, 2001; Klingenberg, 2003a,b, 2015; Bingül et al., 2017).

The fact that the variation in *T. izmirensis* was observed as a distinct, unilateral structural abnormality in only a single individual suggests that it may represent a rare structural anomaly. Such rare structural anomalies manifest as unexpected morphological defects that occur infrequently within a population, typically as isolated cases, and therefore do not exhibit a tendency to recur at the population level. Rare structural anomaly is not recognized as a fourth distinct type of asymmetry alongside the classical categories of *fluctuating asymmetry*, *directional asymmetry* and *antisymmetry*. Rather than representing a separate category within the concept of asymmetry, rare structural anomalies should be considered within a broader conceptual framework. Conversely, the concept of asymmetry itself may be approached in a general context without being strictly divided into subcategories. The term “rare structural anomaly” used in this study has been adopted to refer to distinct structural defects that arise during development and occur infrequently.

CONCLUSION

Setae are known to serve a sensory function in the perception of environmental stimuli in mites (Woolley, 1988; Evans, 1992; Krantz and Walter, 2009; Doğan and Ayyıldız, 2023). The structural defect observed in one *sce* seta of *Tycherobius izmirensis* may cause a decrease in sensory sensitivity on the affected side. However, it is possible that the probable functional defect of this unilateral anomaly found in only one of the symmetrical setae can be compensated for by the normally structured seta on the other side. Therefore, the observed anomaly is unlikely to have a significant negative impact on the mite’s overall ability to adapt to environmental stimuli or its survival success. Consequently, the effect of this anomaly is generally considered to be limited or negligible.

The support of such structural variation observations through studies using larger samples or specimens will enable more precise inferences regarding the morphological diversity and developmental stability of *Tycherobius izmirensis*. Documenting these rare anomaly cases can contribute to the understanding of developmental mechanisms in mites, thereby providing a foundation for more in-depth investigations into the morphological characteristics and adaptive strategies of species.

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SPATIOTEMPORAL PATTERNS OF TRACE ELEMENTS (LI, MN, NI, BA) IN RIVERS FEEDING THE İSTANBUL STRAIT, TÜRKIYE

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ABSTRACT

Sediments in freshwater ecosystems are crucial reservoirs of both essential and toxic elements. While some elements are vital for life, prolonged or high-level exposure, much like with non-essential toxic elements, can be detrimental. The Istanbul Strait, a globally significant marine habitat in northwestern Türkiye, receives substantial input from numerous rivers and lakes. This study investigated the spatiotemporal variations and accumulation of lithium (Li), manganese (Mn), nickel (Ni), and barium (Ba) in the sediments of rivers flowing into the İstanbul Strait. It this research, sediment samples were collected from three key fluvial habitats (Göksu, Kağıthane and Alibeyköy streams) during the dry and wet seasons of 2022–2023. The findings of the study reveal that the average annual concentrations of these elements in the river sediments followed the order: Mn (542.585 ppm) > Li (33.905 ppm) > Ni (21.598 ppm) > Ba (0.425 ppm). Furthermore, significant seasonal differences were observed in trace element accumulations. Notably, average concentrations of Li, Mn, Ni, and Ba in the sediments increased approximately 11, 3, 1.5, and 2 times, respectively, during the wet season.

Keywords: İstanbul Strait, Riverine habitats, Sediment quality, Trace elements

INTRODUCTION

Manganese, an essential element, is crucial for the healthy growth and development of all living organisms. However, much like toxic elements such as lithium, nickel, and barium, an excess of even essential elements can negatively impact the environment. Therefore, maintaining trace elements at concentrations below harmful limits and ensuring a balanced presence of essential elements in aquatic habitats is paramount for environmental protection (Milne, 2000; Belitz et al., 2009; Köse et al., 2013; Çiçek et al., 2013; 2017; Tokatlı and Helvacıoğlu, 2020).

Freshwater sediments play a crucial and dual role in aquatic ecosystems. Not only do they act as transporters and reservoirs for numerous pollutants, but they also offer a rich archive of valuable environmental information (Tokatlı et al., 2014; Yüksel et al., 2024; Varol and Tokatlı, 2024). This makes their study indispensable for understanding the health and history of water bodies. Consequently, the analysis of elemental content in surface sediments is a vital practice for environmental monitoring and assessment (Varol et al., 2022; Ustaoğlu et al., 2022; Tokatlı and İslan, 2022). By examining these elemental signatures, researchers can gain insights into past and present anthropogenic impacts, natural geological processes, and the overall ecological status of freshwater habitats.

The Istanbul Strait stands as a critically important international marine habitat in northwestern Türkiye. Its extensive watershed, teeming with numerous rivers and lakes,

unfortunately also subjects this vital aquatic environment to significant pollution stress (Tokatlı et al., 2023; 2024; Varol and Tokatlı, 2024). In this study, the accumulation and spatiotemporal distribution of lithium (Li), manganese (Mn), nickel (Ni), and barium (Ba) were investigated in the sediments of the rivers that feed directly into the Istanbul Strait and the implications of these findings for ecosystem health in the region were evaluated.

MATERIALS AND METHODS

For this study, three fluvial ecosystems feeding the Istanbul Strait were selected (Figure 1). Surface sediment samples were then collected from the downstream locations of these rivers during both the wet season (end of winter) and dry season (end of summer) of 2022–2023. An Ekman Grab (Hydrobios) was used for sample collection, adhering to the standard protocol (EPA, 2001a).

To determine the concentrations of lithium (Li), manganese (Mn), nickel (Ni), and barium (Ba) in the sediment samples, each sample was first dried at 105 °C for 3 hours. For digestion, 0.25 g of each dried sample was placed into a CEM Mars Xpress microwave device. A mixture of acids, specifically perchloric acid (HClO4) and nitric acid (HNO3) in a 1:3 ratio, was added to the Pyrex reaction vessels. The samples then underwent mineralization by heating at 200 °C for 30 minutes. Following mineralization, the samples were filtered using a 0.45 µm cellulose nitrate filter. After filtration, ultrapure water was added to bring the final volume of each sample to 100 mL. The concentrations of these trace elements in the sediments were then precisely measured using an Agilent 7700 ICP-MS device. All measurements were performed in triplicate at the accredited (TS EN / ISO IEC 17025) central laboratory of Thrace University in Edirne, Türkiye (EPA, 1998; 2001b).

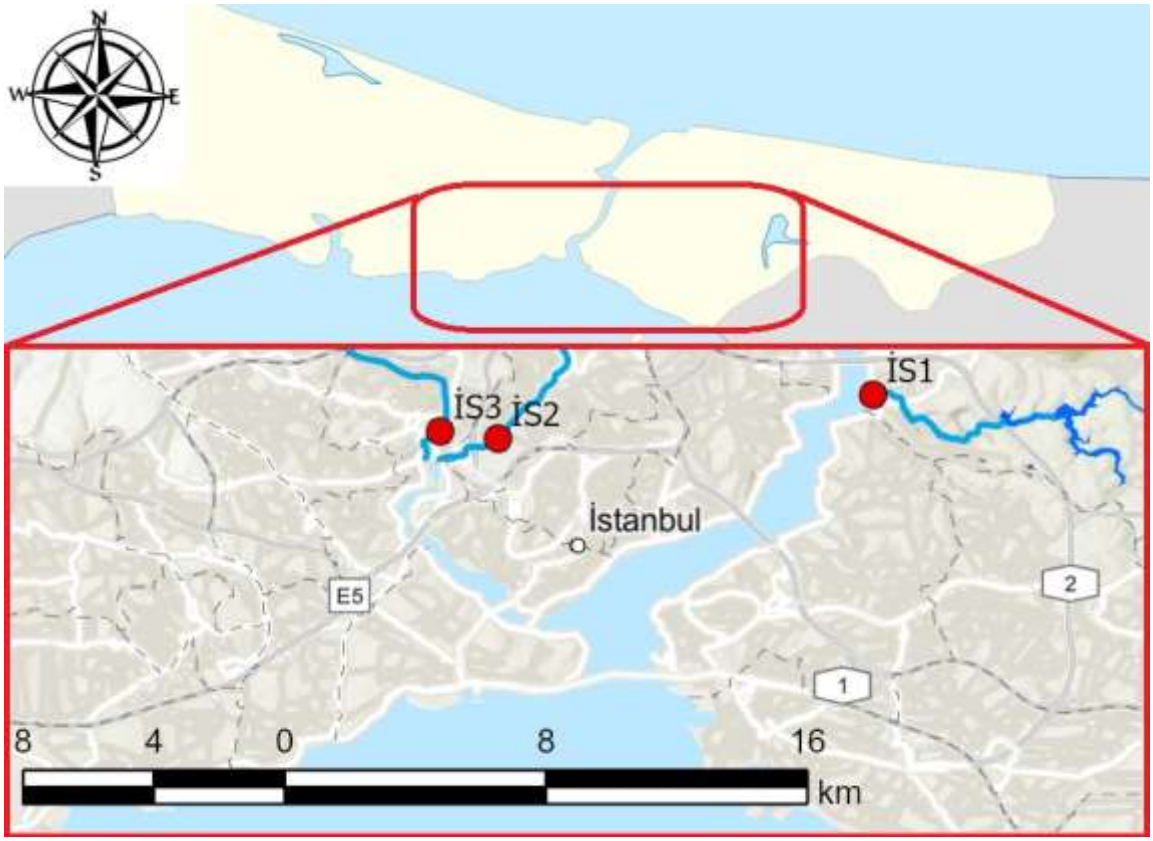

Figure 1. Study area and selected sampling locations

RESULTS AND DISCUSSION

Figure 2 presents the determined concentrations of lithium (Li), manganese (Mn), nickel (Ni), and barium (Ba) in the sediments collected from the designated sampling locations on the Göksu, Kağıthane, and Alibeyköy streams. These values illustrate the spatial distribution of the selected elements, offering valuable insights into the elemental composition of sediments within these fluvial water bodies during the sampling periods.

In the investigated streams, clear seasonal and spatial variations were observed in trace element concentrations. Lithium increased markedly during the wet season, reaching 112.159 ppm in Göksu Stream, compared to much lower values in the dry period (3.044–9.597 ppm). Manganese also exhibited a strong rise in the wet season, with the highest concentration measured in Göksu Stream (1248.808 ppm), whereas dry season levels remained between 234.523 and 359.089 ppm. Nickel showed site-dependent fluctuations, being higher in Kağıthane Stream during the dry season (25.156 ppm) and in Alibeyköy Stream during the wet season (35.766 ppm). Barium, on the other hand, remained at relatively low concentrations (0.183–0.411 ppm in the dry season), though it increased in the wet season, particularly in Alibeyköy Stream (0.764 ppm). These results indicate that wet season conditions, influenced by surface runoff, erosion, and anthropogenic inputs, considerably enhance the mobilization of certain elements, especially Li and Mn.

The pronounced seasonal increases in Li and Mn, particularly during the wet season, suggest that hydrological dynamics play a major role in the mobilization of these elements. Increased precipitation enhances surface runoff and leaching, resulting in the transport of metals from surrounding soils and anthropogenic sources into the streams. Elevated Ni and Ba concentrations in Alibeyköy and Kağıthane Streams further indicate the influence of urban and industrial discharges, which are well-documented contributors to trace metal pollution in densely populated catchments. Similar spatial and temporal patterns in trace metal accumulation have also been reported in previous studies conducted in Turkish aquatic ecosystems (Arslan et al., 2012; Tokatlı et al., 2020; Din et al., 2023; Ustaoğlu et al., 2025; Ali et al., 2025). Overall, the results highlight the combined effects of natural processes and anthropogenic pressures on metal dynamics in stream ecosystems.

CONCLUSIONS

This study demonstrated notable spatial and temporal variations in trace element concentrations in the sediments of Göksu, Kağıthane, and Alibeyköy Streams. Lithium and manganese showed remarkable increases during the wet season, suggesting that hydrological processes strongly influence their accumulation in sediments. Nickel and barium exhibited site-specific patterns, reflecting the impact of local anthropogenic pressures, particularly in urbanized catchments. Since these streams discharge directly into the Istanbul Strait, the observed sediment contamination patterns are of particular concern, as they may contribute to the transfer of trace metals into this ecologically and economically critical marine ecosystem. Therefore, continuous monitoring of these inflowing streams is essential to evaluate and mitigate their potential impacts on the Istanbul Strait.

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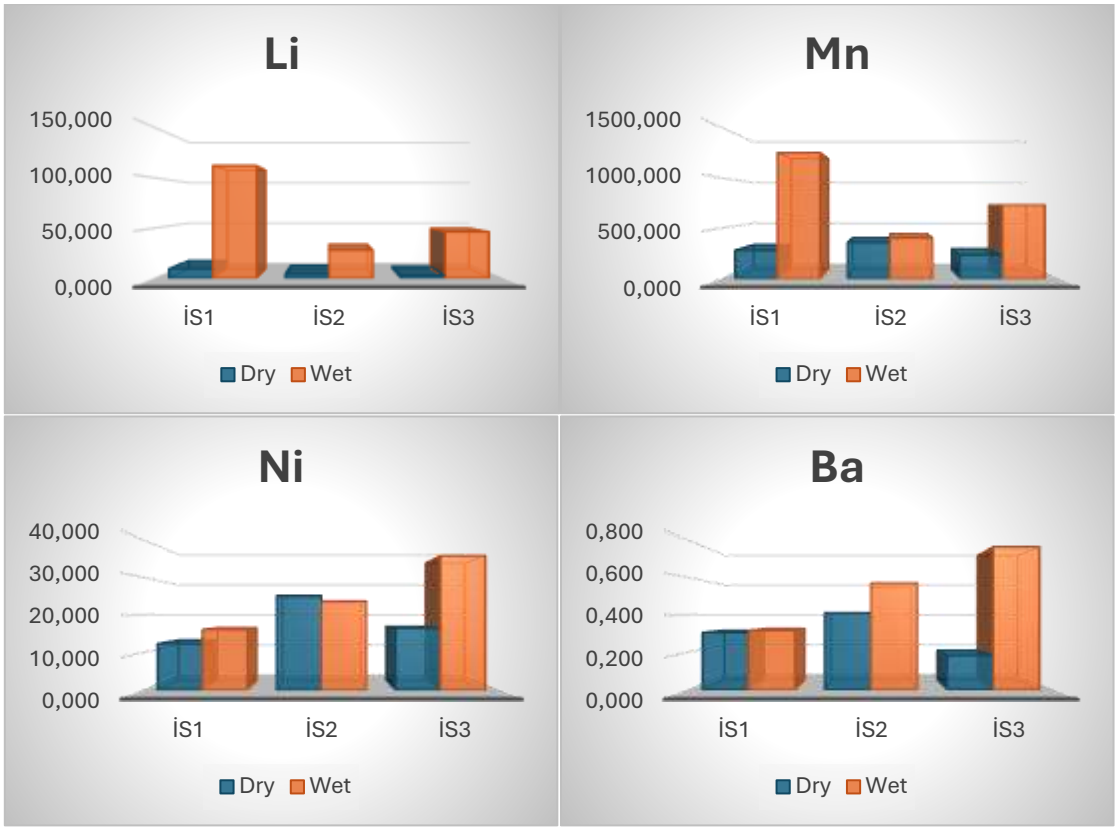

Figure 2. Spatiotemporal distributions of trace elements (ppm)

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PHYSIOLOGICAL EFFECTS OF LOW AND HIGH TEMPERATURE STRESS ON CORN WITH MYCORRHIZAL SYMBIOSIS

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ABSTRACT

In recent years, various methods have been investigated to counteract the negative effects of global climate change that limit corn cultivation. A number of studies have demonstrated the beneficial impacts of mycorrhizal inoculation on the resilience of corn plants when confronted with high or low temperatures. This study examined the alterations in chlorophyll content, leaf relative water content, turgor loss, membrane durability index, leaf protein content, and plant biomass of corn plants treated with mycorrhiza under low and high temperature stress. The study variants consisted of five different mycorrhizae treatments (M0, M1, M2, M3, and M4), seven temperature applications (5°C, 10°C, 15°C, 25°C, 35°C, 40°C, and 45°C), and one corn variety (PL472) with three replications. The chlorophyll content, leaf relative water content, turgor loss, protein content, and plant biomass of corn plants without mycorrhiza (M0) and those treated with different mixed mycorrhizal inoculums decreased under stress. Membrane durability remained at similar levels across all treatments, indicating minimal membrane damage in the plants. The results of the study indicated that the application of mycorrhizae contributed to the preservation of plant parameters such as chlorophyll content, water content, and turgor. M3 mixed mycorrhizal inoculum is the best for preserved to corn negative impacts of low and high temperature stress. The effects of the combined use of Gigaspora sp., Glomus constrictum, and Glomus fasciculatum mycorrhizal species should be thoroughly investigated.

Key words: chlorophyll content; water content; leaf protein content; membrane durability

INTRODUCTION

Corn (Zea mays L.) is a strategically significant plant that is regarded as the "grain of the future" on a global scale. This designation is due to its high nutritional value and the extensive range of applications for its primary and secondary products (Shiferaw et al., 2011). Due to the favorable climatic conditions in Türkiye, corn can be cultivated in nearly every region. However, environmental factors influence corn growth and distribution, with temperature being a primary environmental stress factor that impedes corn development (Durdu, 2013). Low and high temperature stress are critically important in terms of agricultural production feasibility, causing numerous physiological, morphological, and anatomical abnormalities in plants

(Kopecká et al., 2023). Temperature stress, a primary factor limiting corn cultivation, affects proteins, which are macromolecules. In conditions of temperature stress, protein structures become disrupted, and fluctuations in soluble protein content may be detected (Waqas et al., 2021).

In order to mitigate the deleterious effects of extreme temperatures on plant life, it is imperative to utilize specific methodologies that facilitate the activation of the plant's own defensive mechanisms. Mycorrhizal fungi, which are often referred to as "agricultural ecosystem engineers" due to their symbiotic relationship with the roots of naturally occurring plants, play a crucial role in agriculture. They enhance the plant's tolerance to environmental stresses and are foundational to soil fertility, crop yield, productivity, quality, and ecosystem resilience (Jajoo and Mathur, 2021). Mycorrhizal colonization can protect plants against low and high temperature stress by improving water and nutrient use efficiency, water-holding capacity, relative water content, stomatal conductance, and photosynthesis (Mathur and Jajoo, 2020).

This study examined the alterations in chlorophyll content, membrane damage, water content, protein quantity, and plant biomass in corn plants under high and low temperature stress, using four different mixed mycorrhizal inoculums.

MATERIALS AND METHODS

Description of the Plant Material, Experimental Soil, and Mycorrhizal Inoculum

The Ap horizon (0–30 cm) in the experimental research region of Eskisehir Osmangazi University is where the experimental soil was collected. After air drying and filtering through a 2 mm mesh, the soil samples were analyzed for physical and chemical properties. The analyzed soils were calcareous, sandy loam in texture, alkaline (pH=7.99), salt-free, low in organic matter (1.8%), and lacking in manganese (5.64 mg kg^{-1}), zinc (0.24 mg kg^{-1}), and phosphorus (56 kg ha^{-1}), while having adequate levels of potassium (2540 kg ha^{-1}), iron (2.81 mg kg^{-1}), and copper (1.73 mg kg^{-1}).

The corn cultivar PL472, which was acquired from the Polen Seed Company and corresponds to the FAO500 maturity group, was utilized as the plant material for this investigation.

The study utilized four distinct mixed mycorrhizal inoculums. The Diyarbakır Agricultural Control Research Institute provided three of these products, while the multinational commercial corporation Bioglobal provided the fourth, which was a product under the Endo Roots Soluble-ERS name. The following is a list of the species that comprise the mixed mycorrhizal inoculums.

M1-DYB4 (Glomus mosseae, Glomus intraradices)

M2-DYB17 (Glomus intraradices, Glomus constrictum, Glomus microcarpum)

M3-DYB22 (Gigaspora sp., Glomus constrictum, Glomus fasciculatum)

M4-ERS (Glomus intraradices, Glomus aggregatum, Glomus mosseae, Glomus clarium, Glomus monosporus, Glomus deserticola, Glomus brasilianum, Glomus etunicatum, Gigaspora margarita)

Sterilization of Plant Growth Media, Pots, and Seeds

The growing media was composed of a 2:2:1:1 ratio of soil, peat, perlite, and sand. To sterilize the mixture, it was autoclaved for two hours at 121°C and 1.5 atm of pressure. Prior to the studies, the 3-liter plastic pots were carefully cleaned by rinsing them with tap water, then a 10% sodium hypochlorite (NaOCl) solution, and finally three rinses with pure water. After that, they underwent another round of ethanol sterilization prior to planting.

After soaking in 70% alcohol for one minute and 2% sodium hypochlorite for three minutes, the corn seeds were rinsed four times with distilled water, put in 100 ml flasks with water, and incubated for a day.

Plant Cultivation, Mycorrhizal Treatments, And Experimental Setting

A factorial experimental design was used to set up the trial, which had three replications. Seven temperature applications (5°C, 10°C, 15°C, 25°C, 35°C, 40°C, and 45°C) and five mycorrhizal treatments (M0: without mycorrhiza/control, M1, M2, M3, and M4 mixed mycorrhiza inoculums) were applied to the corn variety PL472.

The nutrient-rich fertilizer solutions were then mixed with the sterilized experimental soil. The materials utilized for the preparation of the nutrients included $(NH_4)_2SO_4$ for 200 mg N, KH_2PO_4 for 125 mg K and 100 mg P, Fe-EDTA for 2.5 mg Fe, and $ZnSO_4.7H_2O$ for 5 mg Zn kg^{-1} of soil. A total of 2,000 mycorrhizal spores, comprising a mixture of M1, M2, and M3 mixed mycorrhiza cultures, were introduced into the soil at a depth of three centimeters below the seedbed for each kilogram of soil. The seeds received 2.5 milligrams of the mycorrhiza culture, designated as M4, for every one gram of seed. Eight seeds were then placed in the pots, the potting mixture was added, and the pots were filled to capacity. When the plants emerged, there were only four plants each container. The plants were maintained at 25/17°C, 800 µmol m^{-2} s^{-1}, 65% humidity, and a 14/10-hour light/dark cycle during the experiment. Until harvest, the plants were irrigated with deionized water at field capacity. At the 5–6 leaf stage, 200 mg N kg^{-1} of soil was added as top fertilizer.

Low and High Temperature Applications

The plants were subjected to temperature applications starting at six weeks of age. The optimal time for mycorrhizal colonization is usually six weeks after the corn plants are planted. The temperature range that the corn plant will probably experience in the field at this point is represented by the chosen low and high temperatures. After six weeks, plants with (M1, M2, M3, and M4) and without (M0) mycorrhiza at 25°C were harvested directly for analysis. Plants were kept at 5°C, 10°C, 15°C, 35°C, 40°C, and 45°C for 24 hours (14 hours of light and 10 hours of darkness) in a plant growth cabinet for both low and high temperature applications. The plants were removed from the chamber and harvested for analysis after a 24-hour period at each temperature.

Plant Analyses

The chlorophyll content of plant leaves was determined immediately before harvest by holding the chlorophyll meter (Spectrum Field Scout CM 1000) 30 cm away from the leaves.

The membrane durability index (MDI) in plant leaves was determined by placing a 1 cm leaf section into 5 ml tubes, adding 5 ml of pure water, and shaking the tubes in a shaker for 4 hours. The relative amount of electrolyte (A) that passed into 5 ml of pure water was measured using an electrical conductivity meter. The same samples were then held in a 100 °C water bath for 30 minutes, allowed to cool to room temperature after boiling, and the relative electrolyte amount (B) was measured again. After these procedures, the MDI of the leaves was calculated as a percentage using the formula MDI = (1 - A⁄B) ×100.

The relative water content (RWC) and turgor loss (LT) of the plants were determined by extracting three 2-cm-diameter discs from the leaf samples. Fresh weights (FW) were recorded immediately, followed by turgor weights (TW) after 4 hours in pure water and dry weights (DW) after 24 hours at 70°C. The following formulas were used to determine the RWC and LT values based on the collected data. The results were then reported as a percentage.

$$RWC = (FW-DW)/(TW-DW) \times 100$$

$$LT = (TW-FW)/TW \times 100$$

The quantification of protein was determined by using the Bradford (1976) method. The absorbance of 100 µL of sample and 3 mL of Bradford solution were measured against a bovine serum albumin (BSA) standard prepared at a wavelength of 595 nm using a spectrophotometer. The protein quantity was calculated according to the prepared standard curve.

Biomass of corn plants was measured in gram by weighing the aboveground parts after harvest.

Statistical analyses

All statistical operations were carried out and the figures were produced using the IBM SPSS 26 program. A completely random factorial design was used to analyze the variance of the study's data. To find mean differences, the Duncan multiple comparison test was used. Bar graphs and error bars are used in the figures to display the mean and standard error of the data.

RESULTS AND DISCUSSION

The variance analysis of the physiological parameters of corn plants inoculated with mycorrhiza under different temperatures is presented in Table 1. The findings revealed a statistically significant ($P < 0.01$) impact of mycorrhizal treatments, temperature applications, and their interactions on corn plant chlorophyll content, leaf water content, turgor loss, protein content, and plant biomass. However, the effect of mycorrhizal treatments and interaction with temperature applications on membrane durability index was found to be insignificant.

Table 1. Analysis of variance (F values) for the parameters examined in the study

Source of variance	Chlorophyll content	Membrane durability index	Relative water content	Loss of turgidity	Soluble protein content	Plant Biomass
Mycorrhizal applications (M)	148.144^{**}	1.568^{ns}	26.469^{**}	26.354^{**}	123.760^{**}	485.78^{**}
Temperature treatments (T)	522.099^{**}	23.335^{**}	186.692^{**}	171.705^{**}	259.678^{**}	706.01^{**}
Interactions (M × T)	9.583^{**}	3.608^{**}	2.301^{**}	2.109^{**}	5.660^{**}	18.12^{**}

**:p<0.01, ns: no significant

The corn plant treated with M3 had the highest chlorophyll and protein content, membrane durability index, and plant biomass, the corn plant treated with M4 had the highest leaf water content, and the corn plant treated with M0 had the highest turgor loss, according to the mean values of the physiological parameters of the corn plant after mycorrhizal treatments. All values were found to be at their highest at 25°C, in accordance with the defined standards for temperature applications. The contents of protein, water, and chlorophyll showed a reciprocal trend in tandem with the observed temperature fluctuations (Table 2).

Low and high temperatures cause photosynthesis to decrease (Hajihashemi et al., 2018; Ghani et al., 2023). In the present study, the chlorophyll content of the corn plant was the highest (337.33 SU) at 25°C in the application of the M3, while the lowest (138.33 SU) at 45°C in the M0 (Figure 1a). Previous studies have also reported that chlorophyll content decreases in corn plants under high and low temperature conditions due to temperature fluctuations (Waqas et al., 2021; Baruah et al., 2023; Teng et al., 2025). It was found that the corn plants with mycorrhizal inoculation had a higher chlorophyll content than the group without mycorrhizal inoculation at all temperature applications (Figure 1a). By increasing the uptake of magnesium through their hyphae, mycorrhizae were found to increase the total chlorophyll content, which in turn led to an increase in photosynthesis (Mathur et al., 2018). The hypothesis asserts that the increased photosynthesis rate in plants subjected to stress conditions post-mycorrhizal inoculation is due to the maintenance of chlorophyll structure and the expansion of leaf area, which is a consequence of the corn plant's improved nutrient absorption. Zhu et al. (2017) have reported that mycorrhizal plants are less affected by temperature stress and that mycorrhizal symbiosis protects plants from damage and increases photosynthesis.

Table 2. Average values of physiological parameters of the corn plant treated with mycorrhiza under different temperature level

Treatment	Chlorophyll content (SU)	Membrane durability index (%)	Relative water content (%)	Loss of turgidity (%)	Soluble protein content (mg/gFW)	Plant biomass (g)
M0	180.76 ^{d}	93.81	73.88^{c}	22.64^{a}	9.06^{c}	119.25^{e}
M1	213.86^{b}	93.44	79.85^{b}	16.96^{bc}	11.22^{b}	133.50^{d}
M2	209.81^{bc}	92.88	79.31^{b}	17.57^{b}	11.16^{b}	133.89^{c}
M3	238.76^{a}	93.99	79.82^{b}	17.11^{b}	12.12^{a}	154.31^{a}
M4	203.38^{c}	93.30	82.53^{a}	14.87^{c}	10.82^{b}	144.05^{b}
5 °C	163.40^{e}	90.32^{f}	70.14^{f}	24.85^{b}	8.56^{f}	118.28^{f}
10 °C	187.33^{d}	92.56^{de}	75.02^{e}	21.27^{c}	10.20^{d}	127.78^{e}
15 °C	220.53^{c}	93.67^{cd}	83.85^{c}	13.51^{e}	12.23^{b}	134.62^{d}
25 °C	293.27^{a}	96.56^{a}	92.51^{a}	6.39^{g}	14.17^{a}	168.49^{a}
35 °C	240.20^{b}	96.45^{ab}	87.57^{b}	10.65^{f}	11.57^{c}	151.03^{b}
40 °C	195.33^{d}	94.58^{bc}	80.26^{d}	16.48^{d}	10.21^{d}	147.14^{c}
45 °C	165.13^{e}	91.23^{ef}	64.20^{g}	31.64^{a}	9.20^{e}	118.65^{f}

The initial manifestation of irreversible damage to plants in response to stress is observed in cell membranes and is characterized by alterations in electrolyte leakage (Bajji et al., 2002). An examination of the membrane durability index values calculated based on the electrolyte leakage rate of the corn plant revealed that the highest value (97.03%) was determined for the

M1 treatment at 25°C, and the lowest value (88.79%) was determined for the M2 treatment at 5°C (Figure 1b).

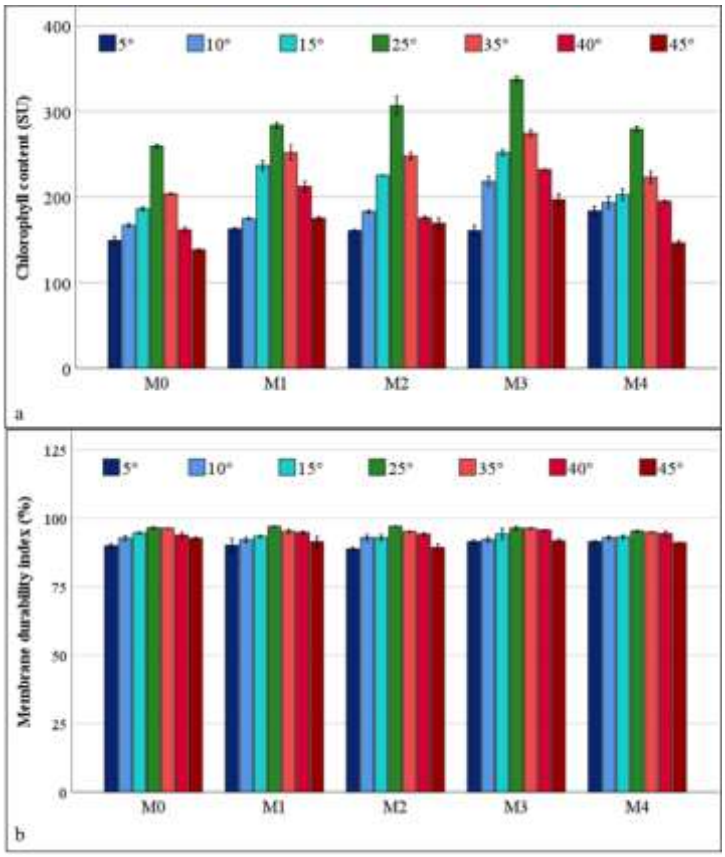

Figure 1. Changes in the chlorophyll content and membrane durability index of the mycorrhizal corn plant under temperature stress

The membrane durability index value was observed to be highest under optimal temperature conditions and decreased with temperature changes. Temperature stress causes significant changes in the cell membrane by affecting the sensors located in the membrane. While plants not exposed to stress have the highest cell membrane stability, membrane permeability is the first to be affected in plants exposed to temperature stress (Kumar et al., 2012). Previous studies have reported that electrolyte leakage increases and cell damage is elevated at low and high temperatures, but cell membrane stability is maintained in mycorrhizal symbiosis, and inoculation with mycorrhiza under stress reduces electrolyte leakage (Shirani and Mohammad, 2020; Malhi et al., 2021). The current study indicates that the influence of mycorrhizal inoculation on membrane durability index values is minimal. The slight rise in electrolyte leakage in plant leaves under temperature stress caused the membrane durability index to change only slightly. This result indicates that the corn variety utilized in this study may exhibit tolerance at the membrane level to temperature variations.

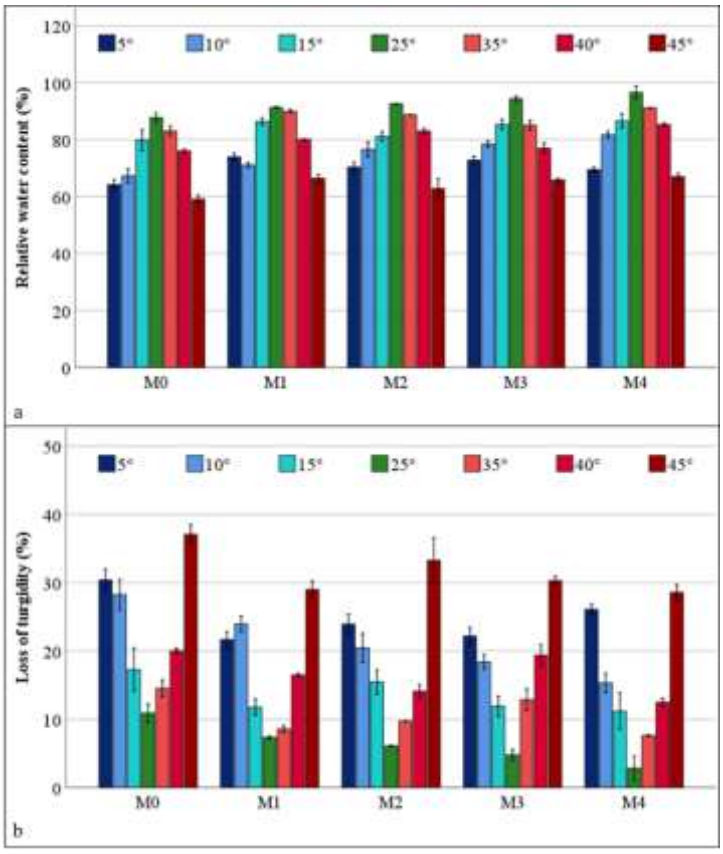

Figure 2. Changes in the leaf relative water content and loss of turgidity of the mycorrhizal corn plant under temperature stress

Plants' development and physiological functions are impacted by their water status. Mycorrhizal and non-mycorrhizal plants have different water statuses (Liu et al., 2015). It was need to recognize the importance of relative water content, turgor loss, leaf water potential, and water conservation capacity in assessing a plant's water status. Water content of detached leaves is used to describe a plant's water-holding ability, showing a clear correlation between higher water content and reduced water loss (Ievinsh, 2023). In this study, mycorrhizal plants showed higher levels of leaf relative water content and turgor at all temperature applications. The maximum water content (96.56%) was observed at 25°C in the M4 treatment, while water content exhibited a decline in both decreasing and increasing temperatures. The lowest water content (59.05%) was found at 45°C with the M0 treatment (Figure 2a).

Turgor loss was also least in plants with higher water content, as expected. Plants with the greatest turgor loss were those exposed to 45°C, with turgor loss exceeding 30%. At 5°C, M1 treatment resulted in higher water content and less turgor loss, while M4 was best at all other temperatures (Figure 2b). Zhu et al. (2010) reported higher leaf water potential in mycorrhizal corn plants during cold stress, suggesting improved water status. It was also reported that high temperature stress affects cell and tissue water content, causing rapid water loss. However, mycorrhizal symbiosis has been shown to increase water conservation in order to prevent dehydration and metabolic disorders (Zhu et al., 2011). This may be due to mycorrhizal fungal hyphae extracting soil water, higher root activity and hydraulic conductivity (Bitterlich et al., 2018).

The protein is very helpful in protecting cells from preventing water loss and ensuring plant life under demanding environments (Samiappan et al., 2021). Protein content was higher at every temperature level with mycorrhizal inoculum. The highest protein content (15.37 mg/gFW) under optimal temperature conditions was observed in the M4 application.

Decreasing or increasing temperature reduced the soluble protein content in corn leaves (Figure 3a). This finding contradicts previous reports indicating that soluble protein content increases in plants under low-temperature stress (Abdel Latef et al., 2011; Turk et al., 2020; Yan et al., 2021). A considerable decrease in photosynthesis, or a reduction in the availability of protein assimilates, which are essential for protein synthesis, could be the cause of the decline in protein content (Chaves et al., 2009). The investigation revealed that the soluble protein content of mycorrhizal corn plants was higher at low temperatures. M3 mycorrhizal plants demonstrated the least protein loss at low temperatures.

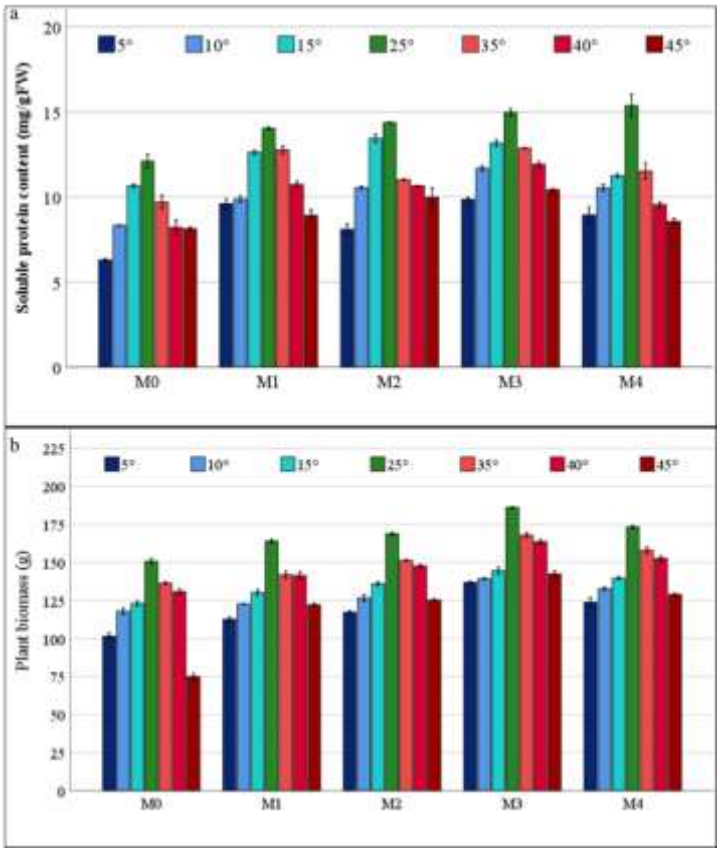

Figure 3. Changes in the soluble protein content and plant biomass of the mycorrhizal corn plant under temperature stress

In a manner analogous to the low temperatures, the protein content of mycorrhizal plants was also elevated at high temperatures, and M3 plants exhibited the highest protein content at all high temperature levels. In a similar finding to the present study, Ayub et al. (2021) and Hu et al. (2023) also reported that high temperature stress reduces protein content in plants. The increase in the kinetic energy of molecules across the membrane is caused by high temperatures, which leads to the loosening of membranes due to protein denaturation. Furthermore, the activity of ribulose bisphosphate carboxylase falls above the optimal temperature range for corn plant (Ristic et al., 2009; Naveed et al., 2014). Nitrate reductase is a pivotal enzyme for nitrogen metabolism. A reduction in its activity has been demonstrated to result in a disruption of protein synthesis and the synthesis of other enzymes, as well as the creation of biomass in plants (Ayub et al., 2021).

The plant biomass values of the corn plant were highest (186.03 g) at the control temperature applied to M3, while the lowest (74.87 g) were determined at 45 °C without (M0) mycorrhizal inoculum (Figure 3b). The application of mycorrhizal inoculum has ensured high levels of plant biomass under all temperature conditions. Despite the observed decline in plant biomass associated with low and high temperatures, the treatment of mycorrhiza has been shown to mitigate this loss. Previous studies have also reported that plant biomass increases with mycorrhizal treatments and that biomass loss decreases in stressed plants (Chandrasekaran, 2022).

CONCLUSION

The present study investigated the effects of mixed mycorrhizal inoculums on chlorophyll content, membrane durability index, water content, soluble protein content, and plant biomass in corn plants under low and high temperature conditions. It was determined that low and high temperature stresses result in chlorophyll loss in plants, leading to a reduction in water content, turgor, protein content, and plant biomass. However, mycorrhizal plants have been observed to maintain chlorophyll, water content, turgor, protein content, and plant biomass under stress conditions. The most favorable outcomes were observed in the M3 group, which received the applied mixed mycorrhizal inoculums. Further detailed studies should be conducted on the effects of the combined use of Gigaspora sp., Glomus constrictum, and Glomus fasciculatum mycorrhizal species in protecting corn plants from temperature fluctuations.

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PROBABILISTIC ASSESSMENT OF FLUORIDE-RELATED HEALTH RISKS IN GROUNDWATER OF THE FELENT STREAM WATERSHED, KÜTAHYA

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ABSTRACT

The Felent Stream Basin (Kütahya) constitutes an important hydrological system in the region, with its groundwater resources playing a key role in meeting diverse local demands. This study aimed (1) to investigate the spatial and temporal variations of fluoride concentrations in the groundwaters of Felent Stream Basin and (2) to evaluate the potential implications of these variations for the dental health of the local population. A total of seven sampling stations were established along the watershed, and groundwater samples were collected during both the wet and dry seasons of 2024. Fluoride concentrations were measured using a spectrophotometric method in accordance with standard analytical procedures. The results demonstrated distinct seasonal fluctuations in fluoride levels. The mean fluoride concentration recorded during the dry season was 0.404 mg/L, which was approximately 2 times higher than the mean value observed in the wet season (0.181 mg/L). The overall average across all samples was calculated as 0.295 mg/L. This increase during the dry period is likely due to reduced dilution and enhanced water-rock interaction. According to the average HQ values, the highest potential risk was observed in children (0.27875), followed by teenagers (0.19676), adults (0.15766), and infants (0.03935). In addition, fluoride-related health risks increased across all age groups during the dry season.

Keywords: Felent Stream Basin, Groundwater, Fluoride accumulations

INTRODUCTION

Fluoride is a naturally occurring element that contributes significantly to dental protection when present at optimal levels. Although drinking water constitutes the most common route of human fluoride intake, additional exposure may occur through the consumption of certain foods, the use of fluoridated medications, and contact with industrial emissions (Andezhath et al., 1993; Güner et al., 2016; Tokatlı and Güner, 2018; Onur and Tokatlı, 2020; Tokatlı et al., 2022; 2023). Nevertheless, chronic exposure to elevated fluoride concentrations can result in various health concerns, most notably dental and skeletal fluorosis.

To better understand and manage such risks, the implementation of health risk assessment techniques has become increasingly widespread across the globe. These approaches are instrumental in evaluating both carcinogenic and non-carcinogenic threats posed by environmental contaminants, offering a scientific basis for risk management and public health decision-making (Ustaoğlu and Aydın, 2020; Tokatlı and Ustaoğlu, 2020; Tokatlı and Varol, 2021a; 2021b; Varol and Tokatlı, 2022; Tokatlı et al., 2022; 2023). Particularly in regions where fluoride levels may vary seasonally or spatially, such assessments are crucial for ensuring the safety and sustainability of water resources.

The Felent Stream Basin (Kütahya) represents a vital hydrological system within its region, with its subterranean water resources playing a pivotal role in supporting various local needs. These crucial groundwater reserves are extensively drawn upon for diverse applications, including industrial supply and irrigation, which are fundamental to the regional economy. Concurrently, the groundwater system is inevitably impacted by anthropogenic activities within the basin. This includes wastewater discharges originating from numerous thermal tourism facilities in the Yoncalı District and domestic wastes from Kütahya Province (Solak et al., 2012; Çiçek et al., 2013; 2017). Furthermore, the interaction between surface and groundwater in this system is complex, as exemplified by the Enne Dam Lake, a significant reservoir on the Felent Stream, which provides 6 hm^3 of drinking water annually to the region (Anonymous, 2022; https://www.dsi.gov.tr/), indirectly influencing the wider hydrological balance.

Considering the critical importance and vulnerability of these subterranean resources, this study specifically aimed (1) to investigate the spatial and temporal variations of fluoride concentrations in the groundwater of the Felent Stream Basin and (2) to evaluate the potential implications of these variations for the dental health of the local population reliant on these sources.

MATERIALS AND METHODS

Surface Water Collection

Figure 1 illustrates the topographic configuration of the Sakarya River Watershed and the Felent Stream Basin, including the spatial distribution of the selected monitoring stations. Table 1 shows the coordinate information of sampling locations. Within the scope of this study, groundwater samples were systematically collected from seven stations selected on the Felent Stream Basin during both the wet and dry seasons of 2024.

Table 1. Coordinates of stations

Sampling Location	Zone of Basin Plains	Zone of Watershed	GPS – North	GPS – East
Ören Village	Köprüören Plain	Upstream	39.52423	29.72003
Köprüören Village	Köprüören Plain	Upstream	39.50928	29.76439
Yoncalı Village	Köprüören Plain	Upstream	39.49303	29.84171
Enne Village	Köprüören Plain	Midstream	39.47439	29.85568
Kirazpınar Village	Kütahya Plain	Midstream	39.46229	29.91116
Kütahya Province	Kütahya Plain	Downstream	39.43557	29.99613
İnköy Village	Kütahya Plain	Downstream	39.45441	29.99842

Fluoride Analysis

Fluoride concentrations were quantified using a spectrophotometric technique, employing a Hach Lange DR 3900 spectrophotometer (wavelength range: 320–1100 nm) in conjunction with the Hach Lange LCK 323 cuvette test (measurement range: 0.1–2.5 mg/L) (https://tr.hach.com/).

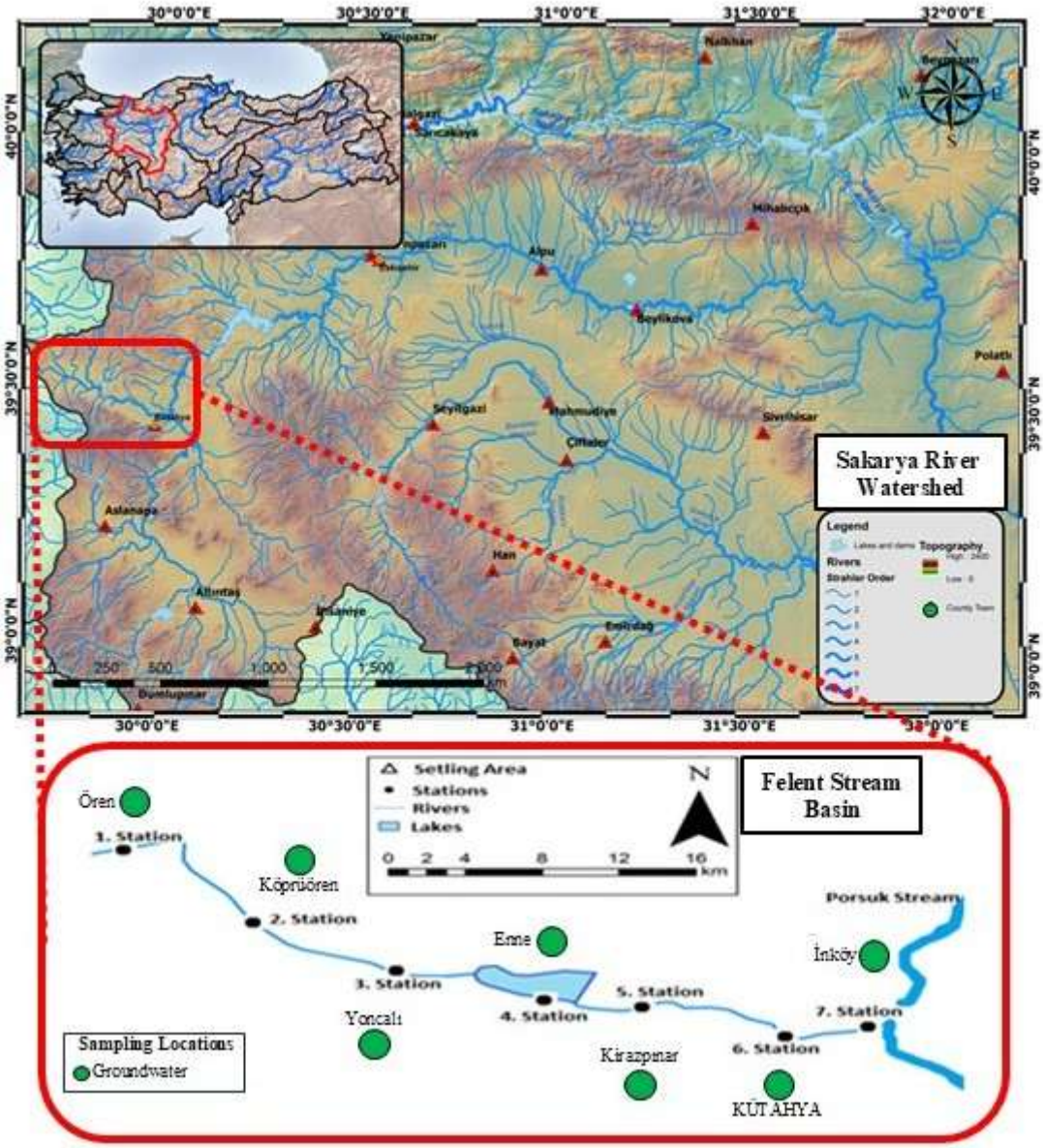

Figure 1. Topographic map of Sakarya River Basin and selected sampling locations

Health Risk Assessment

In the present study, the population was stratified into four age groups based on physiological and behavioral characteristics: infants (<2 years), children (2–6 years), teenagers (6–16 years), and adults (>16 years). Daily fluoride exposure for each group was estimated using Equation 1. The non-carcinogenic health risk associated with fluoride intake was assessed using the Hazard Quotient (HQ), calculated according to Equation 2 (Yousefi et al., 2018). An HQ value below 1 indicates a negligible non-carcinogenic risk, whereas an HQ value above 1 suggests a potential health concern, with an increased likelihood of developing severe fluorosis.

$$EDI = \frac{Cf\ x\ Cd}{Bw} \quad (1)$$

$$HQ = \frac{EDI}{RfD} \quad (2)$$

EDI: Estimation of fluoride consumption – daily (mg/kg/day)

C_f: Fluoride level in the investigated drinking water samples (mg/L)

C_d: Mean daily water intake (L/day) (the mean water consumption rates in infants, children, teenagers and adults were 0.08, 0.85, 2 and 2.5 L/day, respectively)

B_w: Body weight (kg) (body weights of investigated groups were considered 10, 15, 50 and 78 kg, respectively)

RfD: Reference dose (mg/kg/day) (it is 0.06 mg/kg/day for fluoride (EPA, 1992)).

RESULTS AND DISCUSSION

Fluoride accumulations in groundwater of Felent Stream Basin are given in Figure 2. Fluoride concentrations increased during the dry season at most stations, particularly at Ören (0.188 → 0.537 ppm), Köprüören (0.103 → 0.366 ppm), and Yoncalı (0.176 → 0.468 ppm). The highest dry season value was recorded at Ören, while the lowest wet season value was observed in Kütahya (0.069 ppm). Unlike the general trend, a slight decrease occurred at Enne during the dry period (0.294 → 0.273 ppm).

Fluoride plays a dual role in human health; while beneficial for dental integrity due to its strong affinity for tooth enamel, excessive intake can lead to significant adverse health outcomes (Taghipour et al., 2016; Tokatlı et al., 2022; 2023). A primary pathway for human fluoride exposure is drinking water, where optimal concentrations are instrumental in preventing dental caries and promoting overall well-being. Nevertheless, exceeding these beneficial levels can precipitate fluorosis, a widely recognized public health issue affecting dental health (Güner et al., 2016; Tokatlı and Güner, 2018; 2020; Jannat et al., 2022; Din et al., 2024). This study investigated fluoride concentrations in the groundwater of the Felent Stream Basin, finding levels that ranged from 0.069 to 0.537 ppm. Importantly, all examined stations exhibited fluoride concentrations well within the acceptable drinking water limit of 1.5 ppm, as defined by TS266 (2005), EC (2007), and WHO (2011).

Figure 3 presents fluoride exposure risk, showing all the average HQ values well below the acceptable threshold of 1, indicating a low overall risk. Consistent with previous findings, children (mean HQ: 0.27875) continue to exhibit the highest HQ values, underscoring their heightened sensitivity. Furthermore, dry season exposure conditions universally result in higher HQ values compared to wet season conditions, highlighting the significant influence of exposure pathways on risk assessment. Global research widely corroborates that children are particularly vulnerable to chronic health risks posed by fluoride consumption in drinking water (Gao et al., 2013; Narsimha et al., 2018; Dehghani et al., 2019; Qasemi et al., 2020; Tokatlı et al., 2022; 2023). This consistent finding underscores the critical need for meticulous assessment of fluoride exposure in pediatric populations. Supporting these prior observations, the present study likewise determined children to be the most at-risk demographic concerning health implications from fluoride exposure originating from the groundwater of the Felent Stream Basin.

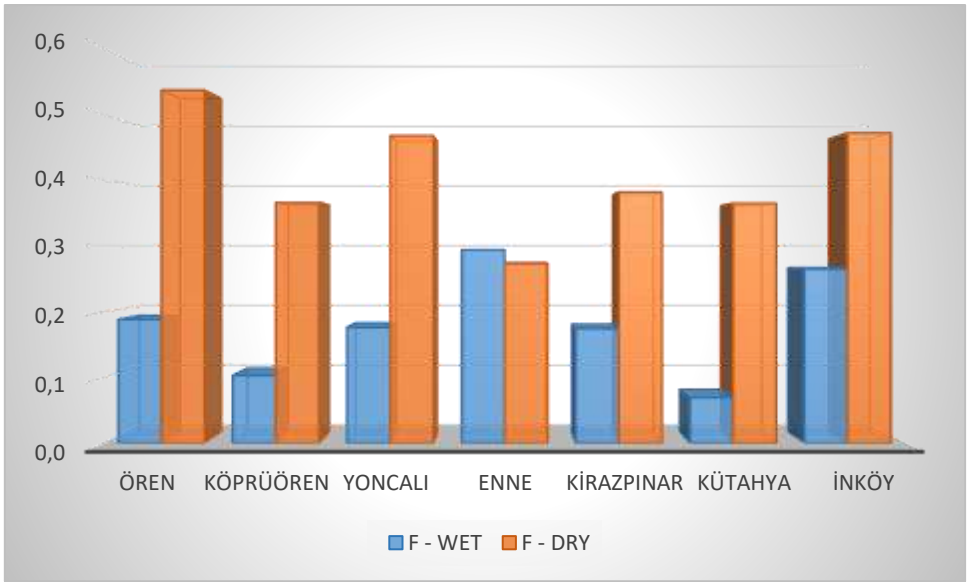

Figure 2. Spatial – temporal fluoride accumulations of groundwater samples (ppm)

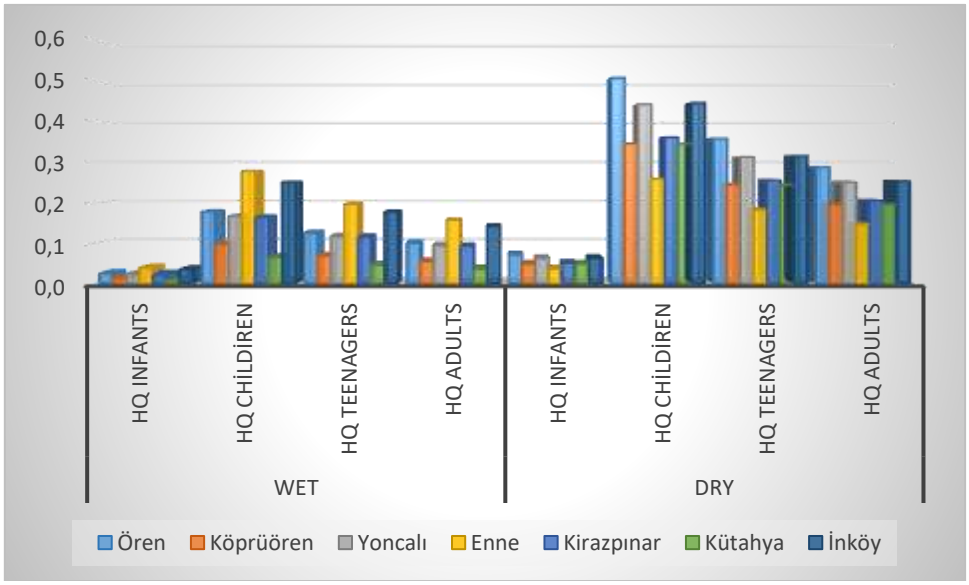

Figure 3. Spatio – temporal HQ values of different consumers

CONCLUSION

This study revealed significant seasonal fluctuations in groundwater fluoride concentrations within the Felent Stream Basin (Kütahya), with dry season levels (mean 0.404 mg/L) approximately double those of the wet season (mean 0.181 mg/L) due to reduced dilution and enhanced water-rock interaction. Health risk assessment consistently identified children as the most vulnerable group (HQ: 0.27875), with fluoride-related risks increasing for all age groups during the dry season. Despite overall levels being acceptable, these findings underscore the critical need for continuous groundwater monitoring, especially in dry periods, and targeted public health interventions to protect the dental health of children in the region.

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SPATIAL–TEMPORAL VARIATIONS OF FLUORIDE IN SURFACE WATERS OF FELENT STREAM (TÜRKIYE) AND ASSOCIATED HEALTH RISKS

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ABSTRACT

Felent Stream, situated in Kütahya Province, is an important component of the Sakarya River Watershed in Türkiye. This study aimed (1) to investigate the spatial and temporal variations of fluoride concentrations in the surface waters of Felent Stream and (2) to evaluate the potential implications of these variations for the dental health of the local population. A total of seven sampling stations were established along the stream, and surface water samples were collected during both the wet and dry seasons of 2024. Fluoride concentrations were measured using a spectrophotometric method in accordance with standard analytical procedures. The results demonstrated distinct seasonal fluctuations in fluoride levels. The mean fluoride concentration recorded during the dry season was 0.749 mg/L, which was approximately 1.5 times higher than the mean value observed in the wet season (0.521 mg/L). The overall average across all samples was calculated as 0.635 mg/L. This increase during the dry period is likely related to reduced water flow and limited dilution, leading to greater accumulation of fluoride in the aquatic environment. According to the average HQ values, the highest potential risk was observed in children (0.59952), followed by teenagers (0.42319), adults (0.33909), and infants (0.08464). In addition, fluoride-related health risks increased across all age groups during the dry season.

Keywords: Felent Stream, Surface water, Fluoride accumulations

INTRODUCTION

Fluoride is an essential trace element that plays a vital role in maintaining dental health. While drinking water is considered the primary source of fluoride intake for humans, other exposure pathways include food, pharmaceuticals, and various industrial activities (Andezhath et al., 1993; Güner et al., 2016; Tokatlı and Güner, 2018; Onur and Tokatlı, 2020; Tokatlı et al., 2022; 2023). However, excessive fluoride intake can lead to adverse health effects, particularly dental and skeletal fluorosis.

In this context, health risk assessment practices have gained significant importance worldwide, especially in recent years. These methods provide a scientific framework for estimating both carcinogenic and non-carcinogenic risks associated with exposure to various environmental contaminants, including fluoride (Ustaoğlu and Aydın, 2020; Tokatlı and Ustaoğlu, 2020; Tokatlı and Varol, 2021a; 2021b; Varol and Tokatlı, 2022; Tokatlı et al., 2022; 2023). The application of such risk assessment approaches is particularly valuable in identifying vulnerable populations, informing regulatory actions, and ensuring the long-term safety of drinking water resources.

The Sakarya River Watershed stands as one of Türkiye's most significant and extensive riverine habitats, and the Felent Stream constitutes a crucial component of this vital system. Extending approximately 35 km in length with an average flow rate of 0.56 m^3/s, the Felent Stream serves multiple purposes within the region. It is extensively utilized for industrial water supply and irrigation, playing a critical role in supporting local economic activities. However, it also functions as a receiving environment for wastewater discharges from numerous thermal tourism facilities located in the Yoncalı District, as well as domestic wastes originating from Kütahya Province (Solak et al., 2012; Çiçek et al., 2013; 2017). Further upstream, the Enne Dam Lake, a significant reservoir on the Felent Stream, contributes substantially to the region's water security by providing 6 hm^3 of drinking water annually (Anonymous, 2022; https://www.dsi.gov.tr/). This dual role as both a valuable resource and a recipient of anthropogenic impacts underscores the ecological and socio-economic importance of the Felent Stream, warranting detailed investigation into its current status and management needs.

Given these multifaceted uses and potential impacts, this study specifically aimed (1) to investigate the spatial and temporal variations of fluoride concentrations in the surface waters of Felent Stream and (2) to evaluate the potential implications of these variations for the dental health of the local population.

MATERIALS AND METHODS

Surface Water Collection

Figure 1 illustrates the topographic configuration of the Sakarya River Watershed and the Felent Stream Basin, including the spatial distribution of the selected monitoring stations. Table 1 shows the coordinate information of sampling locations. Within the scope of this study, surface water samples were systematically collected from seven stations selected on the Felent Stream during both the wet and dry seasons of 2024.

Table 1. Coordinates of stations

Station	Zone of Basin Plains	Zone of Watershed	GPS – North	GPS – East
1. Station	Köprüören Plain	Upstream	39.52341	29.71731
2. Station	Köprüören Plain	Upstream	39.50703	29.76625
3. Station	Köprüören Plain	Upstream	39.48959	29.83703
4. Station	Köprüören Plain	Midstream	39.47417	29.86138
5. Station	Kütahya Plain	Midstream	39.46854	29.91585
6. Station	Kütahya Plain	Downstream	39.44115	29.98189
7. Station	Kütahya Plain	Downstream	39.45076	30.02648

Fluoride Analysis

Fluoride concentrations were quantified using a spectrophotometric technique, employing a Hach Lange DR 3900 spectrophotometer (wavelength range: 320–1100 nm) in conjunction with the Hach Lange LCK 323 cuvette test (measurement range: 0.1–2.5 mg/L) (https://tr.hach.com/).

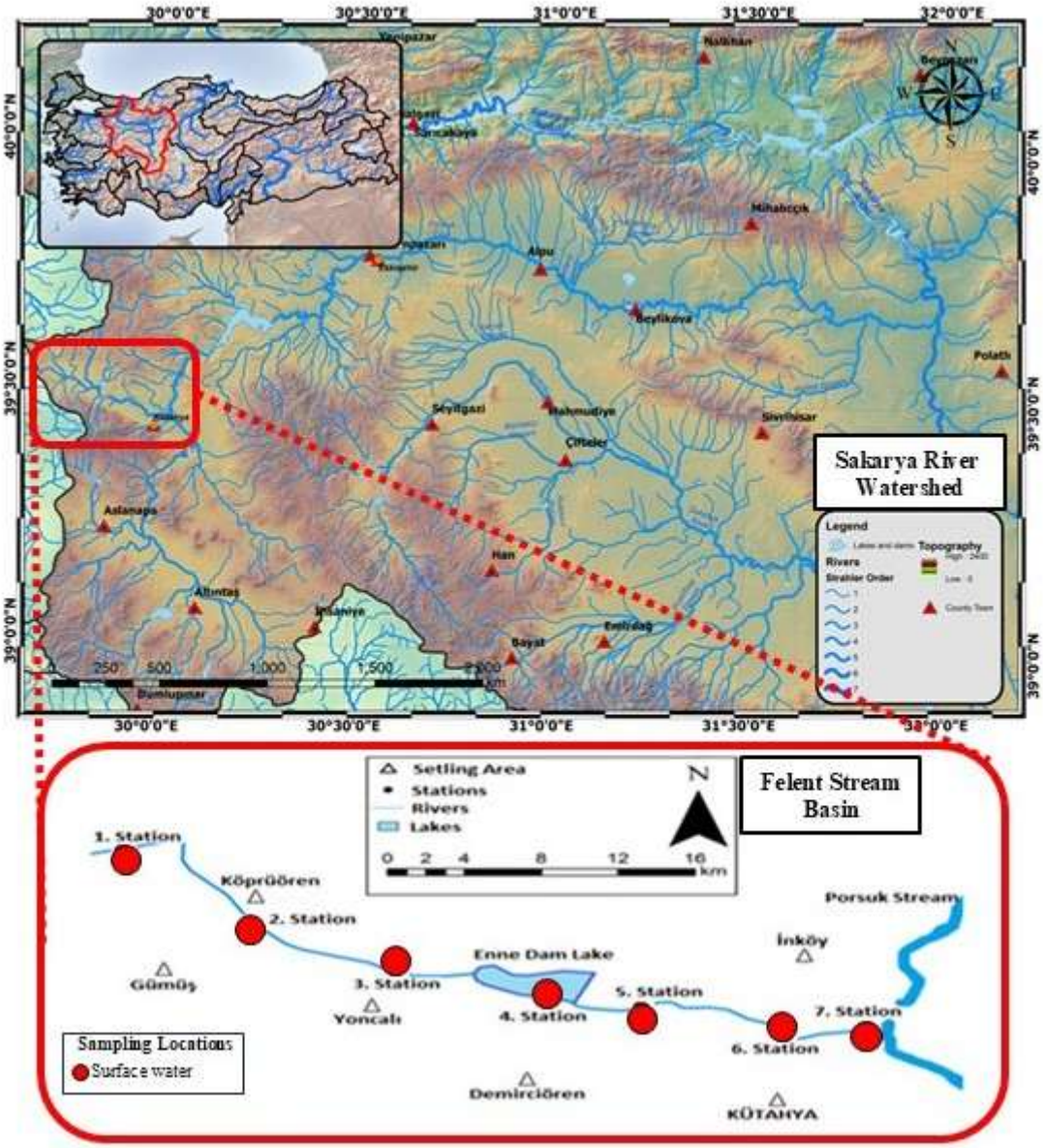

Figure 1. Topographic map of Sakarya River Basin and selected sampling locations

Health Risk Assessment

In the present study, the population was stratified into four age groups based on physiological and behavioral characteristics: infants (<2 years), children (2–6 years), teenagers (6–16 years), and adults (>16 years). Daily fluoride exposure for each group was estimated using Equation 1. The non-carcinogenic health risk associated with fluoride intake was assessed using the Hazard Quotient (HQ), calculated according to Equation 2 (Yousefi et al., 2018). An HQ value below 1 indicates a negligible non-carcinogenic risk, whereas an HQ value above 1 suggests a potential health concern, with an increased likelihood of developing severe fluorosis.

$$EDI = \frac{Cf\ x\ Cd}{Bw} \quad (1)$$

$$HQ = \frac{EDI}{RfD} \quad (2)$$

EDI: Estimation of fluoride consumption – daily (mg/kg/day)

C_f: Fluoride level in the investigated drinking water samples (mg/L)

C_d: Mean daily water intake (L/day) (the mean water consumption rates in infants, children, teenagers and adults were 0.08, 0.85, 2 and 2.5 L/day, respectively)

B_w: Body weight (kg) (body weights of investigated groups were considered 10, 15, 50 and 78 kg, respectively)

RfD: Reference dose (mg/kg/day) (it is 0.06 mg/kg/day for fluoride (EPA, 1992)).

RESULTS AND DISCUSSION

Fluoride accumulations in surface water of Felent Stream are given in Figure 2. Fluoride concentrations reached their highest levels at Station 3 during both the wet (1.65 ppm) and dry (1.92 ppm) seasons. In general, an increase in fluoride levels was observed during the dry season, particularly at Stations 1, 2, 4, and 5. However, an exceptional decrease was noted at Station 7, where fluoride dropped from 0.342 ppm in the wet season to 0.040 ppm in the dry season.

Fluoride, while crucial for dental health due to its propensity for deposition in teeth, can also exert considerable adverse health effects on humans, contingent upon its concentration and exposure levels (Taghipour et al., 2016; Tokatlı et al., 2022; 2023). Drinking water serves as a primary source of fluoride exposure, and maintaining optimal fluoride levels in urban water supplies is known to have a preventive impact on overall body health, particularly in combating dental caries. Conversely, exposure to fluoride exceeding these optimal concentrations can lead to fluorosis, a significant concern for dental public health (Güner et al., 2016; Tokatlı and Güner, 2018; 2020; Jannat et al., 2022; Din et al., 2024). In the present study, fluoride concentrations in the surface water of Felent Stream varied from 0.040 to 1.920 ppm. Notably, fluoride levels at nearly all investigated stations (with the exception of Station 3) were found to be within the acceptable drinking water limit of 1.5 ppm, as stipulated by TS266 (2005), EC (2007), and WHO (2011).

Analysis of fluoride exposure risk, presented in Figure 3, indicates that while all the average HQ values are below the acceptable threshold of 1, children consistently exhibit the highest HQ values (mean 0.59952), suggesting increased susceptibility. Furthermore, dry season exposure conditions generally lead to higher HQ values across all age groups compared to wet season conditions. These findings underscore the critical importance of age-specific risk assessment and the distinct impact of exposure pathways in managing potential fluoride-related health risks. Numerous investigations globally have consistently highlighted that children face a significant chronic health risk from fluoride intake through drinking water (Gao et al., 2013; Narsimha et al., 2018; Dehghani et al., 2019; Qasemi et al., 2020; Tokatlı et al., 2022; 2023). These widespread findings underscore the heightened vulnerability of younger populations to fluoride-related health issues. In alignment with this established body of literature, our current research similarly identifies children as the age group most susceptible to health risks stemming from fluoride exposure via the surface water of Felent Stream.

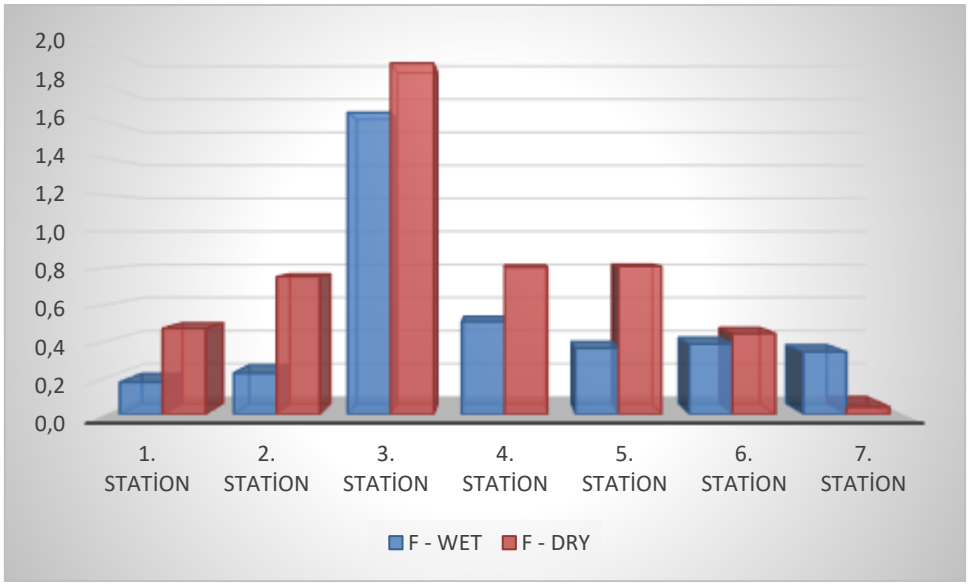

Figure 2. Spatial – temporal fluoride accumulations of surface water samples (ppm)

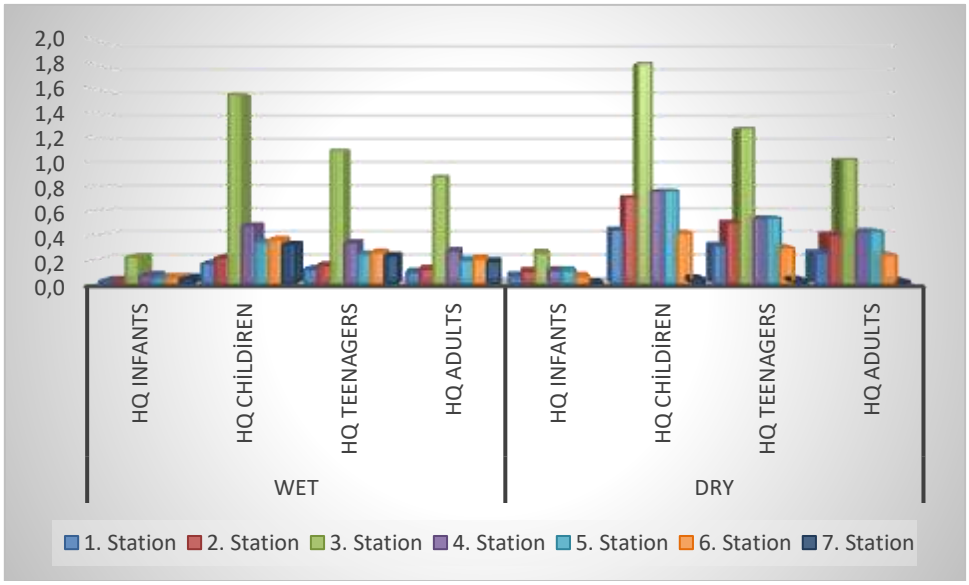

Figure 3. Spatio – temporal HQ values of different consumers

CONCLUSIONS

This study revealed significant seasonal variations in fluoride concentrations within Felent Stream's surface waters, with dry season levels (mean 0.749 mg/L) being notably higher than wet season levels (mean 0.521 mg/L) due to reduced dilution. Health risk assessment confirmed that children face the highest potential risk (HQ: 0.59952) from fluoride exposure, followed by teenagers, adults, and infants, a finding consistent with global observations. Moreover, fluoride-related health risks increased across all age groups during the dry season, directly correlating with elevated concentrations. While overall fluoride levels generally remained acceptable, these findings underscore the critical importance of continuous monitoring and targeted public health strategies, especially for vulnerable populations during dry periods, to mitigate potential dental health impacts.

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ENVIRONMENTAL IMPACTS OF GREENHOUSE GAS EMISSIONS AND PROPOSED SOLUTIONS FOR THEIR REDUCTION

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ABSTRACT

Greenhouse gas (GHG) emissions are among the primary causes of global warming and climate change. Gases such as carbon dioxide (CO_2), methane (CH_4), and nitrous oxides (N_2O) originate from human-induced activities, including fossil fuel consumption, industrial processes, agriculture, and deforestation. The accumulation of these gases in the atmosphere leads to rising temperatures, glacier melting, sea level rise, and an increase in extreme weather events.

This study explores various solutions to mitigate greenhouse gas emissions. Transitioning to renewable energy sources, such as solar, wind, and hydroelectric power, enhancing energy efficiency, promoting the widespread adoption of electric and hybrid vehicles in transportation, implementing sustainable agricultural practices, and initiating forest conservation projects are among the key strategies. Additionally, carbon capture and storage (CCS) technologies, alongside changes in individual consumption habits such as opting for low-carbon footprint products and increasing recycling efforts play a crucial role in reducing emissions.

Keywords: Greenhouse gas, global warming, industrial process, renewable energy

INTRODUCTION

Greenhouse gas (GHG) emissions represent one of the most critical environmental issues confronting the 21st century. This study investigates the ecological consequences associated with GHG emissions and evaluates a range of mitigation strategies aimed at reducing their impact. Particular focus is directed toward carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O), which constitute the primary anthropogenic greenhouse gases. The paper further discusses sustainable mitigation approaches, such as the deployment of renewable energy sources, enhancement of energy efficiency, implementation of carbon capture technologies, and large-scale reforestation efforts (Chen et al., 2023).

Greenhouse gases contribute to the retention of heat within the Earth's atmosphere, thereby exacerbating the greenhouse effect and elevating global temperatures. Although this

natural phenomenon is vital for sustaining life on Earth, anthropogenic emissions have significantly amplified its intensity, culminating in global warming and widespread climate disruption. Key drivers of GHG emissions include industrial processes, deforestation, and agricultural practices (Jensen et al., 2023). The resulting rise in global temperatures has led to an increase in the frequency and severity of heatwaves, as well as accelerated polar ice melt and thermal expansion of ocean waters, collectively contributing to sea level rise and heightened flooding risks (Aghakouchak et al., 2020).

Additionally, coastal marine ecosystems are increasingly threatened by anthropogenic activities, such as industrialization, agriculture, land-sea based aquaculture, and climate change. These pressures have led to coastal eutrophication, which is a major driver of macroalgal blooms (Smetacek and Zingone, 2013).

Macroalgal bloom events such as green and golden seaweed tides—induce profound shifts in both the physical and biogeochemical characteristics of the water column, thereby exerting considerable influence on greenhouse gas (GHG) emissions. Empirical evidence indicates that microalgal blooms can markedly decrease atmospheric carbon dioxide (CO_2) concentrations as a result of intensified photosynthetic activity. Concurrently, these events often stimulate the production of nitrous oxide (N_2O) and methane (CH_4), primarily through mechanisms involving the degradation of particulate organic matter (POM) and the emergence of hypoxic or anoxic conditions within marine systems (Cai et al., 2024).

The accelerating pace of climate change continues to pose serious threats to both human and ecological health (Romanello et al., 2022). In response to this escalating crisis, achieving the targets outlined in the Paris Climate Agreement and the Kigali Amendment to the Montreal Protocol necessitates a substantial and immediate reduction in global greenhouse gas emissions (Intergovernmental Panel on Climate Change, 2023).

According to estimates by the Food and Agriculture Organization of the United Nations (FAO), emissions from the agricultural sector account for approximately 30% of total global greenhouse gas (GHG) emissions. The predominant greenhouse gases emitted from agricultural soils include carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) (Chataut et al., 2023).

In agricultural systems, both natural factors such as soil moisture, temperature, and pH and anthropogenic farming practices exert significant influence over the magnitude of GHG emissions. A wide body of research has investigated various strategies to mitigate these emissions. One promising approach involves the development and cultivation of crop varieties with enhanced capacities for carbon and nitrogen uptake. Such improvements can increase the efficiency of nutrient use, reduce dependence on synthetic fertilizers, and consequently decrease GHG emissions (Johansson et al., 2023).

Furthermore, optimizing fertilization practices including the timing, quantity, and type of fertilizers applied has been demonstrated to be an effective measure for controlling greenhouse gas emissions associated with agricultural activities (Li et al., 2023).

2. Environmental Impacts of Greenhouse Gas Emissions

2.1 Climate Change and Global Warming

The most direct and severe consequence of GHG emissions is global warming. Average global temperatures have increased significantly since the industrial revolution, resulting in more frequent and intense heatwaves, droughts, and wildfires.

Climate change refers to long-term shifts and alterations in temperature, precipitation patterns, wind, and other elements of the Earth’s climate system. Global warming, on the other

hand, is the rise in the average surface temperature of the Earth due to increasing concentrations of greenhouse gases in the atmosphere (Koch et al., 2013).

2.2 Causes of Global Warming

The primary cause of global warming is human activity, particularly the burning of fossil fuels such as coal, oil, and natural gas. These activities release large amounts of greenhouse gases (especially carbon dioxide – CO_2, methane – CH_4, and nitrous oxide – N_2O) into the atmosphere. Other contributing factors include:

-Deforestation, which reduces the planet's capacity to absorb CO_2.

-Industrial processes that emit large quantities of GHGs.

-Agriculture, especially livestock farming which releases methane.

-Waste management practices that lead to the release of methane and other gases (Holgerson and Raymond, 2016).

2.3 Melting Ice Caps and Rising Sea Levels

The warming climate causes glaciers and polar ice caps to melt, contributing to rising sea levels. This threatens coastal ecosystems and human settlements, especially in low-lying countries and island nations.

One of the most visible and alarming consequences of global warming is the accelerated melting of polar ice caps and glaciers. This process directly contributes to rising sea levels, which pose serious threats to both natural ecosystems and human settlements, particularly in coastal and low-lying areas (Ren et al., 2020).

The treason of the melting of the ice caps can be described as; global temperatures rise. This means, Arctic sea ice is shrinking, especially during summer months. Land-based ice sheets and glaciers, such as those in Greenland and Antarctica, are losing mass rapidly. Melting land ice flows into oceans, increasing sea levels. Thermal Expansion means ocean temperatures increase, seawater expands. This thermal expansion accounts for nearly half of the observed sea level rise over the past century. Unlike sea ice, land-based ice adds new water to the ocean when it melts. The vast ice sheets in Greenland and Antarctica are currently the largest contributors to sea level rise from melting ice (Xie et al., 2021).

2.4 Current and Future Threats

Coastal Cities at Risk: Major urban areas like New York, Miami, Jakarta, Mumbai, and Istanbul face increased flooding and infrastructure damage. Saltwater intrusion can also contaminate freshwater supplies and damage agriculture.

Small Island Nations in Danger: Countries such as the Maldives, Tuvalu, and Kiribati are threatened with complete submersion if sea levels continue to rise, potentially creating the world's first "climate refugees."

Coastal Erosion and Habitat Loss: Rising seas accelerate erosion, submerge wetlands, and destroy mangroves and other critical coastal ecosystems.

Climate Migration: As coastal regions become uninhabitable, millions may be forced to migrate inland, leading to social, economic, and political challenges (Liu et al., 2021).

Scientific Projections: Over the 20th century, sea levels rose by approximately 15–20 centimeters (6–8 inches).

According to the Intergovernmental Panel on Climate Change (IPCC), sea levels are projected to rise by 0.6 to 1.1 meters (2 to 3.6 feet) by 2100 if greenhouse gas emissions continue at current levels.

2.5 Solutions and Adaptation Measures

Reducing greenhouse gas emissions can slow down global warming and ice melt. Coastal protection infrastructure, such as seawalls, flood barriers, and artificial islands, can help shield vulnerable areas. Climate adaptation policies must be developed, including strategic urban planning and relocation strategies for at-risk populations (Sheng et al., 2018).

2.6 Ocean Acidification

Excess CO_2 dissolves into oceans, lowering pH levels and disrupting marine ecosystems. Coral reefs, which are home to many marine species, are particularly vulnerable.

Ocean acidification refers to the ongoing decrease in the pH of the Earth's oceans, caused primarily by the uptake of carbon dioxide (CO_2) from the atmosphere. As anthropogenic CO_2 emissions have increased, a significant portion—approximately 30%—has been absorbed by the world's oceans. This process has altered the chemical composition of seawater, posing severe risks to marine biodiversity, ecosystems, and human societies dependent on oceanic resources (Alcaraz-Rocha et al., 2023).

$$CO2(aq)+H2O \rightleftharpoons H2CO3 \rightleftharpoons H++HCO3- \rightleftharpoons 2H++CO3-2$$

This sequence results in the formation of carbonic acid (H_2CO_3), which dissociates to release hydrogen ions (H^+), lowering the pH and increasing ocean acidity. As a consequence, the availability of carbonate ions (CO_3^{2-}) which are essential for marine calcifying organisms to form shells and skeletons decreases.

2.7 Ecological Impacts

Coral Reef Degradation: Coral reefs are particularly sensitive to changes in pH. Ocean acidification weakens coral skeletons and inhibits reef growth, which in turn affects the vast array of marine life that depends on reef ecosystems for habitat and food (Hoegh-Guldberg et al., 2007).

Food Web Disruption: Many planktonic species form the foundation of marine food webs. The decline in their populations due to acidification can have cascading effects on higher trophic levels, including commercially important fish species (Yudhistira et al., 2023).

Impact on Marine Biodiversity and Fisheries: As marine ecosystems become destabilized, biodiversity loss is likely to accelerate. This endangers not only ecological balance but also the livelihoods of millions of people who rely on fisheries and aquaculture (Hollowed et al., 2013).

2.8 Socioeconomic Consequences

Food Security Risks: Communities that depend on shellfish and coral reef fisheries are particularly vulnerable to declining stocks and ecosystem collapse (Sovacool and Mukherje, 2011).

Economic Losses: The global seafood industry may face significant economic setbacks. Coral reef tourism, valued at billions of dollars annually, is also at risk (Kuznet, 2019).

3 Biodiversity Loss

Biodiversity loss refers to the decline or disappearance of biological diversity at the genetic, species, and ecosystem levels. As a result of anthropogenic climate change primarily driven by the emission of greenhouse gases (GHGs) natural habitats are increasingly subjected to environmental stressors that alter ecological balances, disrupt species interactions, and undermine ecosystem resilience. Among these stressors, rising temperatures, altered precipitation patterns, ocean acidification, and habitat degradation are particularly detrimental to biodiversity (Isbell et al., 2013).

3.1 Mechanisms of Biodiversity Loss Due to Climate Change

Habitat Alteration and Fragmentation: Shifts in temperature and precipitation can transform ecosystems, rendering them unsuitable for species that are highly specialized or geographically restricted. Alpine, polar, and coral reef ecosystems are especially vulnerable to these changes. Habitat fragmentation also limits species' ability to migrate in response to changing environmental conditions (Liu et al., 2023).

Consequences for Ecosystem Functioning: Biodiversity plays a critical role in ecosystem stability, productivity, and the provision of essential services such as pollination, water purification, carbon sequestration, and soil fertility. Loss of species richness and genetic diversity can reduce an ecosystem's capacity to respond to environmental changes and recover from disturbances, increasing the risk of ecological collapse (Tong et al., 2019).

4 Agricultural Disruption

Agricultural disruption refers to the adverse effects of climate change on crop production, livestock systems, soil health, and overall agricultural productivity. As global temperatures rise and climatic variability intensifies, agricultur the foundation of global food security and rural economies faces multifaceted challenges. These include altered growing seasons, increased frequency of extreme weather events, changing precipitation patterns, and the proliferation of pests and diseases. Such disruptions threaten the stability of food systems, particularly in regions already vulnerable to environmental and socio-economic stressors (Rees et al., 2014).

4.1 Climatic Stressors Impacting Agriculture

Temperature Increases: Higher average temperatures can accelerate crop maturation, leading to reduced yields. Heat stress also negatively affects livestock health, reproduction, and productivity. For many staple crops such as wheat, rice, and maize, there is a critical threshold beyond which yields decline substantially (Ma et al., 2020).

Extreme Weather Events: More frequent and severe heatwaves, storms, and frosts can cause sudden and severe damage to crops and infrastructure. These events not only reduce agricultural output but also increase production costs and risks for farmers (Wohlfart et al., 2008).

Increased Pest and Disease Incidence: Rising temperatures and humidity create favorable conditions for the spread of pests, fungi, and crop diseases, which were previously confined to specific geographic regions. This increases the vulnerability of food systems and requires greater dependence on chemical inputs (Méndez-Bautista et al., 2009).

Soil Degradation: Changes in temperature and precipitation, along with intensive agricultural practices, contribute to soil degradation, reducing fertility and the capacity to support sustainable farming (Cruz et al., 2020).

Regional Disparities and Vulnerabilities: Developing countries, particularly those in tropical and subtropical regions, are more severely affected due to limited adaptive capacity, high dependence on rain-fed agriculture, and constrained access to technology and infrastructure. Smallholder farmers, who represent a large portion of the global agricultural workforce, face increased livelihood insecurity and food scarcity as a result of climate-induced disruptions (Jia et al., 2018).

4.2 Adaptation and Resilience Strategies

Climate-Smart Agriculture (CSA): CSA practices aim to sustainably increase productivity, enhance resilience (adaptation), and reduce greenhouse gas emissions. This includes crop diversification, agroforestry, conservation agriculture, and efficient water management (nazim et al., 2025).

Technological Innovation: The development and dissemination of drought-resistant and heat-tolerant crop varieties, precision farming technologies, and early-warning systems can support adaptation (Lybbert and Sumne, 2012).

Policy and Institutional Support: Governments and international organizations must invest in agricultural research, rural infrastructure, extension services, and risk insurance schemes to support vulnerable farming communities.

Integrated Land and Water Management: Holistic approaches that conserve natural resources and promote sustainable land use are essential to long-term agricultural resilience.

DISCUSSION AND CONCLUSION

Greenhouse gas emissions pose a critical threat to the environment and to human societies. However, through coordinated global action and the implementation of innovative solutions, their negative impacts can be mitigated. Transitioning to a low-carbon future is not only possible but necessary to ensure the health of the planet for future generations.Multiple factors influence the impact of flooding on greenhouse gas emissions, including soil type, water source, and flood duration.

Agricultural disruption is a critical dimension of climate change with profound implications for global food systems, rural livelihoods, and economic stability. Urgent and coordinated adaptation strategies are necessary to ensure the sustainability and resilience of agriculture in a warming world.

Replacing fossil fuels with renewable sources such as solar, wind, and hydropower is one of the most effective ways to reduce GHG emissions. Improving the energy efficiency of buildings, transportation, and industrial processes can significantly cut emissions while reducing energy costs.

CCS technology captures CO_2 from power plants and industrial sources and stores it underground, preventing it from entering the atmosphere. Trees absorb CO_2 through photosynthesis. Large-scale tree planting can act as a natural carbon sink, helping offset emissions. Practices such as crop rotation, reduced tillage, and efficient fertilizer use can lower emissions from agricultural activities, especially methane and nitrous oxide.

Global cooperation through agreements like the Paris Accord is essential. Governments should implement carbon pricing, subsidies for clean energy, and stricter emission regulations.

In conclusion, the reduction of greenhouse gas emissions should not be confined solely to measures taken by governments and large corporations; rather, it should be recognized as a global responsibility in which individuals must also play an active role. While governments and businesses can make significant contributions by investing in renewable energy, enforcing strict environmental regulations, and implementing policies to limit carbon emissions, individuals can support this process by adopting eco-friendly habits in their daily lives. Key individual actions include conserving energy, opting for sustainable transportation methods, embracing recycling practices, making conscious consumption choices, and preferring products that minimize greenhouse gas emissions. In this context, the adoption of sustainable policies in both production and consumption, the promotion of innovative environmental technologies, and raising public awareness will be crucial in mitigating the devastating effects of climate change.

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FROM SOIL TO SURVIVAL: PGPB-TRIGGERED DEFENSE AND ADAPTATION IN PLANTS

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ABSTRACT

Plants and beneficial soil bacteria engage in dynamic and reciprocal interactions that influence plant development, nutrient acquisition, and stress resilience. Among these, plant growth-promoting bacteria (PGPB) have garnered considerable attention due to their ability to support plant health through a range of biochemical and physiological mechanisms. These bacteria produce a variety of bioactive compounds that enhance plant stress tolerance, improve nutrient availability, and offer protection against phytopathogens.

PGPB influence plant performance through both direct and indirect mechanisms. Direct pathways include the biosynthesis of phytohormones (such as indole-3-acetic acid), solubilization of essential nutrients like phosphate, zinc, and potassium, ammonia production, and atmospheric nitrogen fixation. Indirectly, they contribute by secreting siderophores, lytic enzymes, hydrogen cyanide, and antibiotics, which suppress harmful microorganisms and enhance plant immunity.

These functional traits position PGPB as valuable components in sustainable agriculture, especially for the development of bioformulants including biofertilizers, biopesticides, and biofungicides. Such alternatives reduce dependency on chemical inputs and contribute to environmentally responsible crop management. Nonetheless, despite the expanding repertoire of beneficial strains, the practical implementation of PGPB in agriculture remains challenging. Factors such as microbial survival, strain specificity, plant-microbiome compatibility, and fluctuating environmental conditions can influence their efficacy in field applications.

This review explores recent advances in understanding PGPB-mediated plant adaptations to abiotic and biotic stressors, with emphasis on molecular mechanisms, signaling pathways, and metabolite production. It also discusses formulation strategies and delivery systems designed to maximize their stability and performance. Ultimately, leveraging PGPB potential requires a deeper integration of microbiology, plant physiology, and environmental science to ensure their consistent and scalable use in sustainable agricultural systems.

Keywords: Plant Growth-Promoting Bacteria, stress tolerance, sustainable agriculture, biocontrol

INTRODUCTION

Through the release of bioactive chemical signals, bacteria and plants interact and communicate in a dynamic and ongoing co-evolutionary relationship. Both positive and negative interactions are possible (Wille et al., 2019). The plants gain from positive relationships because they help them acquire minerals, phytohormones, and other nutrients. By releasing a number of bioactive chemicals, these helpful bacterial species can also combat phytopathogens and help plants withstand a variety of stressful situations. Conversely, detrimental interactions are detrimental because infections infiltrate plant tissues, which causes the host plants to die (Dolatabadian, 2021; Adedayo et al., 2022). Utilizing these advantageous microbes can therefore help plants withstand stress that reduces their yield.

As part of the soil ecosystem, microorganisms have a major impact on the nitrogen cycle, soil fertility management, and plant diversity preservation. According to Zhou et al. (2020), a crucial area for important biological interactions between plants and microorganisms is the rhizosphere, a tiny area that surrounds the plant roots. Microorganisms such as bacteria, actinobacteria, fungus, algae, and protozoa aggressively compete for food and space in this productive area (Manghwar et al., 2023). Both the host and the microorganisms benefit from the ability of the plant growth promoting microorganism (PGPM) to live in and interact with plant roots; a population of rhizospheric bacteria and fungi may also serve as a home for other microbes (dos Lopes et al., 2021). The most prevalent of all the helpful microorganisms are bacteria, which are followed by fungus and actinobacteria (Poria et al., 2021). Through direct or indirect methods of nutrient absorption and assimilation, they have a beneficial effect on the plant (Kumari et al., 2018).

Food security is threatened by the world's population growth, which increases the need of fertilizers based on inorganic chemicals, which is bad for the environment and human health (Mitter et al., 2021). Because of the numerous environmental stressors that also contribute to low crop yields, organic farming that relies on microflora such as PGPM guarantees food availability while improving crop quality, productivity, and environmentally friendly farming practices (Jalal, et al., 2023). Therefore, by reducing the use of chemical fertilizers, crop production using PGPM provides sustainability and protects soil biodiversity (dos Lopes et al., 2021).

The majority of plant growth-promoting bacteria (PGPB) are proteobacteria. It includes genera like *Pantoea, Thiobacillus, Pseudomonas, Micrococcus, Rhodococcus, Azospirillum, Azotobacter, Acinetobacter, Acetobacter, Klebsiella, Enterobacter, Alcaligenes, Arthrobacter, Burkholderia, Azorhizobium, Achromobacter, Serratia, Bradyrhizobium, Flavobacterium, Mesorhizobium, Microrhizobium, Streptomyces, Bacillus, Azoarcus, Aeromonas, Azoarcus, Caulobacter, Chromobacterium, Delftia, Frankia, Flavobacterium, Gluconacetobacter, Paenibacillus, Rhizobium* and *Streptomyces* (Table 1).

Table 1. Plant growth promoting bacteria and their common host plant family (from Fanai et al., 2024).

Family	**Plant**	**Plant growth-promoting bacteria**
Fabaceae	*Phaseolus vulgaris*	*Rhizobium acidosoli, R. endophyticum, R. esperanzae, R. etli, R. hidalgoense, R. mesoamericanum, R. tropici, Acinetobacter* sp.
Poace	Rice, wheat, maize, soirghum, sugarcane	*Azospirillum* sp.
Asteraceae	Puticaria	*Bacillus cereus, Agrobacterium fabrum, Brevibacillus brevis, Bacillus subtilis, Paenibacillus* sp., *Acinetobacter radioresistant, Burkholderia* sp.
Solanaceae	*Artemisia annua*	*Brevibacillus* sp., *Bacillus* sp., *Pseudomonas* sp., *Azospirillum* sp., *Klebsiella* sp., *Enterobacter* sp., *Alcaligenes* sp., *Azotobacter* sp., *Streptomyces* sp., *Pantoea* sp., *Bacteroides* sp., *Proteobacteria* sp., *Radiobacter* sp., *Stenotrophomonas* sp.
Brassicaceae	*Brasica oleraceae*	*Pseudomonas* sp., *Enterobacter* sp., *Arthrobacter* sp., *Pantoea* sp.
Crasulaceae	*Echevari laui*	*Erwinia* sp., *Pantoea* sp.

In addition to helping plants tolerate a variety of biotic and abiotic stressors, the PGPB also promotes plant growth by promoting the solubility of various inorganic mineral nutrients, fixing nitrogen, releasing plant growth regulators, and producing a number of other biochemicals that either directly or indirectly increase plant productivity. Figure 1 shows the several ways that bacteria can promote plant growth.

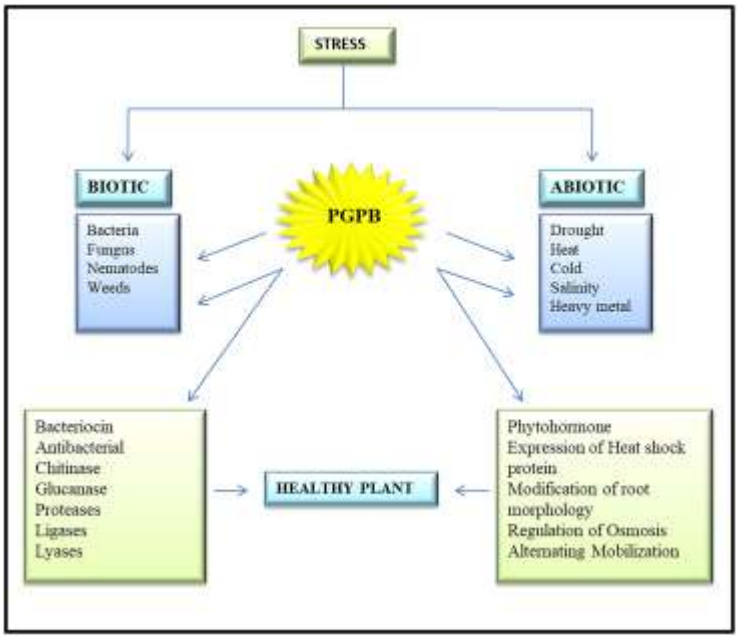


Figure 1. Functions that plant growth-promoting bacteria provide. The impacts of both biotic (caused by living things) and abiotic (induced by environmental variables) stress on plants demonstrate how PGBP helps to lessen the effects of stress. Through processes including siderophores, lytic enzymes, HCN, antibiotics & nitrogen fixation, hormone synthesis, etc., PGBP improve plant development, nutrient absorption, and stress tolerance (from Fanai et al., 2024).

One possible approach to a sustainable future is the use of helpful microorganisms as bioinoculants in agricultural systems. As an effective, environmentally friendly, and productive fertilizer that could help meet the growing demand for food from the world's population, it is a good substitute for chemical fertilizers. They improved the soil microbiota and preserved soil fertility in addition to promoting plant growth.

ABIOTIC AND BIOTIC STRESS FACTORS

Continuously subjecting plants to biotic and abiotic stressors has a negative effect on their growth and development, which lowers yield and quality (Singh et al., 2021). With the help of a naturally occurring PGPB that increases resistance against different phytopathogens by generating biochemicals and improving soil fertility, plants subsequently develop particular kinds of defense mechanisms for stress response (Leontidou et al., 2020). Figure 2 shows the various stressors and bacterial stress tolerance systems.

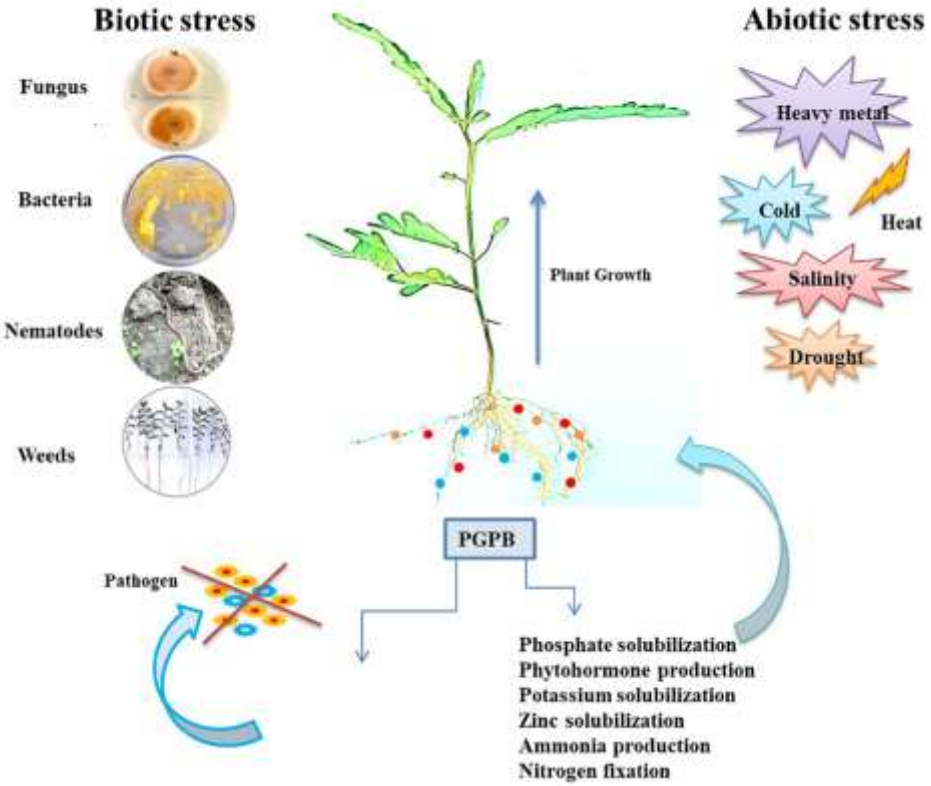


Figure 2. Various stressors and bacteria that support plant growth react to provide plant growth promotion. The impacts of both biotic (caused by living things) and abiotic (induced by environmental variables) stress on plants demonstrate how PGBP helps to lessen the effects of stress. By means of processes like nitrogen fixation, hormone synthesis, and biocontrol, PGBP improve plant growth, nutrient absorption, and stress tolerance (from Fanai et al., 2024).

Drought stress is categorized into hydrological, socio-economic, meteorological, and agricultural types, each leading to soil moisture deficits and the accumulation of reactive oxygen species that damage plant structure and function (Ahluwalia et al., 2021). Traditional mitigation measures—such as biochar amendments, nanoparticle treatments, film farming, and drought-resistant cultivars—have shown only limited benefits in real agricultural systems (Fadiji et al., 2022). By contrast, plant growth-promoting bacteria (PGPB) enhance drought tolerance through improved water uptake, root architecture modification, and production of protective phytochemicals (Khan and Bano, 2019). For example, inoculation with *Bacillus pumilus* and *Pseudomonas putida* improved maize drought resilience and nutrient acquisition (Kálmán et al., 2024), while *Bacillus subtilis* and *Azospirillum brasilense* increased osmolyte synthesis to support wheat germination under water deficit (Ilyas et al., 2020). Strains such as *Pseudomonas lini* and *Serratia plymuthica* enhance soil aggregate stability, and *Bacillus albus* together with *Bacillus cereus* boost seed vigor, pigment concentration, and antioxidant activity, collectively strengthening photosynthetic efficiency and stress defenses (Zhang et al., 2020; Ashry et al., 2022; Saleem et al., 2021). Other notable drought-tolerant bacteria include *Achromobacter xylosoxidans*, *Pseudomonas aeruginosa*, *Lelliottia adecarboxylata*, *Enterobacter cloacae*, *Pseudomonas fluorescens* S3X, and *Staphylococcus sciuri* (Danish et al., 2020; Khalilpour et al., 2021).

A rise in average global temperatures poses a significant abiotic challenge for crop productivity (Desaint et al., 2021). Heat-tolerant microbes—including *Bacillus cereus, Serratia liquefaciens, Pseudomonas putida, P. fluorescens* (Mitra et al., 2021), *Burkholderia phytofirmans, Curvularia protuberata* (Rana et al., 2021), *Paraburkholderia phytofirmans*, and various *Bacillus* and *Pseudomonas* spp. (Ahmad et al., 2023)—mitigate thermal damage by modulating plant hormone levels (e.g., cytokinins, ACC deaminase) and activating antioxidant enzymes that control water uptake and induce heat-shock protein expression (Moumbock et al., 2021). Exogenous application of specific amino acids further alleviates heat-stress effects by enhancing stress-responsive pathways (Santos et al., 2022). Inoculation with *B. cereus* has been shown to boost overall biomass, chlorophyll content, and heat-shock protein accumulation (Khan et al., 2020b), while *Enterobacter* sp. SA187 increased wheat and *Arabidopsis* heat endurance, leading to higher grain yield, plant height, and seed weight. Moreover, *B. cereus* enhances carotenoid, protein, ascorbate peroxidase, chlorophyll, and superoxide dismutase levels (Bisht and Mishra, 2020), and strains such as *B. thuringiensis, B. subtilis, P. brassicacearum* (Ashraf et al., 2019), and *B. velenzensis* further demonstrate the promise of microbial inoculants for improving plant thermotolerance.

Soil salinization, driven by water scarcity and the accumulation of NaCl-rich compost from sewage and waste treatment, and the build-up of soluble ions—bicarbonate, magnesium, sodium, chloride, sulfate, carbonate, and calcium—alters soil composition, diminishes fertility, impairs germination, and disrupts chloroplast structure, leading to reduced pigment synthesis, ion toxicity, and osmotic imbalance (Ahmed et al., 2020; Krishnamoorthy et al., 2022). Salinity stress further reduces plant development, fruit yield, biomass, stomatal conductance, and water movement (Ansari et al., 2019). Plant growth-promoting bacteria counteract these adverse effects by inducing stress-responsive pathways that lower reactive oxygen species production, synthesizing Na^+-binding exopolysaccharides, and producing phytohormones to enhance root growth and water uptake (Subramaniam et al., 2020). For instance, *Bacillus amyloliquefaciens* GB03 releases volatile organic compounds that boost stress mitigation sixfold (Cappellari et al., 2020). Additionally, halotolerant strains of *Streptomyces, Aneurinibacillus aneurinilyticus, Paenibacillus* spp., *Pseudomonas azotoformans*, and other genera such as *Bacillus, Enterobacter, Klebsiella, Agrobacterium*, and *Ochrobactrum* can tolerate NaCl up to 150 g/L, highlighting their promise in reclaiming saline soils (Liu et al., 2021).

Plant growth-promoting microbes (PGPM) suppress biotic stresses from pathogenic fungi, bacteria, nematodes, insects, and weeds by activating systemic acquired resistance (SAR) and induced systemic resistance (ISR). SAR arises when PGPM directly inhibit pathogens, whereas ISR is triggered by bacterial elicitors such as volatile organic compounds (VOCs), microbe-associated molecular patterns (MAMPs), and bioactive secondary metabolites (Dubey et al., 2020). These microbes employ lytic enzymes, antibiotics, and VOCs for direct antagonism and enhance plant defenses indirectly through nitrogen fixation, siderophore production, phosphate solubilization, and phytohormone signaling (Migunova and Sasanelli, 2021). For instance, *Pasteuria penetrans* and *Brevibacillus laterosporus* secrete proteases that inhibit *Heterodera glycines* (Mohan et al., 2020), while *Bacillus megaterium* combats *Meloidogyne graminicola* similarly. Strains like *Pseudomonas aeruginosa, P. cepacia*, and *P. fluorescens* exhibit broad-spectrum antimicrobial and anthelmintic activities (Bhavya and Geetha, 2021), and *Bacillus subtilis* and *B. pumilus* produce chitinases and antifungal VOCs that disrupt nematode chitin synthesis. *Bacillus amyloliquefaciens* FZB42 and *Arthrobacter nicotianae* release bacteriocins and VOCs that inhibit *Meloidogyne* spp. By colonizing the rhizosphere and producing siderophores, phytohormones, and antibiotics, PGPM also inhibit soil-borne fungi, solubilize phosphate, and promote plant growth via indole-3-acetic acid synthesis. These combined actions outcompete pathogens for nutrients and induce ISR against

bacterial genera—such as *Erwinia, Pectobacterium, Pantoea,* and *Xanthomonas*—that cause wilts, blights, galls, and root rots (Nazari and Smith, 2020; Nabila and Kasiamdari, 2021).

Table 2. Overview of Plant Growth-Promoting Rhizobacteria functions (from Priyanka et al., 2020)

Function of PGPR	**Description**	**PGPR strain**
Nitrogen fixation	Conversion of free atmospheric nitrogen into oxide form that can be used	*Enterobacter, Klebsiella, Burkholderia,* and *Stenotrophomona*
Phosphate solubilization	Conversion of insoluble phosphate into the soluble form absorbed by the plants	*Achromobacter, Agrobacterium, Bacillus, Enterobacter, Erwinia, Escherichia, Flavobacterium, Mycobacterium, Pseudomonas* and *Serratia*
Potassium solubilization	Conversion of potassium in the useful form	*Acidothiobacillus ferrooxidans, Bacillus edaphicus, Bacillus mucilaginosus, Burkholderia, Paenibacillus* sp. and *Pseudomonas*
Production of siderophore	Enhancing the source of iron in the soil that can be absorbed by the plants	*Aeromonas, Azadirachta, Azotobacter, Bacillus, Burkholderia, Pseudomonas, Rhizobium, Serratia* and *Streptomyces* sp.
Phytohormone production	Production of phytohormones such as auxin, cytokinins, gibberellic acid and ethylene	*Azotobacter*sp., *Rhizobium* sp., *Pantoea agglomerans, Rhodospirillum rubrum, Pseudomonas fluorescens, Bacillus subtilis* and *Paenibacillus* sp.

DIRECT AND INDIRECT MECHANISMS OF PGPB IN ENHANCING PLANT PERFORMANCE

Indirect mechanisms

Siderophore production: Bacteria secrete ferric-specific ligands (hydroxamates, phenolates, carboxylates) to solubilize iron for themselves and host plants, enhancing root colonization and outcompeting pathogens; common producers include *Bacillus, Chryseobacterium, Phyllobacterium,* and *Pseudomonas* spp. (Bhatt et al., 2019).

Protease secretion: Microbial proteases (alkaline, acidic, neutral)—notably from *Bacillus* spp.—hydrolyze pathogen proteins and generate antifungal metabolites (e.g., serine proteases, subtilisins), serving as an indirect plant defense; activity is screened on skim-milk agar by clear halo formation (Castaldi et al., 2021).

Catalase activity: Catalase-positive strains (e.g., *Bacillus marinus, B. sphaericus, Staphylococcus aureus*) detoxify H_2O_2 at root interfaces, mitigating oxidative damage under stress; detected by bubble formation in 3% H_2O_2 (Talaiekhozani, 2022).

Amylase production: Alpha-amylases from endophytes like *Bacillus amyloliquefaciens* and *B. licheniformis* degrade pathogen cell walls and contribute to industrial applications (detergents, stain removal); screened on starch agar via iodine-stained halos (Ismail et al., 2021).

Urease activity: Ureolytic bacteria hydrolyze urea into plant-available ammonium and nitrates, supporting nitrogen nutrition and biomineralization (soil enrichment, concrete repair); measured by pH-dependent color change in urea broth (Cui et al., 2022).

Hydrogen cyanide (HCN) release: Rhizobacteria (mostly *Pseudomonas* and *Bacillus* spp.) emit HCN to inhibit fungi, nematodes, insects, and weeds without harming the host plant; detected via alkaline-picrate colorimetric assay (Alemu, 2016).

Table 3. Mechanisms and functions of PGPB (*adapted from Olanrewaju et al. (2017) and Fanai et al. (2024))*

Mechanism	**Category**	**Primary Function**	**Reference**
Indole-3-acetic acid (IAA)	Direct	Stimulates root initiation, cell division, lateral root formation	Olanrewaju et al. (2017)
ACC deaminase activity	Direct	Degrades precursor of ethylene; alleviates abiotic-stress inhibition	Olanrewaju et al. (2017)
Cytokinin production	Direct	Promotes shoot growth, delays leaf senescence	Fanai et al. (2024)
Nitrogen fixation	Direct	Converts atmospheric N_2 into bioavailable NH_4^+	Fanai et al. (2024)
Phosphate solubilization	Direct	Releases organic acids to convert insoluble P into plant-available forms	Fanai et al. (2024)
Siderophore production	Indirect	Sequesters Fe^{3+}, suppresses pathogen growth, improves iron nutrition	Olanrewaju et al. (2017)
Lytic enzyme secretion	Indirect	Produces chitinases/proteases to degrade fungal and nematode walls	Olanrewaju et al. (2017)
Hydrogen cyanide (HCN) emission	Indirect	Volatile compound that inhibits soil-borne pathogens and weeds	Olanrewaju et al. (2017)
Antibiotic metabolite release	Indirect	Synthesizes antimicrobial compounds against bacteria and fungi	Olanrewaju et al. (2017)
Induced systemic resistance (ISR)	Indirect	Elicits plant defense pathways via MAMPs and VOCs	Fanai et al. (2024)

Direct mechanisms

Phytohormone production: Plant growth–promoting bacteria (PGPB) synthesize cytokinins, gibberellins, and especially indole-3-acetic acid (IAA), which regulate cell division, root initiation, and stress responses. Strains such as *Paenibacillus polymyxa, Rhizobium leguminosarum, Pseudomonas fluorescens* produce cytokinins (Mekureyaw et al., 2022), while *Bacillus pumilus* and *B. licheniformis* generate multiple gibberellins (Gutiérrez-Mañero et al., 2001). IAA producers, including *Azospirillum, Arthrobacter, Bradyrhizobium, Pantoea, Rahnella*, and *Enterobacter*, enhance root elongation and nutrient uptake (Rehman et al., 2020).

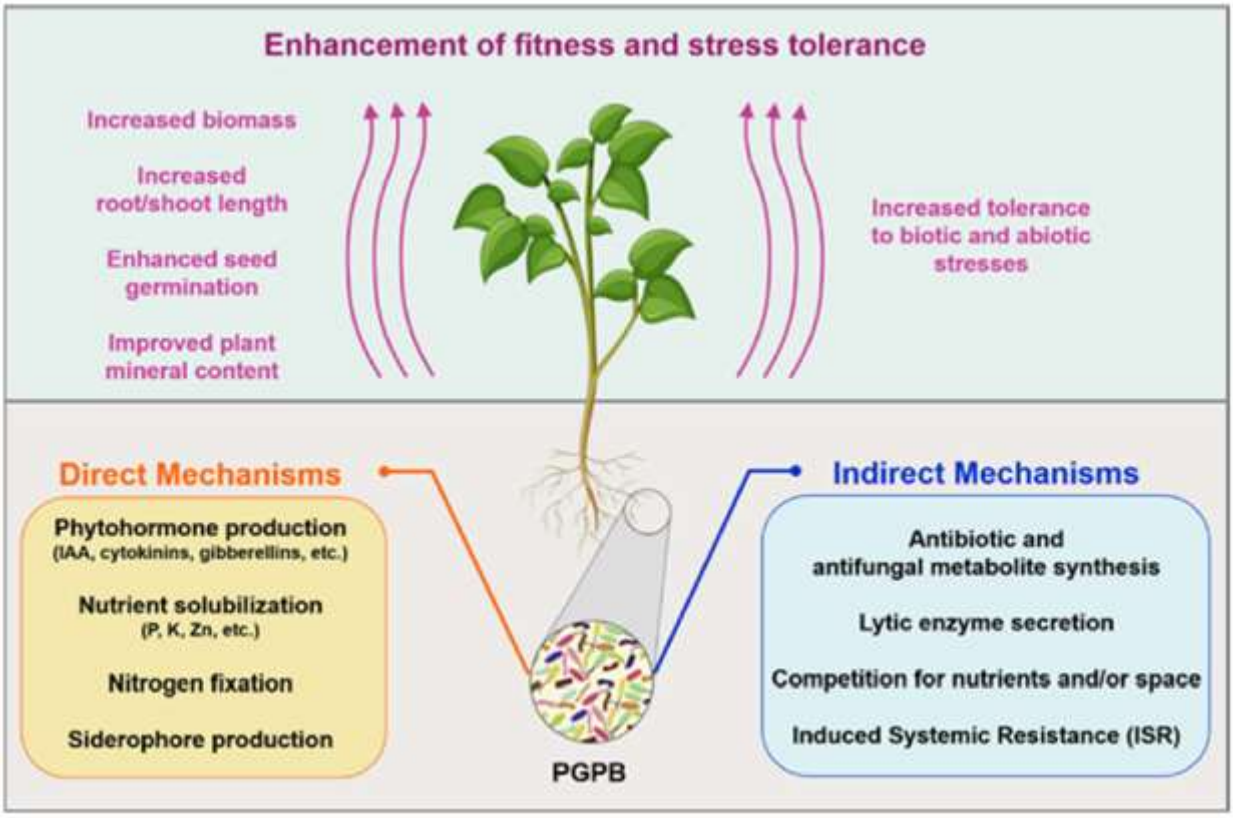
Phosphate solubilization: PSB release organic acids (citric, gluconic) that chelate cations and convert insoluble phosphates (tricalcium, rock phosphate) into bioavailable forms. Key taxa include *Pseudomonas putida, Azospirillum lipoferum, Bacillus firmus, B. polymyxa, Serratia marcescens*, and *Arthrobacter aureofaciens*. PSB inoculation boosts phosphorus

availability and activates stress-responsive genes in crops like potato and sugarcane (Lin et al., 2023).

Ammonia production: Ammoniagenic PGPB such as *Pseudomonas putida*, *Klebsiella* spp., and *Enterobacter asburiae* generate NH_3, raising soil pH to suppress pathogens and supply inorganic nitrogen, which enhances root/shoot growth and biomass (Gohil et al., 2022).

Nitrogen fixation: Symbiotic genera (*Bradyrhizobium*, *Mesorhizobium*, *Sinorhizobium*, *Azorhizobium*, *Neorhizobium*) and free-living diazotrophs (*Azotobacter*, *Azospirillum*, *Burkholderia*, *Pseudomonas*, cyanobacteria) convert atmospheric N_2 into plant-available forms, supporting growth and yield (Basile and Lepek, 2021). Microbial consortia of *B. subtilis* and *A. brasilense* enhance root/shoot development, gas exchange, and grain yield in wheat, while co-inoculation of *Bradyrhizobium* and *Bacillus* improves nodulation and *Vigna unguiculata* productivity (Galindo et al., 2024; Gaspareto et al., 2023).

Zinc solubilization: Zinc is a vital enzyme co-factor, with optimal plant tissue levels at 30–100 $mg \cdot kg^{-1}$; deficiency impairs growth and causes necrotic lesions. Zinc-solubilizing



bacteria secrete organic acids and iron-chelating enzymes to lower rhizosphere pH and release Zn^{2+} (Eshaghi et al., 2019). Key taxa include *Pseudomonas aeruginosa*, *Gluconacetobacter diazotrophicus*, *P. striata*, *P. fluorescens*, *Burkholderia cenocepacia*, *Serratia liquefaciens*, *S. marcescens*, *Bacillus thuringiensis*, *B. aryabhattai*, *B. subtilis*, *Thiobacillus thioxidans*, and cyanobacteria. *Rhizobium*, *Pseudomonas*, and *Bacillus* spp. enhance Zn translocation and grain biofortification, improving wheat quality via exopolysaccharide and siderophore production (Jalal et al., 2024). Co-inoculation of *B. subtilis* or *P. fluorescens* with foliar ZnO further boosts maize chlorophyll, amino acids, glutelin, and prolamin content. Bacterial Zn nanoparticles inhibit biofilm formation in pathogens and augment human oral microbiome antimicrobial activity (Lallo da Silva et al., 2019).

Figure 3. Overview of the main mechanisms used by PGPB to improve plant growth (from Vuolo et al., 2022)

Potassium solubilization: Although abundant in soils, K often exists in insoluble forms; PGPB release organic acids (citric, oxalic, tartaric) to chelate and solubilize K^+, facilitating uptake (Olaniyan et al., 2022). Potassium activates over 60 plant enzymes, regulates stomatal dynamics, enhances disease resistance, fiber strength, and produce quality, and mitigates water-stress sensitivity (Rawat et al., 2022). Notable K-solubilizers include *Acidithiobacillus, Burkholderia, Pseudomonas* spp., *Bacillus megaterium, Arthrobacter, Pantoea ananatis, Rahnella aquatilis, Enterobacter* sp., *Bacillus/Paenibacillus mucilaginosus, Bacillus licheniformis, P. azotoformans, B. edaphicus,* and *Pseudomonas putida* (Bakhshandeh et al., 2017).

CONCLUSION

Plant growth–promoting bacteria represent a cornerstone of sustainable agriculture by naturally supplying key metabolites—phytohormones, siderophores, lytic enzymes, hydrogen cyanide, and ammonia—and by solubilizing essential nutrients (P, Zn, K) or fixing atmospheric nitrogen. Their multifaceted modes of action not only enhance crop yield and quality under both biotic and abiotic stresses but also reduce reliance on synthetic agrochemicals, thereby protecting soil health and the broader environment.

To translate this promise into practice, future work must prioritize the development and field-ready formulation of robust biofertilizers and biocontrol agents tailored to specific crops and stress conditions. Equally critical is the systematic evaluation of PGPB–host compatibility to safeguard beneficial traits during root colonization, as well as the design of microbial consortia whose members coexist synergistically without compromising individual functionality. Addressing these challenges will pave the way for precision applications of PGPB, driving both productivity gains and ecological resilience in modern farming.

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TOXIC DIALOGUES: HERBICIDES, MICROBIAL VOICES, AND THE ECOSYSTEM'S RESPONSE

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ABSTRACT

Herbicides, long heralded as precise tools in agricultural management, leave behind echoes that reverberate through the most intimate layers of ecological networks. This study explores how herbicidal interventions—especially those involving compounds like glyphosate—silently alter microbial communities that anchor essential ecosystem functions. These non-target effects begin at the microscopic scale, where shifts in soil microbiome composition interrupt vital processes such as nutrient cycling, rhizosphere signaling, and plant–soil feedback mechanisms.

Framed through a microbiome-centric lens, we present a synthesis of recent studies alongside conceptual models illustrating how herbicide residues serve not only as chemical stressors, but as agents that restructure the metabolic dialogues among microbes and their hosts. Alterations in microbial diversity and function affect plant vitality, reduce resilience to biotic and abiotic stresses, and cascade upward to influence animal performance, herbivory dynamics, and pollination success. As herbicides disrupt these finely tuned interdependencies, we observe potential tipping points that may reshape ecosystem trajectories and even influence microbial and host evolution.

More than collateral damage, microbial responses to herbicidal exposure represent a silent rebellion—one with the power to mediate or magnify ecological instability. We advocate for integrative strategies that acknowledge microbiome health as a central criterion in agrochemical risk assessments. Understanding these subterranean symphonies is essential for mitigating the long-term consequences of anthropogenic disturbance and for crafting sustainable, microbe-conscious stewardship of ecosystems.

Keywords: microbiome, herbicides, soil microbial networks, environmental resilience, agrochemical impact, microbe-mediated risk

INTRODUCTION

Herbicides have revolutionized modern agriculture by enabling targeted and efficient weed control, contributing significantly to crop productivity and food security. Among these, **glyphosate** stands as one of the most extensively used broad-spectrum herbicides worldwide due to its effectiveness and non-selective inhibition of the *5-enolpyruvylshikimate-3-phosphate synthase* (EPSPS) enzyme in the shikimate pathway (Duke and Powles, 2008). However, while glyphosate's primary mode of action is aimed at plants, this same biochemical pathway is present in many soil microorganisms, raising growing concerns about its unintended effects on non-target species (van Bruggen et al., 2018).

Soil microbial communities, particularly those inhabiting the **rhizosphere**, play a pivotal role in maintaining ecosystem functions. These communities mediate **nutrient cycling, organic matter decomposition**, and intricate **plant–microbe interactions** that influence plant growth, health, and resistance to environmental stressors (Berg et al., 2020). Disturbances to microbial composition and diversity—whether through direct toxicity or altered plant exudation patterns—can ripple through the entire ecosystem. Emerging evidence suggests that herbicide residues can suppress **mycorrhizal colonization**, shift **bacterial community structures**, and increase plant susceptibility to **soil-borne pathogens** (Zaller et al., 2014; Newman et al., 2016; Johal and Huber, 2009).

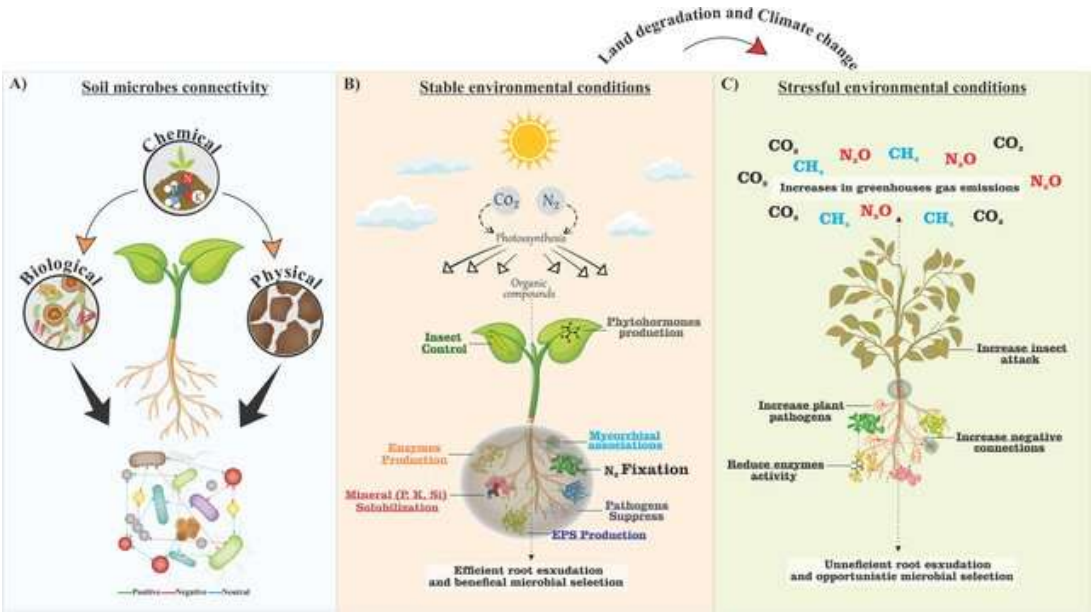

Figure 1. The intricate connections between soil biodiversity and plants. a These interactions are influenced, including chemical, physical, and biological variability. b In a stable ecosystem with no or low human disturbances, plants can effectively recruit their microbial communities, primarily facilitated through rhizosphere exudation and selection processes. This plant-soil biodiversity interactions catalyze multiple ecosystem processes such as enhanced enzyme activity, solubilization, nitrogen fixation, and disease suppressiveness. However, land degradation and ongoing climate changes disrupt these interactions, leading to an increased negative correlation among taxa. c As a result, plants become more vulnerable to harmful and opportunistic agents like insects and pathogens (from Pedrinho et al., 2024).

Importantly, these microbial alterations are not merely short-term or incidental. Herbicides may induce long-term **restructuring of microbial networks,** with implications for microbial evolution, functional redundancy, and overall soil resilience (Timmis et al., 2019). Furthermore, **chronic exposure** to glyphosate and similar compounds may drive microbial **adaptation or resistance,** potentially reducing the soil's capacity to buffer abiotic stressors such as drought or nutrient depletion (Schlatter et al., 2017).

Given the central role of the microbiome in **agroecosystem stability**, there is an urgent need to reconsider how herbicides are evaluated in terms of ecological risk. Traditional assessments focusing solely on crop yield or visible phytotoxicity fall short of capturing the subtle but profound consequences on **microbial health.** Integrating a **microbiome-centric perspective** into agrochemical regulation offers a more holistic understanding of ecosystem sustainability and resilience (Mitter et al., 2019).

This paper synthesizes recent findings on the impact of herbicides—especially glyphosate—on soil microbial communities. It aims to elucidate the biochemical, ecological, and evolutionary consequences of microbial disruption, and to advocate for frameworks that prioritize **microbial integrity** as a cornerstone of sustainable agriculture.

HERBICIDES AND THEIR LEGACY

Herbicides have revolutionized modern agriculture by enabling efficient weed control, reducing labor costs, and increasing crop yields. Since the introduction of synthetic herbicides like 2,4-D in the 1940s, over 270 active ingredients have been commercialized, shaping global farming practices and food systems (Mesnage et al, 2021; U.S. EPA, 2004). However, their legacy is complex—marked by ecological disruption, resistance evolution, and persistent residues in soil and water. Herbicides have played a central role in modern agricultural intensification, with compounds like **glyphosate, atrazine**, and **2,4-D** becoming dominant tools in weed control (Duke and Powles, 2008). Glyphosate, in particular, has been praised for its broad-spectrum action and post-emergent use. However, its primary target—the *shikimate pathway*, absent in animals but present in most plants and microbes—raises significant concerns about **non-target effects** on microbial communities (van Bruggen et al., 2018). While herbicides were initially believed to degrade rapidly in soil, increasing evidence shows that **residues persist** and interact with complex biotic and abiotic soil components, affecting ecosystem function far beyond weed suppression (Silva et al., 2019). Herbicides such as glyphosate, atrazine, and dicamba have become staples in conventional farming due to their broad-spectrum activity and compatibility with genetically modified (GM) crops (Parven et al., 2024). Systemic and pre-emergence herbicides offer targeted control, reducing the need for repeated applications and mechanical weeding.

Despite their utility, herbicides pose significant risks, as s**oil degradation** (long-term use alters microbial communities, reduces biodiversity, and impairs nutrient cycling), w**ater contamination** (runoff and leaching introduce herbicide residues into aquatic ecosystems, affecting non-target organisms like amphibians, fish, and invertebrates) and r**esistance development** (overuse has led to herbicide-resistant weed populations, complicating management and increasing chemical dependency (Mesnage and Antoniou, 2017).

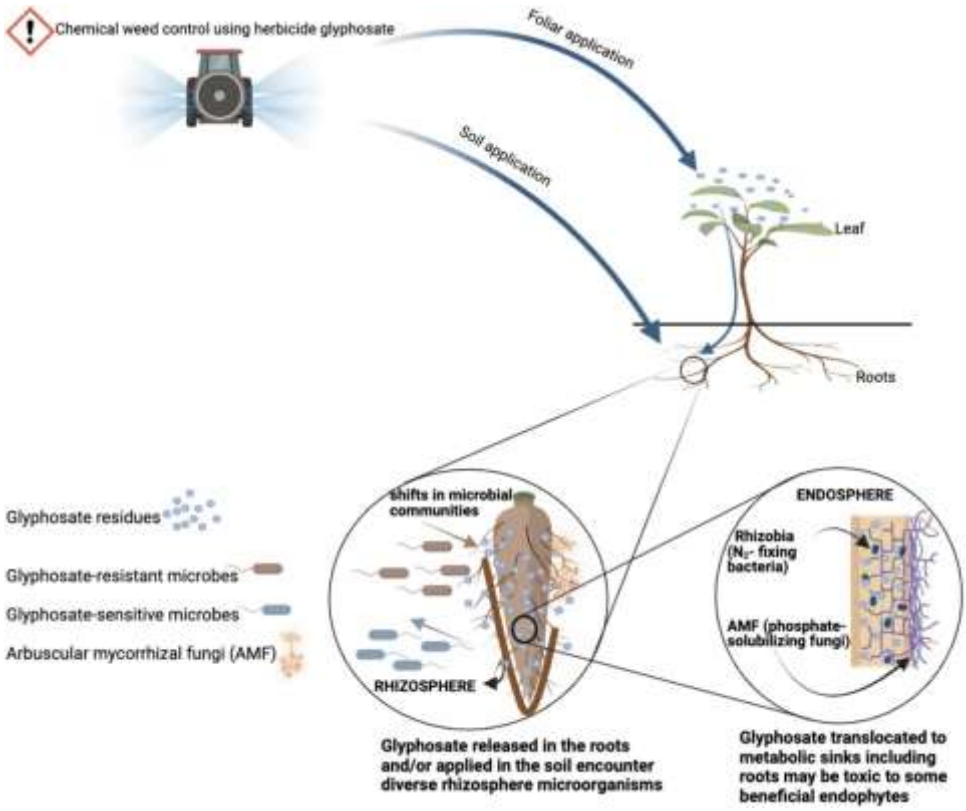

Figure 2. Possible interference of glyphosate with microbial communities in the rhizosphere and endosphere (from Sibalekile et al., 2025).

THE RHIZOSPHERE MICROBIOME: KEYSTONE OF SOIL HEALTH

The **rhizosphere microbiome**—microorganisms that inhabit the narrow zone surrounding plant roots—plays a foundational role in **nutrient cycling, stress response, and plant immunity** (Berg et al., 2020).

The specific functions of soil microbes can be divided into seven major groups (Berendsen et al., 2012; Cheng et al., 2022; Kumar et al., 2022):

- Decomposition: Microbes—bacteria and fungi—break down organic matter such as crop residues, insect carcasses, animal manure, and other organic materials in the soil. Microbes secrete enzymes that help break down complex organic compounds into simpler forms, releasing nutrients that plants can use. Microbes contribute to humification as they break down organic compounds. Humification produces stable, mature organic compounds that increase the organic matter content of the soil. This offers additional benefits to crops, such as increased water-holding capacity, nutrient retention, and carbon sequestration.
- Nutrient cycling: Microbes are involved in the circulation of essential nutrients in the soil. They can “unlock” nutrients that are locked up in forms inaccessible to the plant and therefore promote the availability of nutrients for plant absorption. Nitrogen fixation (mineralization) is one example of how many beneficial soil microbes convert organic nutrients into inorganic forms that can be absorbed by plants. Soil microbes also unlock nutrients from inorganic “bound” forms.
- Soil structure and aggregation: Certain bacteria and fungi produce sticky substances such as polysaccharides that bind soil particles together, forming aggregates. Soil

aggregates improve soil structure, porosity, and water-holding capacity, allowing for better root penetration and aeration.

- Disease suppression: Certain beneficial bacteria and fungi called biocontrol agents can suppress soil-borne pathogens and pests. Biocontrol agents suppress pathogens and pests through a variety of processes, including: producing antimicrobial compounds, consuming or parasitizing pests and pathogens, or outcompete pathogens for soil niches. The term "disease-suppressing soils" is often used to refer to fields that experience little or no impact from soil-borne diseases, even when the diseases are widespread throughout the surrounding region or minimal crop protection products are used. Microbial biocontrol agents are known to play a key role in the ability of disease-suppressing soils to mitigate the impacts of pathogens.
- Phytohormone Production: Some soil microbes can produce plant hormones in the soil, many of which are called "plant growth regulators" or PGRs. Hormones such as auxin, cytokinin, and gibberellin are known to support plant growth, yield, and stress tolerance when applied as foliar products. However, their routine application can be very expensive. Microbial hormone producers in the soil can also offer these benefits and have been found to support crop resistance to stressors such as drought and activate plant defense systems to ward off pathogens.
- Symbiotic relationships: Many plants form beneficial symbiotic relationships with soil microbes. Mycorrhizal fungi form associations with plant roots, extending their reach for water and nutrients in exchange for microbial “food” in the form of carbon produced by the plant through photosynthesis. Mutually beneficial bacteria, known as endophytes, live within plant tissues and promote plant growth by performing beneficial processes such as nitrogen fixation and carbon uptake through root exudates. This symbiotic relationship improves plant nutrient uptake, overall plant and soil health, and the efficiency of agricultural systems. However, common agricultural practices can disrupt these relationships, leading to a decline in beneficial microbes and increased dependence on agricultural inputs.
- Salinity regulation: Some microbes have adapted to survive in saline environments and possess mechanisms to tolerate high salt concentrations. They can actively colonize and thrive in saline soils where other organisms struggle. Salt exclusion is when certain microbes have mechanisms to exclude salts from their cells or actively limit their uptake. By excluding salts, microbes can ensure proper cellular function and growth in high salinity conditions. Salt metabolism is when certain microbes possess enzymes that can metabolize or break down specific types of salts. They can convert salts into less harmful forms or compounds that other organisms can use.

In short, balancing microbiome disruptions with practices that support plant-microbe symbiosis, such as cover crops and conservative tillage, is key to optimizing soil health processes and harnessing the benefits of soil microbes.

These microbial consortia are sensitive to external chemical inputs and serve as early indicators of soil disturbance (Kurenbach et al., 2021; Yang et al., 2023; El-Helow et al., 2024). Herbicides can alter:

- **Microbial richness and evenness**
- **Community composition** (e.g., shifts from *Proteobacteria* to *Actinobacteria*)
- **Enzyme activity**, such as dehydrogenase and phosphatase
- **Plant–microbe communication**, such as root exudate signaling

Soil microbial consortia—interactive communities of bacteria, fungi, archaea, and protists—are highly sensitive to herbicide exposure. Although herbicides are designed to target weeds, their biochemical specificity often extends to non-target organisms, leading to unintended disruptions in soil microbial dynamics. Also, herbicides can disrupt microbial communities by following mechanisms (Kurenbach et al., 2021; Yang et al., 2023; El-Helow et al., 2024):

- **Enzyme inhibition:** Common herbicides like glyphosate inhibit the shikimate pathway, which is present in bacteria and fungi. This hampers the synthesis of vital aromatic amino acids such as tryptophan, tyrosine, and phenylalanine, affecting microbial growth and metabolism.
- **Loss of microbial diversity:** Herbicide exposure can shift community composition by reducing beneficial groups like nitrogen-fixing bacteria, phosphate-solubilizers, and symbiotic fungi. This undermines key soil functions like nutrient cycling and plant-microbe interactions.
- **Metabolic shifts and functional decline:** While some microbes adapt to herbicides, this often comes with altered metabolic activity or reduced efficiency in organic matter decomposition and other soil biochemical processes.
- **Antibiotic resistance proliferation:** Studies show that prolonged herbicide use can increase the abundance of antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs), potentially facilitating the spread of resistant pathogens through horizontal gene transfer.

ADAPTIVE RESPONSES AND MICROBIAL RESISTANCE

Repeated herbicide exposure can drive **microbial adaptation**, including d**egradation capability evolution** (e.g., *Pseudomonas* acquiring glyphosate degradation genes), s**hift toward resistant or resilient taxa or horizontal gene transfer** of resistance traits. While such adaptations may improve degradation efficiency, they may also alter **microbial network interactions**, affecting ecological balance (Rousidou et al., 2013). Microbial shifts may feed back into **plant susceptibility**, nutrient cycles, and carbon dynamics, especially under climate stress.

Microbes adapt to herbicides through several strategies, such as e**fflux pump activation**, m**embrane modifications** (like changes in membrane permeability help microbes limit herbicide uptake and increase resistance to antibiotics), s**tress response pathways** (e.g. herbicide exposure triggers oxidative stress, DNA damage, and SOS responses, which can lead to mutagenesis and horizontal gene transfer) and c**ross-resistance evolution** (mutations that confer herbicide tolerance may also increase resistance to antibiotics, especially when stress pathways overlap) (Liao et al., 2021; Hill, 2017). These adaptive responses have cascading effects s**oil microbiome disruption, antimicrobial resistance and food chain contamination, like r**esidual herbicides and resistant microbes can enter crops and livestock, posing risks to human health (Daram et al., 2021; Paul and Mandal, 2019).

HERBICIDE EFFECTS ON ECOSYSTEM-LEVEL PROCESSES

While the rhizosphere is a well-studied hotspot of microbial activity, recent research reveals that microbial alterations extend far beyond root zones, influencing **macroecological processes** such as nutrient cycling, carbon sequestration, biodiversity patterns, and ecosystem resilience. These changes are not confined to local soil patches—they ripple across landscapes and biomes, shaping ecological trajectories at multiple scales (Wang and Zou, 2024; Mohanram and Kumar, 2019). Microbial communities in bulk soil, litter layers, and aquatic interfaces

respond dynamically to environmental perturbations, including herbicide exposure, land-use change, and climate variability. These shifts can a**lter biogeochemical cycles**, m**odulate greenhouse gas emissions** and i**Influence plant community assembly (**Pantigoso et al, 2022; Ruuskanen et al., 2022).

Microbial alterations often involve **cross-kingdom interactions**, such as fungal networks facilitating bacterial dispersal or protists regulating microbial grazing. These interactions contribute to s**oil organic matter formation**, t**rophic cascades** and to h**abitat connectivity**.

Beyond the rhizosphere, microbial alterations influence **macroecological processes, such as carbon sequestration** via changes in microbial respiration, n**itrogen and phosphorus cycling and soil aggregation and erosion resistance. S**everal studies report that herbicide-induced microbial imbalance can decrease **soil organic matter**, reduce **greenhouse gas buffering capacity**, and lower **crop resilience to drought and pests** (Ghimire et al., 2020). These indirect impacts challenge the long-held belief in herbicide neutrality toward ecosystem services.

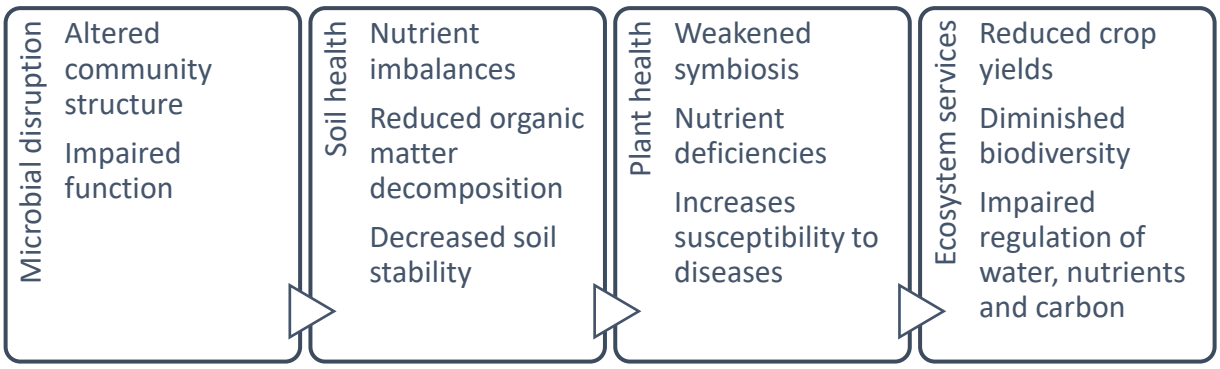

Figure 3. Ecosystem model showing cascading impacts of microbial disruption—soil health, plant health, insect interactions, and ecosystem services.

Herbicides have long been regarded as indispensable tools in modern agriculture—cost-effective, targeted, and efficient. Yet, their ecological footprint reaches far beyond weed suppression. Current herbicide risk assessment frameworks primarily focus on chemical persistence, toxicity to plants and animals, and residue levels in food and water. What remains largely overlooked is the impact of herbicides on the **soil microbiome**, a critical engine of nutrient cycling, plant health, and ecosystem stability (Sweeney et al., 2025; Kodikara et al., 2022).

Soil microbes—especially those in the rhizosphere—play foundational roles in maintaining agroecological functions. However, these organisms are almost entirely absent from conventional toxicological testing. Risk assessments typically rely on short-term, single-organism studies that fail to reflect the complexity and dynamism of microbial communities, which are deeply intertwined with numerous ecosystem services (Zhao et al., 2025; Wend et al., 2024).

An additional blind spot lies in **microbial genetic mobility**, where herbicide-induced stress can activate antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs). This not only jeopardizes soil health but also raises concerns about public safety via the

potential horizontal transfer of resistance traits to pathogenic microbes (EFSA, 2023; Liao et al., 2021).

Table 1. Gaps in Herbicide Risk Assessment.

Assessment Domain	**Current Gaps**
Soil Health	Focused mainly on chemical residues and physical structure
Non-target Effects	Limited consideration of microbial collateral damage
Temporal Dynamics	Assessed via static endpoints (e.g., residue half-life)
Bioindicator Integration	Rarely utilizes microbial indicators
Interaction Effects	Ignores chemical synergy or antagonism with microbial networks
Resistance Development	Focused on weed resistance only
Ecosystem Services Impact	Underrepresents microbial contributions to services
Standardized Protocols	Lacks harmonized microbial toxicity assays

CONCLUSIONS

Herbicides, while instrumental in modern agriculture, pose complex ecological challenges that extend far beyond weed control. Their influence on soil microbial consortia—especially in the rhizosphere—triggers cascading effects that alter nutrient cycles, suppress beneficial taxa, and potentially promote antibiotic resistance. These microbial disruptions reshape plant–soil interactions, reduce ecosystem resilience, and compromise vital services such as pollination, pest regulation, and carbon sequestration.

Conventional herbicide risk assessments often overlook these microbiome-mediated effects, focusing narrowly on chemical residues and plant toxicity endpoints. Integrating microbiome-centric metrics into regulatory frameworks—such as microbial diversity indices, functional group profiling, and gene transfer monitoring—offers a path toward more holistic ecological stewardship.

A microbiologically informed approach to weed management can help sustain soil health, foster agroecosystem stability, and safeguard public and environmental well-being. The microbial voice, long silent in regulatory discourse, must now become a guiding force in rethinking agricultural sustainability.

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THE IMPORTANCE OF MARKER ASSISTED SELECTION IN BLACKBERRY

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ABSTRACT

Plant breeding is a scientific process based on genetic principles that aims to import desired traits to plants. The development of molecular marker technologies and the application of Marker Assisted Selection (MAS) have accelerated and improved the reliability of breeding efforts compared to traditional methods. The ability to select fruit species based on genetic information, especially in the early stages, offers substantial time and cost savings in the breeding processes. Although genetic maps using molecular markers have been created for many important agricultural species, this data is rarely integrated into commercial breeding programs. The blackberry (*Rubus* sp.) is an example of this due to its high ploidy level and complex genetic structure. In this species, which is difficult to breed conventionally, genetic mapping and genome analysis must first be developed in order to effectively utilize MAS. Widespread wild blackberry populations in Turkey are an important source of genetic diversity and offer the potential to develop local varieties by integrating them into breeding efforts. To benefit from this potential, it is crucial to develop genomic and mapping studies, increase research on MAS, and effectively evaluate genetic resources. In this review, the importance of MAS studies in blackberry breeding and molecular studies on this subject are included.

Anahtar Kelimeler: Blackberry, Marker-assisted selection, Molecular markers

INTRODUCTION

Plant breeding is the development of new plants that possess desirable heritable traits by utilizing differences in genetic structure and natural distribution (Kanlıtepe and Duman, 2010). Although advances in agricultural technologies have contributed to yield improvement for many years, changing farming practices, environmental conditions, the evolution of pests, and consumer demands continuously compel breeders to develop new and adaptable cultivars. Traits such as fruit color or disease resistance can be targeted in newly developed varieties. These traits can be transferred to desired cultivars through hybridization from individuals within the gene pool. The acquisition of target traits through hybridization often relies on the broad allelic diversity found in genetic resources such as wild relatives and local varieties; therefore, the conservation and availability of these resources are of great importance for the sustainability of breeding programs. While traditional breeding continues to contribute to yield enhancement, new approaches such as biotechnology are needed to improve its success. In this context, DNA marker technology, based on molecular genetics and genomic studies, holds the potential to increase the accuracy and efficiency of breeding. Molecular markers enable the identification and conservation of individuals with distinct genetic characteristics. The use of this technology

is referred to as marker-assisted selection (MAS), which constitutes one of the fundamental elements of molecular breeding (Tanksley and McCouch, 1997; Gülşen and Mutlu, 2005; Collard and Mackill, 2008).

Molecular markers enable the identification of genes associated with important traits in horticultural crops, and the utilization of this information in breeding programs is achieved through marker-assisted selection (MAS). For a successful breeding program, a large number of progenies must be produced and evaluated; the appropriate number of hybrids depends on the program's objectives, parental quality, and screening methods such as pre-field MAS or greenhouse phenotyping. In many cases, thousands or even tens of thousands of individuals need to be screened, although successful cultivars have also been selected from smaller populations (Caruso et al., 2020; Rojas, 2015). While conventional breeding programs are capable of generating large seedling populations, they often lead to high costs and low efficiency. In contrast, MAS enhances breeding effectiveness by facilitating selection for traits that are otherwise difficult to identify at early stages or those that are recessive (Rojas, 2015). Although MAS provides the opportunity to combine desirable traits more rapidly and with fewer losses, its application in the improvement of complex traits remains limited due to low heritability and environmental effects. Therefore, the integration of large-scale genotyping into breeding systems is critical for the transfer of multiple genes (Xu and Crouch, 2008).

In *Rubus* species, studies aimed at identifying commercially important traits such as resistance to diseases and pests, as well as postharvest durability and processing quality, have facilitated the development of molecular markers that can be employed in marker-assisted selection. However, the limited morphological variation in these species complicates the identification of germplasm and cultivars; therefore, DNA markers serve as an essential tool both for cultivar identification and for the assessment of genetic diversity (Antonius-Klemola, 1999; Ryu et al., 2017). The use of molecular tools in blackberry breeding is of great significance for shortening the breeding process and enhancing production. Nevertheless, the autotetraploid structure of erect and semi-erect blackberry cultivars intended for fresh consumption poses challenges to genetic studies (Foster et al., 2019). In molecular breeding studies of autopolyploids, the high heterozygosity, multivalent inheritance, and difficulties in determining allele dosage in autotetraploid cultivars complicate genetic analyses. In recent years, however, software tools have been developed to overcome these challenges, and a reference genome for diploid *Rubus argutus* as well as high-throughput genotyping platforms have been established. Collectively, these advancements have rendered molecular breeding more feasible in tetraploid blackberries (Johns et al., 2025).

Among berry crops, blackberry is a species in which phenotypic selection can be slow and costly due to polyploidy and its complex hybrid pedigree. However, with the rapid expansion of genetic and genomic resources in recent years, it has become an increasingly strong candidate for marker-assisted selection (MAS) (Foster et al., 2019; Brůna et al., 2023; Paudel et al., 2025). Furthermore, studies in berries have demonstrated that wild relatives and local cultivars provide valuable allelic sources for traits such as resistance and quality, which further reinforces the strategic importance of MAS in blackberry breeding (Migicovsky et al., 2022).

Blackberry (*Rubus fructicosus* L.)

Blackberry, belonging to the genus *Rubus*, is classified within the order Rosales, and the family Rosaceae in general identification the name *Rubus fruticosus* is used (Yıldız, 2017). *Rubus* is well known for its taxonomic complexity (Sochor et al., 2022). The basic chromosome number in *Rubus* species is seven, with ploidy levels ranging from 2x to 14x. Diploids are predominantly sexual, while polyploids may exhibit apomictic reproduction. Moreover, factors such as incompatibility genes and vegetative propagation further complicate research on this

group (Thompson, 1997). Since limited morphological variation makes the reliable identification of germplasm and cultivars difficult, studies focusing on commercially important traits such as disease and pest resistance, as well as postharvest durability and processing quality, have encouraged the development of molecular markers for use in MAS. Consequently, DNA markers have become an essential tool for both cultivar identification and the assessment of genetic diversity (Antonius-Klemola, 1999).

The relatively limited genetic variation in *Rubus* has led to slow progress in breeding, thereby increasing the importance of interspecific hybridization and mutation breeding (Ryu et al., 2017). While genetic diversity studies have been more concentrated in raspberry, where interspecific relationships have been elucidated through molecular markers, the use of DNA-based methods in blackberry has also become widespread. While genetic diversity studies have focused mostly on raspberry and interspecies relationships have been revealed with molecular markers, the use of DNA-based methods in blackberry has become widespread, but difficulties such as limited genetic structure information, genotype discrimination, and the inability to determine heterotic groups persist; moreover, the cost and labor requirements of traditional breeding have made DNA-based analyses a more suitable option (Ochieng et al., 2018). Blackberry is widely cultivated among berry crops in temperate and subtropical regions (Umarusman et al., 2020). Most cultivars, largely originating from North America, are tetraploid (2n = 4x = 28) and are extensively grown in the United States (Clark et al., 2007). Nevertheless, the native range of blackberry is considered to be Southern, Western, and Central Europe. Commercial cultivars have been developed in erect, semi-erect, thorny, thornless and, trailing forms, with widely cultivated varieties in both the world and Turkey, including Arapaho, Bursa-I, Bursa-II, Bursa-III, Boysenberry, Black Satin, Cherokee, Chester, Nessy, Dirkson, Darrow, Lawton, Navaho, and Thornless (Zenginbal and Gündoğdu, 2020; Kırıt et al., 2023). Blackberry, a significant crop in Mexico and the United States, is notable for its early fruiting and high economic returns. Its richness in phenolic compounds enhances its nutritional value (Milosevic et al., 2012; Balcı and Keleş, 2019), yet postharvest issues such as softening and leakage complicate transport and storage, thereby limiting its commercial value (Godwin, 2021). The development of large-fruited and high-yielding cultivars through breeding has attracted the interest of small producers, while the expansion of production into different regions has enabled year-round supply. Moreover, the transition from red raspberry to blackberry cultivation has provided advantages for growers due to its lower input requirements (Altunbaş and Pırlak, 2019; Godwin, 2021).

Molecular Markers

Advances in genetics and genomics, particularly through the use of molecular markers, have facilitated the development of genetic mapping studies in crop plants. As a result, more comprehensive linkage maps have been established, extending beyond those based solely on morphological traits. Molecular markers have been successfully applied in various areas, including the assessment of genetic diversity, phylogenetic analyses, gene identification, genome mapping, and marker-assisted selection (Güleç et al., 2010; Iordăchescu et al., 2021). SSR markers are particularly preferred in commercial species due to their high levels of polymorphism and reproducibility, whereas SNPs are notable for their low mutation rates and evolutionary stability (Iordăchescu et al., 2021). However, in blackberry and other *Rubus* species, such studies have progressed more slowly compared to red raspberry, owing to their complex genetic structure and lower economic priority (McCallum et al., 2018).

In linkage mapping studies, various molecular markers such as AFLP, RAPD, SSR, and SNP have been employed to construct genetic maps, thereby enabling the identification of genes or QTLs associated with target traits (McCallum et al., 2018). These developments have contributed to the advancement of genetic research by facilitating the association of traits with

specific chromosomal regions. Since the identification of genes or genomic regions affecting agriculturally important traits relies on examining the relationship between DNA markers and phenotypic traits, this forms the foundation of marker-assisted selection applications. Today, in line with environmental changes and regulatory frameworks, the need for developing traits aimed not only at quality but also at sustainability and environmental resilience has increased (Adhikari et al., 2017; McCallum et al., 2018).

Marker-Assisted Selection (MAS)

In fruit breeding, the improvement process generally takes 20-25 years, which delays the development of new cultivars (Aksu and Çevik, 2015). With the development of molecular markers and genetic maps targeting QTLs in plants, marker-assisted selection (MAS) enables the identification of individuals carrying genomic regions associated with desirable traits, thereby facilitating the transfer of target genes or QTLs into elite germplasm and allowing the process to be completed more efficiently and in a shorter time (Aksu and Çevik, 2015; Karakaş and Bayrıl, 2024).

In MAS applications, marker selection is made by considering factors such as reliability, DNA quality and quantity, technical complexity, level of polymorphism, and cost. The use of markers closely linked to the target gene region increases analytical accuracy. In practice, SSRs are widely used, reliable and polymorphic tools, although they present certain technical and economic limitations, while alternative markers such as STS, SCAR, and SNPs can also be effectively utilized (Collard and Mackill, 2008).

The success of MAS depends on the strength of the marker-QTL/major gene association, the level of recombination, and the capacity to screen large numbers of individuals. However, limitations related to technical infrastructure, cost and labor requirements, and the lack of knowledge on appropriate and cost-effective strategies restrict its application (Güleç et al., 2010; Wannemuehler, 2018). For the effective implementation of this method, genetic maps in which marker sequences are associated with phenotypic or biochemical traits are required and such maps also allow the identification of quantitative traits such as QTLs (Kanlıtepe et al., 2010; Vidyagina et al., 2021). Compared to phenotypic screening, MAS is more practical and reliable, as it enables studies independent of environmental influences, saves time and resources for traits that are difficult to identify and allows for early selection by distinguishing between homozygous and heterozygous individuals. Additionally, it is effective in the identification of genetic resources, prevention of varietal confusion, and protection of commercial rights (Yorgancılar et al., 2016).

In marker-assisted selection, the process generally begins with the selection of appropriate parents, at least one of which carries the marker alleles for the target trait. Subsequently, the F1 population is generated, and false hybrids are eliminated through marker screening. In the F2 population, individuals are analyzed with markers, and those carrying the desired alleles are selected. In the subsequent F2:3 and later generations (F3, F4, F5), marker screenings continue, with a particular focus on identifying homozygous individuals and superior lines. In advanced generations (e.g., F5:6), phenotypic evaluations are also considered alongside marker data. In the final stage, selected lines are subjected to comprehensive trials for traits such as yield, quality, and disease resistance. This approach accelerates the breeding process by enabling the early identification of desirable genotypes (Jiang, 2013).

Today, MAS is widely applied, particularly in backcross breeding, where markers facilitate the selection of traits that are difficult to identify phenotypically, enable the early detection of rare individuals linked to the target gene and eliminate the need for an additional selfing step in the transfer of recessive genes. This approach ensures the effective selection of the target region, reduces linkage drag, and simplifies the identification of recurrent parents (Güleç et al., 2010; Xu and Crouch, 2008; Yorgancılar et al., 2016).

Marker-Assisted Selection (MAS) Studies in Blackberry

The initial steps toward marker-assisted selection (MAS) applications in blackberry began with the establishment of marker infrastructure. Lewers et al. (2008) developed hundreds of SSR markers from an EST library of 18,432 clones prepared from "Merton Thornless" tissue, reporting that approximately 30% of these markers were polymorphic. This study laid the foundation for subsequent genetic mapping and MAS applications in the following years.

Subsequently, one of the most important structural traits in blackberry breeding, thornlessness, was introduced into cultivation through the "Merton Thornless" cultivar developed in the United Kingdom and became the primary source transferred into modern cultivars (Clark and Finn, 2011). The genetic basis of this trait was later examined in greater detail through a linkage mapping study conducted in a tetraploid population, in which the thornlessness locus was mapped to LG4 and the primocane-fruiting locus to LG7 (Castro et al., 2013). Thus, the foundation of MAS for these two traits was established. More recently, thornlessness has been further refined by Johns et al. (2025) using a genome-wide association analysis, narrowing its position to the 30.48-36.04 Mb interval on chromosome Ra04, where five candidate genes, including *HOX3* were identified. Moreover, two KASP markers developed in this study predicted the phenotype with 96-97% accuracy across 626 genotypes. This advancement has enabled the rapid and reliable selection of thornless individuals at the seedling stage.

Studies on quality traits also highlight the importance of MAS. In an analysis conducted across the Rosaceae family, 173 alleles associated with sweetness (soluble solids content, SSC) were identified in blackberry, of which 92 were tested using KASP and 48 were validated. Furthermore, a QTL designated *qSSC-Ruh-ch1.1* was reported to increase SSC by +1.5 °Brix (Zurn et al., 2020). Subsequently, in a GWAS performed by Godwin et al. (2025) on 301 genotypes, the heritability values for SSC, pH, and titratable acidity (TA) were found to be 61%, 67% and 70%, respectively; significant regions were identified on Ra02 for SSC and on Ra05 for pH and TA. Candidate genes reported in these regions included *MYB1*, *PEPC*, malate synthase, and *ALMT9*. Similarly, in Godwin's master's thesis (2021), six QTLs and 16 candidate genes were identified across four chromosomes in 307 genotypes. These findings demonstrate that sweetness and acidity traits can be effectively supported through MAS.

In terms of postharvest quality, fruit firmness and red drupelet reversion (RDR) represent major challenges. In a GWAS conducted on 300 tetraploid genotypes, Chizk et al. (2023) reported heritability estimates of 68% for firmness and 34% for RDR and identified a negative correlation between these two traits. Among the SNPs associated with firmness, markers were located near genes encoding polygalacturonase and pectin methylesterase. These findings suggest that MAS can serve as a valuable tool in the development of cultivars with firm texture and improved durability.

In addition, diversity and parental selection also contribute indirectly to MAS. AFLP analyses conducted on 81 *Rubus* genotypes collected from Japan distinguished between cultivated and wild materials and demonstrated that hybrid individuals diverged from their parents (Miyashita et al., 2015). Furthermore, RNA-Seq-based SSR and SNP studies performed on *Rubus glaucus* cultivated in the Andes have enabled the evaluation of traits such as thorn presence and anthracnose tolerance in relation to parental selection, thereby demonstrating that MAS can also be integrated into different *Rubus* species (López et al., 2019).

CONCLUSION

Blackberry is a significant species among berry crops, distinguished by both its nutritional content and economic value. Owing to its significance in fresh consumption and its processability in industry, its global production has been steadily increasing each year. However, blackberry breeding is more challenging compared to other berry species. The primary reasons for this include variable ploidy levels, a complex hybrid pedigree, and long generation times. These factors render breeding through conventional methods both time-consuming and costly.

Marker-assisted selection is regarded as an important tool for overcoming these challenges. However, MAS studies conducted on blackberry to date have remained limited. The reasons for this include the insufficient development of large mapping populations, the scarcity of high-density genetic maps, and the relatively recent adoption of high-throughput genotyping approaches. Nevertheless, in recent years, studies on traits such as thornlessness, primocane fruiting, fruit firmness, red seed return (RDR), sweetness and acidity have provided significant progress in this field. In particular GWAS and QTL mapping studies have enhanced the understanding of the genetic basis of these traits and laid the foundation for marker-assisted breeding.

All these developments indicate that MAS may find much broader applications in blackberry breeding in the future. With the diversification of genetic resources, the establishment of larger populations and the use of advanced molecular techniques, MAS will facilitate early selection in blackberry, reduce time and cost losses, and enhance the efficiency of breeding programs.

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SOFT CHEMISTRY NANOCOMPOSITE OF MGO DOPED PVC FABRICATION FOR PHOTOCATALYTIC APPLICATIONS

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ABSTRACT

The objective of this research is to explore the photocatalytic properties of poly (vinyl chloride) (PVC) films, both pure and doped with varying concentrations of nanoscale magnesium oxide (MgO), fabricated by the soft chemistry method and deposited using spin-coating technique. The concentrations of MgO used were 3%, 7%, and 10%. Various analytical methods were employed to characterize the samples. The presence of MgO nanocrystals within the PVC films was confirmed through X-ray diffraction, infrared and UV-visible spectroscopies. X-ray diffraction analysis revealed an average particle size of 22.29, 23.77 and 29.68 nm for the MgO with concentration of 3%, 7%, and 10% respectively. FTIR infrared spectroscopy confirmed the presence of peak corresponding to the Mg–O–Mg bond vibration. Optical analysis revealed distinct differences between the spectra of pure and doped matrices. Increasing in doping led to decreased absorption in visible region. Thus, the transmittance drops from 90% (undoped PVC matrix) to 80% (10% MgO doped PVC matrix). Slight redshifted of absorption edge compared to the bulk semiconductor. The optical band gap of the nanocomposites decreased with increasing MgO concentration, ranging from 3.75 to 3.69 eV. Atomic force microscopy also shows a change in the roughness of the nanocomposites film with increasing MgO nanoparticles percentage. Photocatalytic activity assessments demonstrated that the inclusion of MgO nanoparticles enhanced the efficiency of the nanocomposites under UV light. The (PVC/10% MgO) sample exhibited a 65 % efficiency in removing methyl blue (MB) within 150 min, comparable to other samples with lower efficiency. The photodecomposition reaction of MB followed first-order kinetics, characterized by a rate constant (k) of 0.00526 min^{-1}.

Keywords: MgO; PVC films; Xray; FTIR; Doped; Semiconductor; Gap.

INTRODUCTION

Nanocomposites embedded in polymer matrix have gained a lot of interest and thus a numerous research work has been devoted to it. Single polymer matrix is one of the most attractive classes of materials in the world, due to their interested properties and applications. Polymer nanocomposites offer advanced in new technology in various uses and has no environmental benign effects. Moreover, the effective properties of polymer nanocomposites rely on the nature of the components, the shape of the particles, the size and arrangement of the inclusions, the concentration, the volume fraction of the components, extent of dispersion of nanoparticles and interfacial bonding in the polymer matrix. Generally, Inorganic particles in polymer matrix improve density, mechanical, thermal, electronic, magnetic, and redox properties of nanocomposites [1-4]. Among oxides, MgO nanoparticles is a well-known refractory oxide, which has potential advantages with wide bandgap around 7.8 eV. MgO is interesting inorganic substance which has FCC type crystal packing, abundantly used raw material because of its high thermal stability, biodegradability and low toxicity. MgO is a chosen material due it's potential application in chemical industry as it is used as air pollutant scrubber for gases, catalysis, optoelectronics, insulating material filler, refractory materials, and as a catalyst support.[5,6] In photocatalytic application MgO is seen one of the promising photocatalyst and have been successfully applied for methylene blue dye degradation. Doping of MgO is an effective method to increase photocatalytic activity of MgO catalyst. Reports have revealed insertion of silver nanoparticles in the crystal lattice of MgO enhance the photocatalytic activity for dye degradation. [7] The doping of Ag results in lower energy region absorption of light and thus makes possible to extend the photocatalysis in the visible part of electro-magnetic spectrum. Polymers are the dominant materials widely used, owing to their low cost and ease of processing, so they are considered to be versatile, beneficial and economical for their wide spread applications. [8] Polymers like PVC has attained tremendous interest in various life aspects. The interest in PVC have arisen because of its good properties like excellent mechanical properties, highly rigidity than other general thermoplastics materials. The advantage of PVC is that it breaks down easily and does not produce dioxins under natural conditions. PVC also has found good applications in antibacterial and industries. [9, 10]

In this work, an attempt has been made to synthesized MgO nanoparticles and MgO/PVC nanocomposites and investigate their photocatalytic activity. we have also given well full details about the structural, optical, and morphological properties of MgO/PVC nanocomposites film.

MATERIAL METHOD

2.1 Synthesis of Magnesium oxide (MgO) nanoparticles (NPs)

A sol-gel technique was employed to synthesize MgO NPs. Mg $(NO3)_2.6H_2O$ was taken as a precursor of magnesium and treated with C_2H_5OH. The solution was stirred for 24h until white gel was obtained. After that the mixture was filtered, washed with distilled water and ethanol several times and dried at 100 ° C for 24h.Theobtained powder was annealed at 950° C for 36h. Finally the powder was grinded and used for further analysis. The annealing phase results in formation of desired product.

2.2 Photocatalytic procedure

A UV light source (VL -215.LC, 15W), with emission maximum at 365 nm was employed to check the photocatalytic activity of MgO/PVC. 10 ml MB solution with an initial concentration of 10^{-5} mg/ml was taken in a glass beaker. A thin glass strip (5×2.5 cm) containing the films of MgO/PVC nanocomposites was put in the beaker containing MB. The beaker was kept undervisible light at a distance 20 cm emitted by a 300 W iodine tungsten lamp (Philips and Co). The whole system was covered by cloth so that natural white light would have no role in catalysis. During irradiation the aliquots of solution was taken out after every 30 min from the reactor and checked by analyzed by a 723 UV–Vis spectrometer (UV-1800 Shimadzu LC 2010-HT).

RESULTS AND DISCUSSION

3.1 Structural study

3.1.1 X-ray diffraction (XRD) analysis

Fig 1shows the XRD patterns of pure PVC and MgO/PVC nanocomposites films (3, 7 and 10 in wt% n of MgO). From, XRD of pure PVC film, polymer chain have disordered structure indicating amorphous nature [11, 12]. The MgO/PVC nanocomposites film exhibits characteristic peaks of MgO, which appear at 2θ angle 36.23°, 43.04° corresponding to the reflection of of (111) and (200) correspond to the cubic phase of MgO (JCPDS card N° 45-0946). The prominent (111) peak of MgO exists but very less intense. From table 1 it can be inferred that intensity and area of MgO/PVC under the peaks at 2θ = 39.3° and 43.04° shifts to right when MgO/PVC loading is increased as compared with that of MgO/PVC, this decrease in lattice parameters reflects formation of mixed phase of MgO/PVC. So it can be inferred that there is change in crystal structure due to varying of MgO/PVC dopant ratio. The interplaner distance d_{hkl} of MgO/PVC were determined using Bragg's law. The change in the interplaner distance d_{hkl} from 2.29 for MgO/PVC (3%) to 2.36 for highest loading of MgO/PVC (10 %) clearly indicates the increase in the lattice size upon doping, which demonstrating a possible extension of MgO lattice. Additionally, to these observations, after increasing doping, the peak of (111) has a high intensity value. Compared to the JCPDS card N° 45-0946, MgO present a texture along the (111) plane.

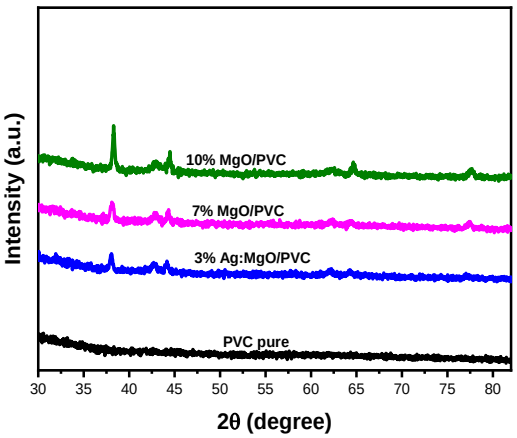


Fig 1: X-ray diffraction spectra of Pure PVC, MgO/PVC and Ag :MgO/PVC nanocomposites thin films.

3.1.2 The grains size

Debye Scherer's formula was used to calculate size of crystals [18], and the values calculated are recorded in **Table 1:**

$$D = \frac{0.9\lambda}{\beta cos\theta} \quad (1)$$

Where β = FWHM, θ is the diffraction angle, λ is the X-ray wavelength (1.5405 Å). As the dopant MgO/PVC increases the for MgO/PVC samples the crystallite size (D) also increases.

Table.1. : Structural parameters MgO/PVC nanocomposites thin films

Samples	2θ(°)	(hkl)	FWMH (rad)	d	D (nm)
3% MgO/PVC	38,03	(111)	0,4	2,35	22.29
7% MgO/PVC	38,16	(111)	0,35	2,35	23.77
10% MgO/PVC	38,3	(111)	0,28	2,36	29.68

3.1.3 FTIR Analysis

In the range of 4000-400 $cm^{-1}$of the FTIR spectra of pure PVC, PVC/MgO and MgO/PVC/PVC nanocomposits films were recorded as shown in Fig .2. Sharp peak were recorded in the all films at 756 and 904 cm^{-1}. This was attributed to the PVC Bond. With the increase in MgO/PVC loading there is a clear shift towards lower frequencies indicating MgO interactions with C-Cl, C-H bonds of PVC. matrix, due to interaction between MgO and C-Cl, C-H bonds of PVC. [19] The spectrum of MgO/PVC nanocomposites film appears three distinct band is observed in the wave number 615, 1324,1434 cm^{-1}, indicating the bending vibrations of Mg–O–Mg, C-H and C=C, respectively. [20] The spectrum of the MgO / PVC nanocomposite film displayed at 2360 cm^{-1} were assigned to the typical the CH_2 bending vibration mode [21].

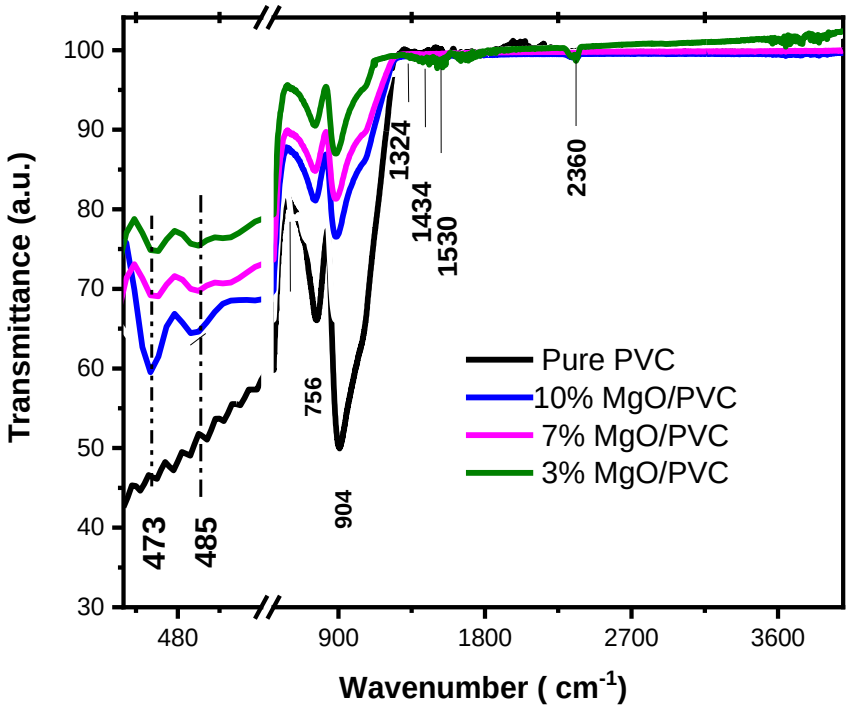


Fig 2: FTIR spectrum of PVC and MgO/PVC nanocomposites.

3.2 Optical study

3.2.1 UV–Vis analysis

To determine the optical properties of MgO/PVC nanocomposite thin film UV–Vis Spectroscopy was employed. The UV-VIS absorbance spectra in the region 200–800 nm for doped and undoped films are shown in Figure 3 , the pure PVC is completely fully transparent (90%) and has a steep absorption edge at 300 nm. The absorption of the films gets decrease with the increase in MgO amounts concentration as shown in Fig. 3. The absorption region(300-400nm) is probably due to electron transition from valence band to the conduction band. For MgO/PVC composite films this absorption band is quite prominent and wider than PVC pure and MgO/PVC. There is a slight shift in UV absorption intensity in MgO/PVC films. To obtain effective UV absorption the concentration of metal ions needs to be optimized as there is change in absorption with addition of MgO/PVC NPs in PVC matrix [22].

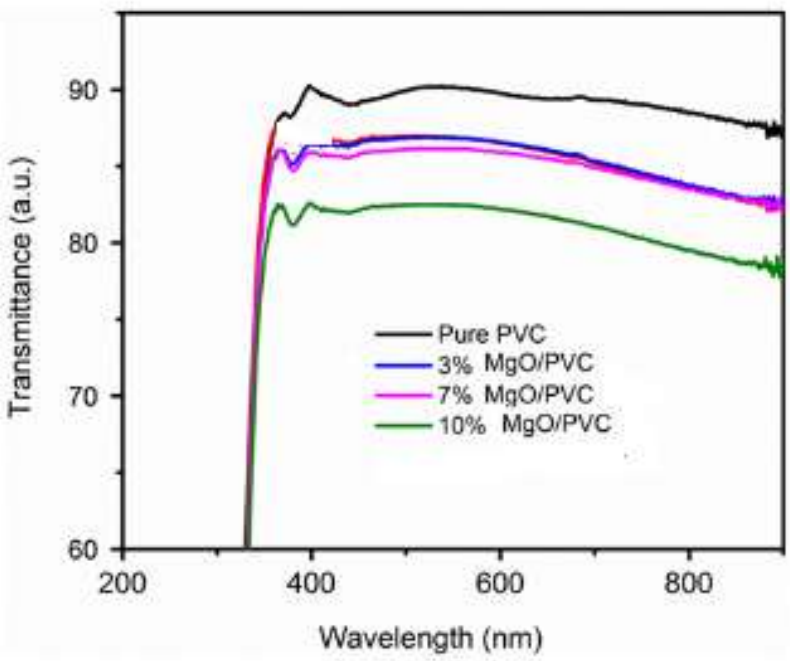


Fig .3: Optical transmission spectra in the UV–Visible region of Pure PVC and MgO/PVC nanocomposites thin films .

3.2.2 Determination of Optical Band

Tauc' plot method was employed to determine the optical band gap of PVC, MgO /PVC and MgO/PVC nanocomposites films. Using the classical Tauc approach, the band gap (Eg) was calculated fromthe plot of $(\alpha h\nu)^{1/2}$ vs. photon energy ($h\nu$), Figure **4** shows direct optical band gap for neat PVC and MgO/PVC nanocomposites. The optical band gap for PVC was found ~4.1 eV. Incorporation of MgO in PVC has great influence on direct transition in nanocomposites as optical band gap gets decreased with increase in concentration of Ag-doped MgO nanoparticles. This is credited to formation of new local levels in the bottom of conduction band which aid in electron transport from valence band through local levels and then to conduction band [23]. These results revealed that the incorporation of PVC served as accepter for MgO/PVC which reduced the band gap (~3.69eV) for 10% MgO/PVC nancomposite with red shift confirming p-type conductivity.

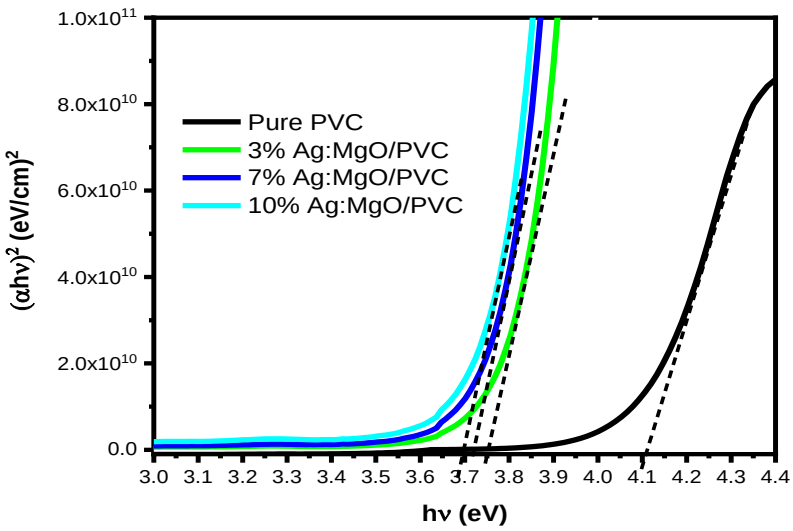


Fig .4: The plot $(\alpha h\upsilon)^2$ versus incident energy (hυ).

3.3 Morphological study

3.3.1 Atomic Force Microscopy (AFM) analysis

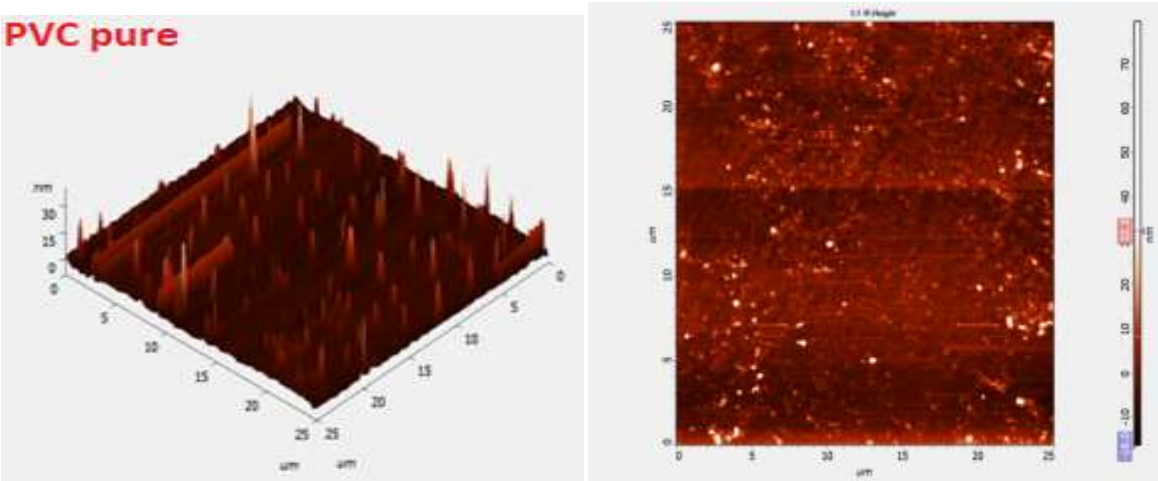
AFM is one of the important analyses for surface characterization. To investigate the topography and roughness of the thin films, AFM 3-D micrographs were recorded. This measurement technique presents digital images allow the quantitative measurements of roughness average, the root mean square and diameter of pores [24] and are estimated and tabulated below.

The change in surface morphology and roughness due to incorporation of Ag doped MgO nanoparticles in PVC membranes is characterized. The mean roughness (Ra) and root mean square roughness (Rq) parameters were measured for these thin films and are displayed in Table 1. Both parameters increased with increase in the Ag :MgO doping weight percentage. It can be seen from Table 1 that mean roughness (Ra) is varied between$1.933 \leq Ra \leq 35.592$ and the root-mean squared roughness (Rq) is varied between $4.117 \leq Rq \leq 644.643$ .It is clear that the RMS increased with Ag :MgO content, indicating that PVC/MgO-Ag nanocompositesfilms have a rougher surface than PVC/MgO nanocomposites.This is an acceptable concurrence with XRD results proposing a debasement of crystallinity and a huge change in MgO/PVC nanocomposites film microstructure. Appropriately, 7% and 10% MgO/PVC/PVC nanocomposites film has the most elevated permeable, which prompts a huge explicit surface region.Thusly, this permeable structure film most promising methods to improve the photocatalyst activity and make the recycling safety.

Samples	Avg. Diameter of pores	Roughness average	Root mean square
Pure PVC	36-360	1.93	4.12
PVC/3%-MgO	25-500	28 .49	34.76
PVC/7%-MgO	12.5-375	31.33	37.29
PVC/10%-MgO	31-375	35.59	44.64

Table.2 : Measurements of roughness average, the root mean square and diameter of pores of samples prepared .

Fig.5 shows the two-dimensional and three-dimensional AFM images of the surface topography of PVC pure and MgO/PVC nanocomposites films of different concentrations. It can be seen that the number, diameter of the pores augment with increasing the MgO nanoparticles. may be due to the formation of agglomerates of nanosheets on the membrane surface during the phase inversion process. This is due to increase in the aggregation of tiny particles at some points that contributed in the increase in the roughness parameters [25]. As shown by AFM micrographs and their line profiles, 7%, 10% MgO/PVC nanocomposites films have high porous structure as compared to MgO/PVC. This indicates that Ag doping leads to an increased porosity and as a result lead to a large specific surface area.



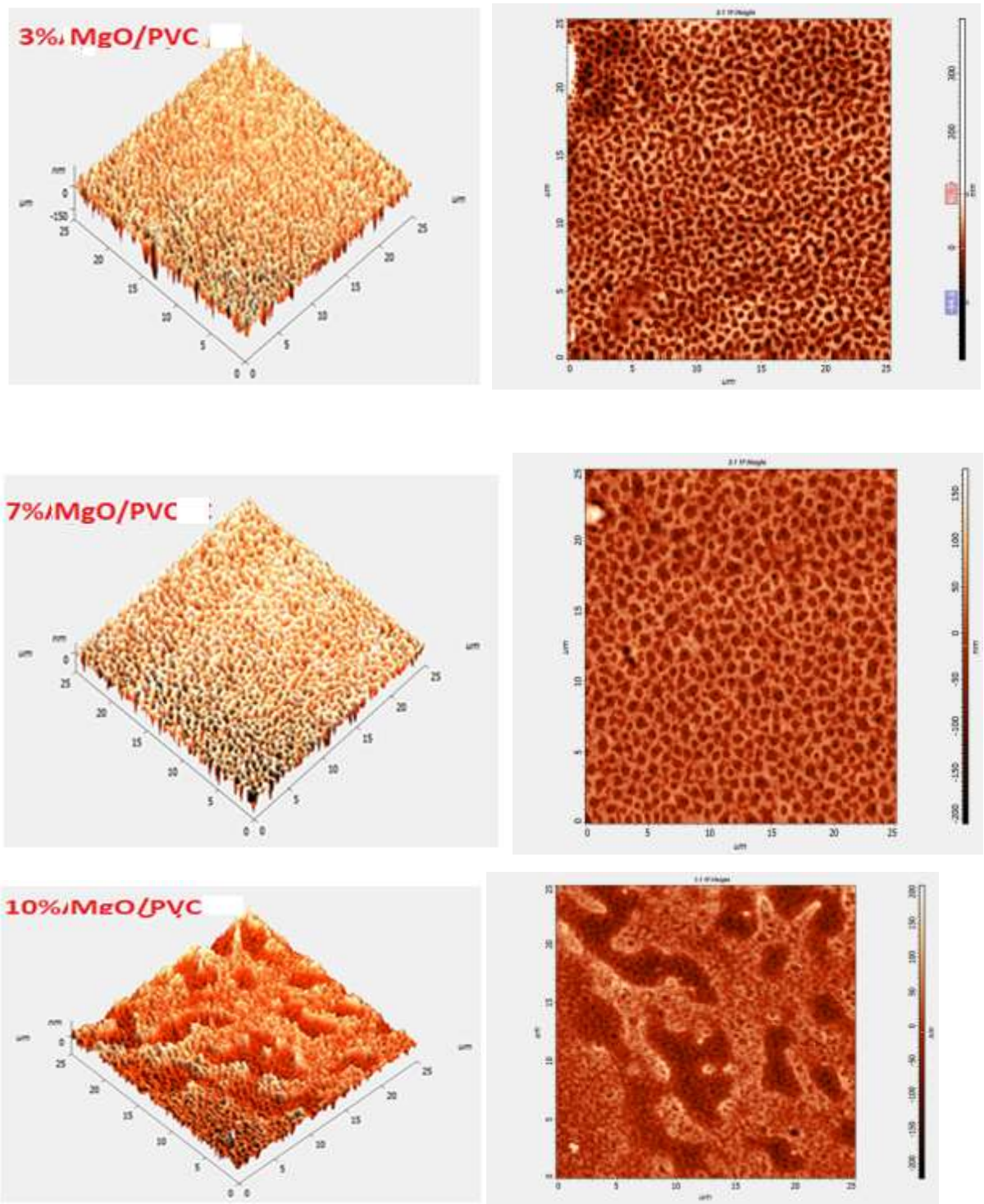
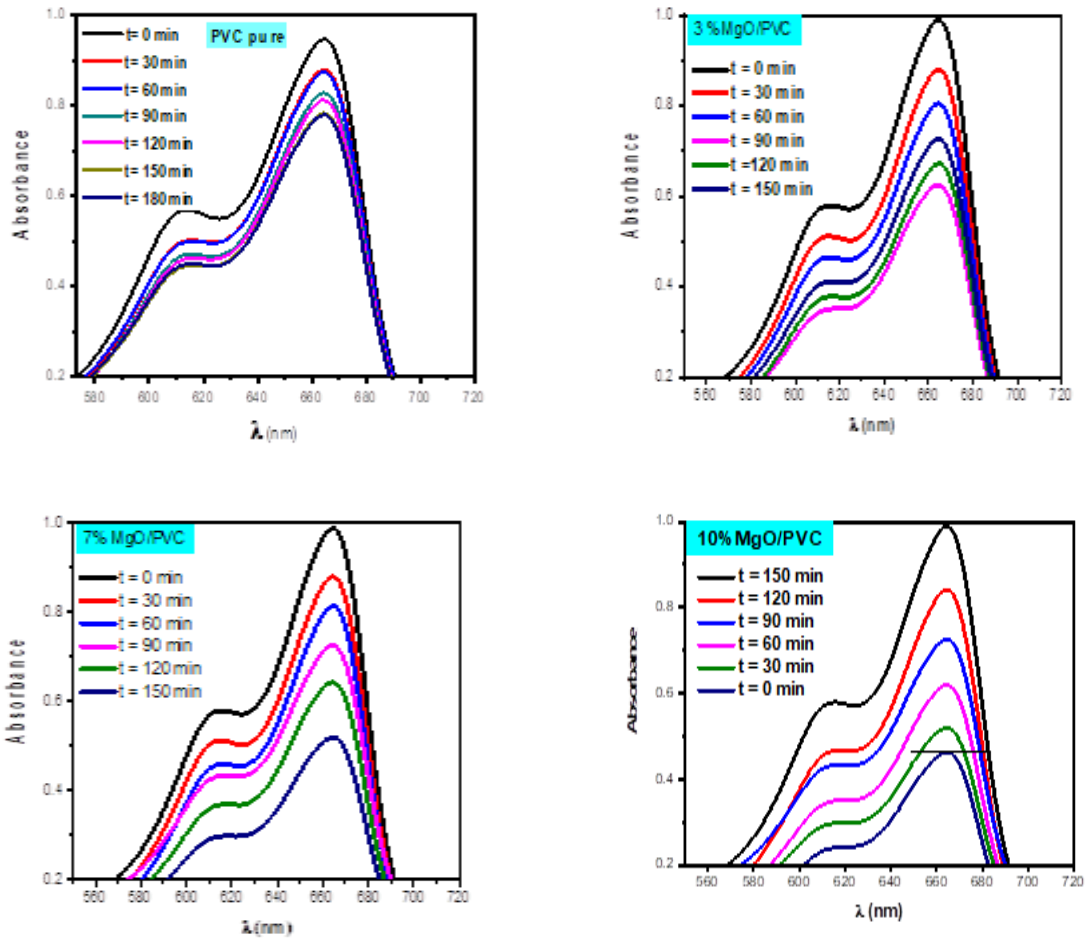


Fig .5:AFM for pure PVC and MgO/PVC nanocomposites.

3.5 Photocatalytic tests

The photocatalytic degradation of methylene blue by PVC pure and MgO/PVC nanocomposites are shown in the Figure (6). The methylene blue degradation efficiency increases continuously with increases of time. After two hours of test period, the methylene blue degradation is found to be 17%, 35%,46% and 52% for PVC and MgO/PVC with concentrations 3%,7%,10% respectively . Comparing pure PVC and MgO/PVC the highest activity is observed for the 10% MgO /PVC nanocomposite can be assigned to the most efficient charge separation. The presence of defect content in MgO can reduce electron-hole recombination and increase the photocatalytic activity.



Fiure 6. Potocatalytic degradation and **(b)** the plots ln (C_0/C) versus time; **(c)** degradation rate of MB dye under UV light irradiation of PVC and MgO/PVC nanocomposites thin films.

Figure.7 shows the kinetic plot between ln(C/C0) and irradiation time " t " for the photodegradation of methylene blue as a function of irradiation time for pure PVC and MgO/PVC nanocomposites at different concentrations and a linear relationship is observed.The rate constant (k) was calculated from the slopes of MgO/PVC(3%,7%,10%) nanocomposites and it was found to be 1.64×10^{-3} min^{-1}, 3.50×10^{-3} min^{-1} ,4.24×10^{-3} min^{-1}, 6.25×10^{-3} min^{-1} respectively. This shows that MgO/PVC has enhanced photocatalytic degradation than pure PVC. In the case of MgO/PVC, the photocatalytic activity increases due to the doping level has

increased. This may be due to the highest porous structure, which provides more active sites for the adsorption of pollutant molecules. The diminution of the band gap (Eg) allow the absorption of more UV light energy and higher generation of electron-hole pairs [26]. This promotes the •OH and $O_2^{\bullet -}$concentrations and thus improves the effectiveness of the photocatalyst. As a result, more electrons and holes can contribute in the photoreaction and so rise the removal of organic molecules [27].

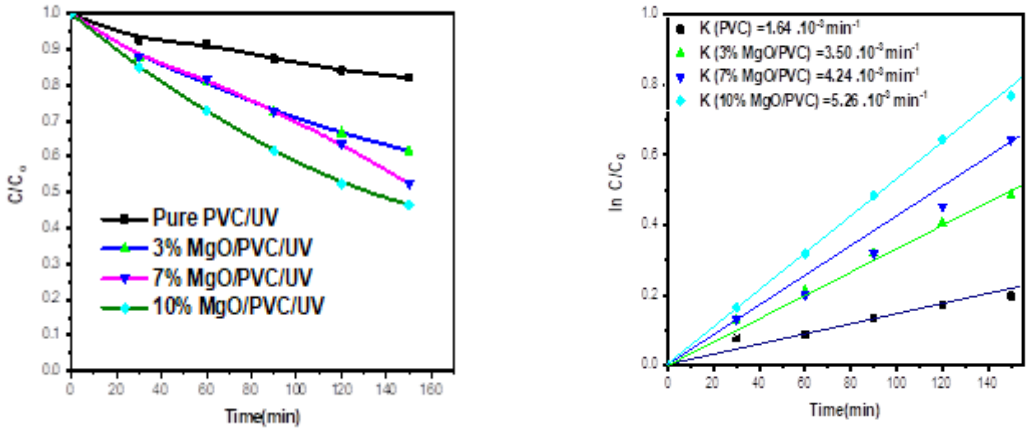


Fig .7: Decomposition of the dyes (MB) under UV light(a) C/Co vs time (b) ln C/Co vs time (c) % dye degration vs time.

3.6 Photodegradation Mechanism

Photocatalytic degradation of MgO/PVC/PVC nanocomposites film is initiated by degradation of MgO. Since the degradation of PVC starts indirectly through oxidative radicals generated on doped MgO [28].

In general, when Ag doped MgO is irradiated by UV light, a photoelectron moves from valence band of the semiconductor and iel is the intermediate energy levels generated after doping process .This photon has the energy (hυ) equal to or greater than the band gap. The semiconductor absorbs this radiation and one electron can be excited from the doping donor level to conduction band of the semiconductor and forms cationic radicals (eq ((2)).

$$MgO + h\upsilon\ (E \geq E_g) \longrightarrow MgO^{*}\ (e_{cb}^{-} + h_{vb}^{+}) \quad (2)$$

The photogenerated holes (h_{vb}^{+}) react with water molecules to produces the hydroxyl radicals •OH. While the electrons in (CB) conduction band meanwhile reacts with O2 and generate superoxide radicals anions $O_2^{\bullet -}$(eq(3)). the oxygen molecules adsorbed on the semi conductor surface accelerates oxidation process and also prevent any further e- / holes recombination by trapping electrons.

$$h_{vb}^{+} + H_2O \longrightarrow \bullet OH \quad (3)$$

$$e_{cb}^{-} + O_2 \longrightarrow O_2^{\bullet -} \quad (4)$$

These reactive species like H2O2, $O_2^{\bullet -}$ and •OH radicals produced ,for ultimately help to degrade target pollutants present around it (eq (5,6,7)).

$$O_2^{\bullet -} + H_2O \longrightarrow O_2^{\bullet}H + OH^{-} \quad (5)$$

$$2O_2^{\bullet}H \longrightarrow H_2O_2 + O_2 \quad (6)$$

$H_2O_2 + h\upsilon \longrightarrow 2^{\bullet}OH$ (7)

Photocatalytic degradation of PVC is only excited by UV light due to presence of C-C and C-H bonds in polyvinyl chloride. It is proposed that in PVC/MgO nanocomposites, on MgO nanostructures - MB dye generates holes in ground state which takes part in degradation of PVC matrix also with $^{\bullet}OH$ and $O_2^{\bullet}H$. These oxygenated species are initiators of PVC degradation by attacking the polymeric chain.

$-(CH_2CHCl)-^{*} + {}^{\bullet}OH \longrightarrow (^{\bullet}CHCHCl)- + H_2O$ (8)

$-(CH_2CHCl)-^{*} + {}^{\bullet}O_2H \longrightarrow (^{\bullet}CHCHCl)- + H_2O_2$ (9)

The oxygen molecules (O_2) have an important central role in the degradation reactions of PVC matrix. Oncethe carbon-centered radicals included in the PVC polymer chain, their successive reactions lead to the chain cleavage with the O_2 incorporation and CO2 evolution.

$-(^{\bullet}CHCHCl)- + O_2 \longrightarrow -(CH(OO^{\bullet})CHCl)-$ (10)

$-(CH(OO^{\bullet})CHCl)- + -(CH_2CHCl) \longrightarrow (CH(OOH)CHCl)- + -(^{\bullet}CHCHCl)-$ (11)

$-(CH(OOH)CHCl)- + h\upsilon \longrightarrow (CH(O^{\bullet})CHCl)- + {}^{\bullet}OH$ (12)

$^{\bullet}OH + \text{MB Dye} \longrightarrow CO_2 + H_2O$ (13)

CONCLUSION

A spin-coating approach was used used to deposit MgO/PVC/PVC nanocomposites films. A detailed investigation of optical, morphological and structural properties was also carried out. The investigations revealed by FTIR, XRD indicate in MgO NPs. The studies also reveal a clear dispersion of MgO NPs in matrix of PVC. The increase in MgO content also enhanced the crystallinity and grain size. According to AFM we deduce that 7% , 10% MgO/PVC nanocomposites films are porous structure. Films of MgO/PVC nanocomposites are transparent and transmission optical band gap decrease from 4.1 eV to 3.69 eV. The application of MgO/PVC nanocomposites thin films was checked for photocatalysis. The improvement in photocatalytic activity was noticed for the degradation test of organic pollutants under UV light, MgO NPs enhanced photocatalytic efficiency of PVC film.

**** The ZnO is widely used.***
**** It is a very interesting material.***
**** It has various advantages:***

- ***Non-toxic,***
- ***Abundant on earth***
- ***It is a wide gap semi-conductor (3.37eV),***
- ***Piezoelectric,***
- ***It has a large binding energy of exciton (60meV),***
  G***ood catalyst and can be used as a gas sensor.***

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VALORIZATION OF FOOD INDUSTRY WASTES FROM CEREALS AND PULSES INTO FUNCTIONAL FOOD ADDITIVES

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ABSTRACT

The rising demand for sustainable food systems has led to increased interest in the valorization of agro-industrial waste, especially from cereals and pulses. Despite their abundance of bioactive components, these staple crops produce significant by-products during processing, such as bran, husks, hulls, and seed coats, which are frequently underutilized. The residues comprise valuable constituents, such as dietary fibers, proteins, lipids, vitamins, minerals, and phytochemicals exhibiting antioxidant, antibacterial, and anti-inflammatory effects. Efficient recovery procedures, ranging from traditional techniques such as solvent extraction and alkaline hydrolysis to innovative green technologies including enzyme-assisted extraction, ultrasound, and pulsed electric fields, enable the isolation of functional components. Recent studies highlight their successful applications in food systems, nutraceutical formulations, and biodegradable packaging. For instance, protein hydrolysates from rice husk exhibit anticancer activity, while arabinoxylans from bran display promising prebiotic effects. Furthermore, fermentation and enzymatic processes enhance the bioavailability and functionality of these compounds, while encapsulation technologies safeguard sensitive molecules throughout storage and processing. The processing residues of cereals and pulses have also been investigated for their potential to improve gluten-free product formulations, augment the nutritional and functional properties of baked goods and dairy products, and act as meat and fat replacers in processed foods. However, challenges remain, including the presence of anti-nutritional factors, microbial instability, and undesirable changes in sensory and physicochemical properties. Ultimately, the valorization of cereal and pulse waste offers a promising strategy for resource-efficient, sustainable food production. This review provides an overview of recent developments in recovery technologies, practical applications, and current limitations, while highlighting future directions for incorporating these underutilized materials into high-value food and packaging innovations.

Keywords: Bioactive, By-products, Cereal and pulses, Food waste valorization, Functional food additives, Sustainability.

INTRODUCTION

In recent years, there has been a lot of interest in agro-food waste valorization strategies to establish more sustainable management approaches. Cereals and pulses are grown in great quantities across the globe due to their economic significance as staple foods and dietary sources of energy. The FAO's most current figures show that the global output of cereals and pulses has reached high levels in recent years, and food consumption will continue to rise (OECD-FAO., 2022).

The primary cereals produced are maize, wheat, rice, barley, rye, sorghum, and oat crops (Cardoso et al., 2021), whereas global pulse production is dominated by beans, peas, and chickpeas (Karaca and Nickerson, 2022). However, the food manufacturing industry releases substantial and inevitable waste. Various processing methods (i.e., milling, germination, malting, soaking, peeling, blanching, fermentation, heat processing, drying, canning, freezing, and malting) yield a substantial number of fractions as by-products and wastes, including bran, germ, hull, husk, fiber, seeds, broken grain, leaf, peel, stem, and other elements of the crop. These fractions are underutilized sources of many bioactive components and nutrients. Cereal wastes are abundant in dietary fibers (lignin, β-glucans, hemicellulose), carbohydrates, bioactive peptides, lipids, enzymes, vitamins (mainly vitamin E and vitamin B), minerals, and phytochemicals (phenolic acids, phenols, procyanidins, γ-aminobutyric acid, γ-oryzanol, phytic acid, ferulic acid, etc.) (Skendi et al., 2020). Regarding the pulses, residues provide proteins (mainly from seeds), edible fibers, and bio-functional molecules like minerals (calcium, magnesium, iron, manganese, phosphorus) phenols, carotenoids, and phytosterols to be valorized in different industrial areas (Susmitha et al., 2022; Tassoni et al., 2020). Hence, besides the economic and environmental issues, cereal and pulse processing waste represent a significant loss of biomass with nutritious and functional components. In that respect, the recovery and incorporation of these components as dietary supplements, food additives, coatings, and nutraceuticals gain importance and create new gates for varied technologies. The present section aims to provide an overview of some recent potential applications in the field and the drawbacks associated with the utilization techniques.

RECOVERY AND EXTRACTION OF FUNCTIONAL COMPOUNDS

Bioactive components and fractions can be recovered using conventional and novel techniques consisting of several gradual separation methods. As a common approach, these follow pre-treatment/classification, macro-micro molecule separation, extraction, isolation, and purification pathways which can then be extended by value-added new product development. It is noteworthy to know the structure and physicochemical characteristics of target compounds to determine the appropriate method (Galanakis, 2015; Tassoni et al., 2020). The choice of recovery process primarily depends on the physicochemical properties of the prominent constituents and bioactive components in the waste product.

Many reviews are available with regards to the principle and parameters crucial to the recovery, in particular to yield and composition of the extracts obtained from cereals and pulses, for example, by the applications of supercritical fluid extraction (SFE) (Santana and Meireles, 2023), ultrasound assisted extraction (UAE) (Wang et al., 2021), microwave assisted extraction (MAE) (Duke et al., 2024), and pulsed electrical field (PEF) (Kumari et al., 2018). The detail of recovery, therefore, will not be included in this section. Current status of using these emerging technologies is to determine the most effective/suitable approach, either alone or in combination, for the recovery of different functional ingredients from different matrices.

The milling process separates grain kernels into flour, germ, and bran. By dry fractioning (milling), it is possible to obtain physical-functional bulk fractions that are rich in

proteins, essential amino acids, and minerals (Kamani et al., 2020), but in most cases, further separation stages are needed. Extraction is a particularly important phase of the bioactive recovery process. Conventional methods include alkaline and acid hydrolysis (Buranov and Mazza, 2009), and solvent extraction (Smuda et al., 2018). On the other hand, supercritical carbon dioxide extraction (Benito-Román et al., 2019), enzyme-assisted (Zuorro et al., 2019), ultrasound & microwave-assisted (Ochoa-Rivas et al., 2017), and pressurized aqueous solution extractions (Povilaitis et al., 2015) are some of the innovative, low-cost, green, and sustainable practices being developed.

The utilization of proteins from cereals and pulse wastes is a smart alternative to meet nutritionally well-adjusted consumption demand. Alkaline extraction coupled with isoelectric precipitation stays the most common protein extraction method (Contreras et al., 2019). Besides, enzymatic hydrolysis (Prandi et al., 2021), ultrafiltration (des Marchais et al., 2011), and bioprocessing designs with fermentation (Arte et al., 2019) are some of the other applications well applied for this purpose. In the case of solvent extraction, the type of solvent is critical for efficiency as it yields significantly different antioxidant capacities and bioactive contents (Smuda et al., 2018). In a recent study regarding the hot water extraction of rice husk, peptides in the protein hydrolysate of rice husk have proven an anticancer potential with a cytotoxic effect (Ilhan-Ayisigi et al., 2021). From dietary fibers, arabinoxylans (AXs) which have protective effects against free radicals and exhibit prebiotic potential (Tassoni et al., 2020), were recovered at high yields from rice and wheat brans using water and enzyme extractions (xylanase, cellulase, β-glucanase, α-amylase), one of which was enhanced with extrusion pre-treatment (Fadel et al., 2018; Toft Vangsøe et al., 2019). A significant portion of the dry matter of oats, rye, and barley is composed of β-glucans, which are valuable for metabolic bioactivity (i.e., prebiotic activity, lowering cholesterol). That can be a leading reason for their recovery from the waste of these crops. (Fărcaș et al., 2022). On the other hand, water extraction of xylan from agricultural wastes (brans and seed coats) of different cereals and legumes was achieved. Further enzymatic hydrolysis of xylan (with endoxylanase) produced non-digestible xylooligosaccharides, which are non-cariogenic, low-calorie sugar substitutes with promising applications in the food industry (Palaniappan et al., 2023).

Phytochemicals that exhibit many antioxidative, antimicrobial, antiviral, and anti-inflammatory features (de Villa et al., 2023; Rochfort and Panozzo, 2007; Smuda et al., 2018) are high-value substances abundantly present in the processing residues of cereals and pulses. Recently, it was proven that total phenolics in both wheat and oat brans were shown to increase by +22.5% and +25.8%, respectively, after being subjected to thermal treatment coupled with ultrasound extraction (Călinoiu and Vodnar, 2020). Also, obtaining polyphenols possessing antioxidant activity from industrial cereal solid wastes can be significantly altered by the addition of lactic acid and specific amino acids (glycine or alanine) but also choline chloride (Kottaras et al., 2017). As an emerging technology, pulsed electrical field (PEF)-assisted extraction was effective to exploit these wastes as a source of bioactive molecules. It provides a faster way to recover beneficial compounds while using less energy and solvent (Velusamy et al., 2023). Through fermentation with different cultures, improved structures can be obtained in terms of antioxidants, vitamins, and dissolved or branched protein fractions from by-products and wastes (Ghamry et al., 2023). Then these structures can be used as useful components such as nutraceuticals, coatings, supplements, and packaging materials. Recently, studies on solid-state fermentation, in which microorganisms (*Lactic acid bacteria, Aspergillus* spp.*, Saccharomyces cerevisiae, and Bacillus subtilis)* digest solid substrates in low moisture conditions, took place as an efficient bioprocessing procedure to increase the bioavailability of bound phenolic components and therefore the antioxidant activities of grain-stream wastes. (de Villa et al., 2023). Because research is currently scant, additional investigations i.e., in vitro analysis, on the applicability are required.

Rice bran oil and wheat germ oil may be used to supplement diets due to their immune-boosting qualities some of which come from unsaturated fatty acids, sterols, tocopherols, and carotenoids (Meriles et al., 2022; Saad et al., 2022). Mingyai et al. suggested environment-friendly supercritical CO_2 extraction as a good option for obtaining bran oils (Mingyai et al., 2017). However, during recovery and storage stages precautions should be taken against lipid oxidation due to enzymatic activity.

THE APPLICATIONS IN FOOD INDUSTRY

Developing value-added products from agro-industrial wastes has attracted interest on a global scale. Waste can be used as they are, or it can be valorized to obtain bioactive components for generating novel substances with nutritional and functional advantages. In the course of waste valorization, the functional chemicals in cereals and pulse wastes may become sensitive and unstable when exposed to environmental and process conditions. The encapsulation process is a promising practical option for the stability and protection of bioactive compounds. With this regard, freeze-drying using maltodextrin was found to be the best efficient method to encapsulate the phenolics (protocatechuic acid, ρ-coumaric acid, vanillin, salicylic acid, and catechin) in cornsilk extract (the maize waste) compared to spray-drying and microwave-drying (Pashazadeh et al., 2021). In a recent study examining the effects on health, the rice husk protein hydrolysate was encapsulated with chitosan (Ilhan-Ayisigi et al., 2021). Thermally stable nanoparticles obtained have shown cytotoxic effects promising to produce anticancer peptides and new food supplements that can be derived from them. On the other hand, compounds such as nutraceutical proteins from wastes (pigeon pea milling) can be used as an encapsulation material for water-insoluble bioactive compounds like curcumin (Tapal et al., 2019).

Agricultural waste can be a low-cost alternative to gluten-free food ingredients. Several initiatives have been launched to overcome the nutritional deficiencies of gluten-free products (bread, cake, pasta) and to improve their quality in terms of bioactive compounds and functional properties using rice bran, broken bean flour, chia seed cake, hemp seed cake, okara (soybean process by-product), and chickpea hull (Difonzo et al., 2022). The increased utilization of wastes as replacers and carrier materials can be linked to the development of novel emulsion systems. For example, β-glucan extracted from oat and barley side streams showed great emulsion stability (>80%) and behaved like a viscosifier (Karp et al., 2019). In another study, it was shown that both impure soluble and insoluble oat side streams could be used as innovative bio-based emulsifiers in suspensions, without any further separation or chemical modification (Valoppi et al., 2021). Moreover, as suggested by Tapia et al. 2019 (Tapia-Hernández et al., 2019), prolamin nanoparticles such as zein, gliadin, and kafirin, which are by-products of the cereal starch isolation process, have great potential as pickering emulsion stabilizers once their wettability characteristics are altered. It is possible that oxidative stability, (natural) antioxidant capacity, microbial stability, color, texture, and physicochemical properties of beef, sausage, and mayonnaise-type emulsions can be improved by the incorporation of various extracts from bean hulls and cereal brans or seed coats (Elsebaie et al., 2022; Herrera-Balandrano et al., 2019; Velur Balasubramaniam et al., 2022). In terms of replacement studies, black-gram protein-rich fractions (with germ and aleurone) and soybean oil oleogels structured with rice bran wax worked well as meat and fat substitutes in patty and sausage formulations (Kamani et al., 2021; Wolfer et al., 2018).

The addition of waste products could cause a wide variety of changes in the structural, functional, nutritional, and sensory characteristics of bakery products. Many beneficial effects of fortification or enrichment with waste products from cereals and pulses were observed, such as increased dietary fiber content and technological quality of bread with wheat bran (Chaari et al., 2022), increased nutritional value, phenolics, and antioxidant capacity of crackers with

barley bran (Gangopadhyay et al., 2019), improved syneresis and stability of yogurt with rice bran (Wu et al., 2023), the enhanced profile of flavonoids in rice-based snacks with pea seed coats (Bresciani et al., 2023), and enrichment in a fundamental vitamin, folate, with pulse by-product(Ruggeri et al., 2022). Unutilized, downgraded lentils were found to be a good source of ingredients, contributing positive compositional and functional attributes to the extruded final product (Portman et al., 2020). The ingredients have a strong impact on the structure and quality properties of newly developed products. Color darkening, low specific volume, low hardness in bakery products such as bread (Ni et al., 2020), off-flavor, low digestibility, and presence of antinutrients are some of the negative effects of the grain and pulse waste products that limit their use in the formulations and necessitate alternative solutions (Skendi et al., 2020; Tassoni et al., 2020). Thus, applications like enzymatic treatment, fermentation, and extrusion of underutilized cereal or pulse wastes offer final products with highly soluble materials, micronutrients, and low antinutrients, leading to better digestibility and sensory acceptance (Aktas-Akyildiz et al., 2020; Chakraborty et al., 2022; Kamani et al., 2021). Processing by-products through fermentation enables novel value-added food products. Diverse metabolites can be produced through the fermentation of wastes such as organic acids, natural preservatives (sorbic and benzoic acids), enzymes, alcohols, volatiles, esters, and bioactive peptides (de Villa et al., 2023; Skendi et al., 2020). Also, it was well reviewed that extrusion of some cereals and legumes in the form of a sole or mixture allows producing a wide range of snacks and flours with different structural, nutritional, and functional characteristics, i.e., expansion rate, hardness, crispiness, dietary fiber content, protein content, phenolics, and antioxidant capacities (Grasso, 2020).

Plastics take many years to degrade, threatening the ecology. In this regard, biodegradable polymers and packaging created by the use of grain by-product extracts have become economically important in recent years. Their applications in the field include beneficial structural (pores), mechanical (flexibility), nutritional (fibers, protein), and functional (antioxidants, phenolics) changes, as well as antimicrobial effects (Bilo et al., 2018; Wang et al., 2019; Xu et al., 2022). As a stunning example, the integration of brewers' spent grain extracts into biofilms to generate active packaging enabled antibacterial and antifungal action against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* (Moreirinha et al., 2020).

CHALLENGES AND PROSPECTS FOR THE FUTURE

The use of waste from the cereal and pulse industries presents some challenges. Although these products can be an attractive source for an assorted number of functional compounds or fractions, the process costs and efficiency remain the primary economic and technical obstacles. Now, as well as being economical, sustainable, and environmental-friendly techniques for separation, extraction, and isolation are required to be explored. As new waste sources and purification methods are explored, alternative or combined technologies can be developed for the protection of the sensitive bioactive compounds extracted from these resources. Incorporation attempts for nutritional development can result in alterations in the structural and physiochemical properties of the final product. Aside from the undesired changes in quality, another handicap for the use of cereal and pulse sources is the presence of anti-nutritional factors that cause off-flavors and low bioavailability. Future research on improving sensory features and bioavailability, including the removal or masking of off-flavors, is critical in this context. On the other side, agricultural wastes are biologically unstable considering their microbial load. This can hasten microbial spoilage from the first days of storage and impact product safety. Therefore, the integration of further feasible cleaning and sterilization studies into product development or biosynthesis processes still needs to be addressed to ensure final product safety.

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THE ROLE OF ORGANIC FERTILIZER ON CROP PRODUCTION IN AFGOI-SOMALIA

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ABSTRACT

The study used a descriptive approach and had 400 participants from Afgoi District Lower-Shebelle, Somalia. A non-simple random selection was used to acquire information from farmers, educators, and workers. To clarify the effect of naturally occurring fertilizer on crop productivity, the data were evaluated using correlation analysis. The majority of responders (56%) indicated significant agreement with the study's findings that green manure improves crop productivity by directly adding nitrogen to the soil, increasing soil fertility. The majority of responders (47.5%), however, were in agreement that plants grown in green manure receive nutrients from deeper levels of soil and release them in the topsoil. On the other hand, the majority of respondents (54%) firmly concur that green manure improves nutrient availability, both macro and micro. The majority of respondents (51%) firmly concur that green manures are environmentally benign under other circumstances. In addition, the majority of responders (57.5%) highly concur that green manure raises the caliber of products and crops. The majority of respondents (51.5%) indicated high agreement with the finding that adding manure to cropland can help keep or raise the level of soil organic matter. However, the majority of respondents (38%) agreed that animal manure includes both macro and micronutrients in both organic and inorganic forms that are essential for crop production. On the other hand, the majority of responders (45.5%) strongly concur that animal dung from a locally accessible source is cheaper than inorganic fertilizer when needed. Similar to this, the majority of respondents (41%) firmly concur that poultry manure is the best animal manure. However, the majority of respondents (51%) strongly concur that adding animal manure to the soil improves its quality. The majority of respondents (55%) said firmly that crop farming is the primary means of subsistence for Afgoi communities. However, the majority of respondents (37.5%) agreed that improvements have been made to the production system over the past two years. On the other hand, the majority of respondents (49.5%) agreed that using organic fertilizer increases crop output per unit of surface area. Similar to this, the majority of respondents (52.5%) strongly concur that fertilizer quality affects crop production.

Keywords: Animal Manure, Crop Production, Green Manure, Organic Fertilizer

INTRODUCTION

Organic fertilizers are crucial for the growth of plants. Organic fertilizers are typically more economical and easily accessible from local sources than chemical fertilizers (Solomon et al. 2012). The foundation of fertile soil is organic matter (Aboudrare 2009). Organic fertilizers are made from biological or living substances. These fertilizers provide nutrients to the soil gradually over time. Whereas artificially created fertilizers, or those obtained via the mining of non-living materials, are known as inorganic fertilizers. Inorganic fertilizers also referred to as chemical fertilizers, are utilized by plants very quickly. There are many different types of organic fertilizers, including Livestock manure from animals, including cows, poultry, goats, and others. Green manure is made from young plants, especially different kinds of legumes. Compost is made from organic agricultural waste like sorghum, maize stalks, or decaying garbage. Organic fertilizers such as sheep manure from farms and chicken manure may aid in crop growth as an alternative to artificial fertilizers (Khan et al. 2005). Organic fertilizer can improve soil fertility and aid in plant growth. Positive actions, including ammonium (NH_4^+), potassium (K^+), and magnesium (Mg_2^+), are held in biochar, which keeps these nutrients in the soil for an extended period of time and releases them to plants as needed (Lal 1982). The manure from the farm had a long-lasting influence on maize when administered to wheat-based in a wheat-maize crop combination. In soils that had been impaired by erosion, it also performed better at restoring crop yield (Jadoon et al. 2003)). In a field environment, consideration is given to the phosphorus content of maize derived from organic manures, such as crop residue, bio-organic matter, chicken manure, and pressing mud. Researchers discovered that maize includes a significantly high amount of phosphorus (42.68%) compared to other sources, such as poultry manure. When utilized as a source of phosphorus, farmyard manure is substantially more successful than other treatments at boosting the accessibility of phosphorous from soil (Meena et al. 2007). The output of maize grain varies substantially as a result of the addition of organic manures. Although grain production for manure was equal to or, on occasion, greater when phosphorus was obtained from inorganic sources, applying poultry manure produces the highest grain output when compared to another method (Jama et al. 2007).

When applied in place of mineral fertilizers, organic manures increase the grain production of maize (Reddy et al. 2007). Compared to other organic sources, poultry dung offers greater phosphorus that is readily available (Garg et al. 2007). Organic manure sources enhance the accessibility of organic matter, phosphorous, and potassium, as well as plant uptake of these nutrients. Organic amendments also enhance the amount of P and K in the overall plant, as is the height of the plant, LAI, dry matter manufacturing, and LAI. When it comes to plant P and K concentrations, poultry dung offers the best benefits. According to research, increasing the availability of nutrients in the soil from organic manures is what causes improved soil concentrations of nutrients and plant growth (Adnan 2021).

Somalia's agricultural sector faces several challenges, such as low soil fertility, inadequate access to modern farming techniques, and reliance on traditional farming practices. Due to these factors, yields of crops have remained low, and the nutritional security of the population has declined. Organic fertilizers may be used to surmount these obstacles and improve agricultural

output in Somalia. However, more study is needed to determine the effectiveness of organic fertilizers in Somalia and how they affect crop productivity. Little is known and appreciated about organic fertilizers among Somali farmers. The vast majority of farmers in Somalia still use outdated methods and need more access to education and training in contemporary agriculture. Farmers are also not fully informed about the benefits of organic fertilizers and how to use them due to a lack of agricultural extensions and instructional programs. Due to growers' inadequate knowledge and understanding of organic fertilizers, their potential impact on crop output may be restricted, given how seldom they are now employed. The availability of organic fertilizers is a benefit of their use. Organic fertilizers could be hard to come by in Somalia, and farmers might not have the resources or expertise to create them themselves. This might promote the use of costly chemical fertilizers that harm the environment. Due to their limited accessibility and availability, organic fertilizers are challenging to utilize and may not have a significant impact on crop output. The unique climate and soil of Somalia may affect the efficacy of organic fertilizer in addition to challenges with knowledge and accessibility. The effectiveness of organic fertilizers may vary depending on factors such as soil pH, nutrient content, and rainfall patterns.

This research aims to investigate how organic fertilizer affects crop production in Afgoi District-lower Shebelle, Somalia. The general purpose of this study is to identify the role of Organic fertilizer on crop production at Afgoi District-lower Shebelle, Somalia. To Identify the role of green manure on crop production at Afgoi District-lower Shebelle, Somalia. To Determine the role of animal manure on crop production at Afgoi District-lower Shebelle Somalia.

MATERIAL AND METHOD

The research approach of the study was the main focus of this chapter. The chapter includes research design, target population, sample size, sampling procedures, research instruments, and Research quality involves collecting the data, collecting the data, interpreting the data, and finally limiting the study while addressing ethical considerations.

Research Design

The study was conducted using a descriptive design. In order to discuss and examine the impact organic fertilizer has on crop production in Somalia's Afgoi District Lower Shebelle. Utilizing A data obtained from the questionnaire. The target population can be easily reached, the design is efficient, and it is less expensive, all of which make it the best option for this study.

Research Population

The target population of the study was 400 from Afgoi District- lower Shebelle, Somalia.

Sample Size

The researchers used Solvent's formula to calculate the sample size, with a maximum acceptable error of 5%.

$$n = \frac{N}{1+Ne^2}$$

N: Stands for the population

n. Stands for the sample size

e. Stands for acceptable error

$n = \frac{400}{1+400(0.05)^2}$ = so the sample was 200

Sampling Procedure

The sampling method was non-random sampling anon-simple random sampling was selected to collect information from the accessible populations of farmers, Agro-students, and workers.

This study's findings about the role of organic fertilizer on crop production have been established in a number of ways. The research's conclusions and findings from a structured questionnaire are discussed in this section. This study's overarching goal is to determine how organic fertilizer affects crop output in Somalia's Afgoi District (lower Shebelle). In contrast, the objective of the study is to identify the role of green manure on crop production at Afgoi District-lower Shabelle, Somalia. to determine the role of animal manure on crop production at Afgoi District-lower Shebelle, Somalia.

RESULTS AND DISCUSSION

The Role of Organic Fertilizers

It is known that soil quality and productivity are increased with organic fertilizer application. For this purpose, many organic fertilization materials are widely used (Figure 1). According to the study's findings, the majority of participants (56%) indicated high agreement that green manure improves crop output by directly aerating soil fertility. In contrast, the majority of respondents (47.5%) thought that plants grown in green manure receive nutrients from deeper soil layers and leave them in the topsoil. The majority of responders (54%) strongly disagree, however, that green manure enhances the availability of both macro and micronutrients. Most poll respondents (51%) completely agree that green manures are environmentally favorable in other contexts. Additionally, the majority of respondents (57.5%) firmly concur that green manure raises the standard of produce and crops.

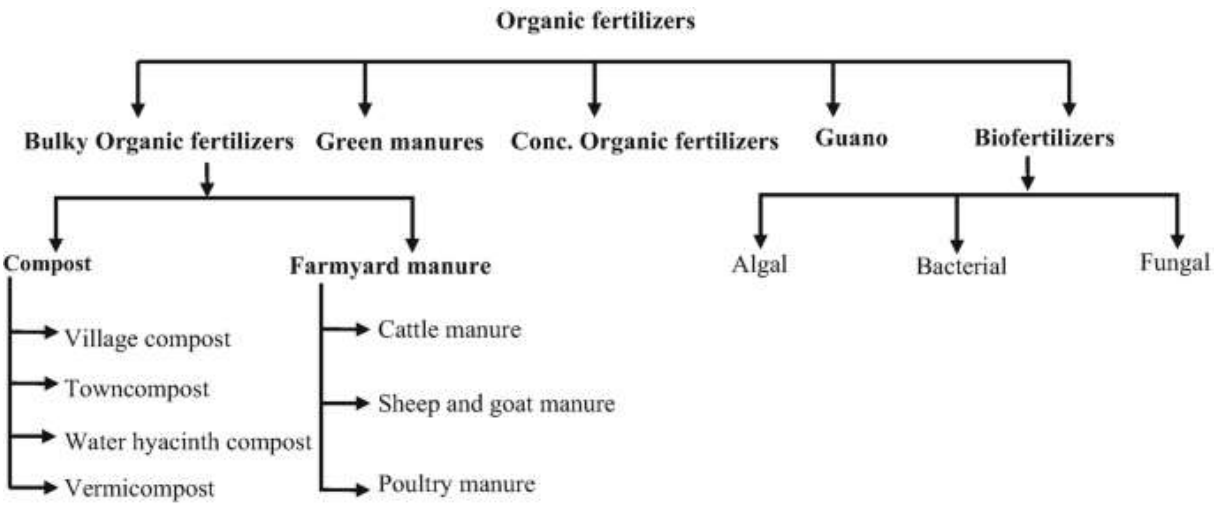

Figure 1. Organic fertilizers used in improving soil fertility (Singh et al. 2020)

Animal Manure

The majority of respondents (51.5%) answered that they strongly agreed with the claim that spreading manure to croplands can help maintain or raise the level of soil organic matter. On the other hand, a large majority of the participants (38%) said they completely concurred with the assertion that animal dung from locally accessible sources contains both micro and macro nutrients needed for crop productivity in organic as well as inorganic forms.

Crop Production

Organic fertilizers are among the most important inputs in plant production. In fact, it is reported that organic fertilizers can be a good alternative in combating stress factors in plant production (Figure 2).

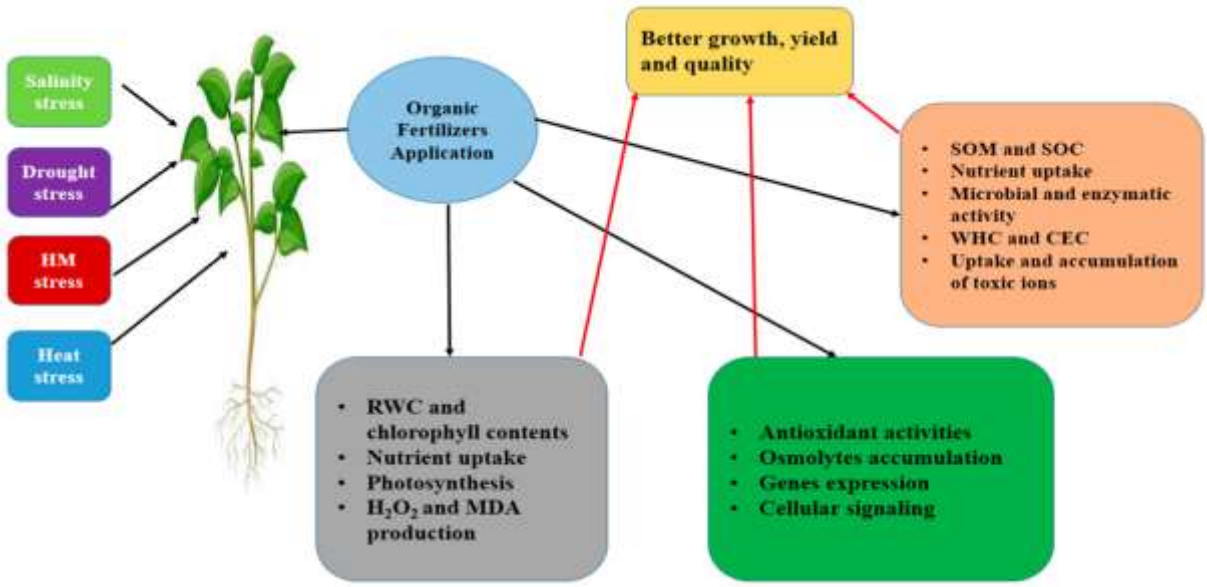

Figure 2. Effects of organic fertilizer application against stress factors in plant production (Liu et al. 2024)

The majority of respondents (55%) said firmly that crop production is the primary means of subsistence for Afgoi communities, according to the data. Conversely, most respondents (37.5%) believed that the production system had improved during the previous two years. Alternatively, the majority of responses (49.5%) agreed that the application of organic fertilizer increases crop productivity per unit of surface area. In a similar vein, the majority of responders (52.5%) strongly concur that fertilizer quality affects crop productivity.

With regard to the result of this study and based on its objectives, the following could be concluded: The objective of the role of Organic fertilizer on crop production indicates that the use of green manure is widely believed to increase soil fertility through the direct addition of nitrogen, as well as by absorbing nutrients from deeper soil layers and leaving them in the surface soil. Respondents also strongly agree that green manure increases the availability of both macro and micronutrients, is environmentally friendly, and improves the quality of crops and produce.

The objective of the role of animal manure on crop production shows that the use of animal manure can help maintain or improve soil organic matter levels and that it contains both macro- and micronutrients needed for crop production in organic and inorganic forms. Respondents also strongly agree that animal manure from locally available sources is cheaper than inorganic fertilizers and that the best animal manure is from poultry.

Crop productions conclude that crop production is the livelihood strategy for Afgoi communities, with most respondents strongly agreeing with this statement. While a smaller percentage of respondents agreed that the production system has improved in the past two years, the use of organic fertilizers is widely believed to increase crop production per unit of surface area. Respondents also strongly agree that the quality of fertilizers is an important factor in crop production.

CONCLUSIONS

To improve crop production and soil fertility, increase the application of organic fertilizers like green manure and animal faces. To educate farmers about the advantages and appropriate methods for the application of organic fertilizers, services for extension should be made available. Develop and implement operational policies that encourage the creation and application of organic fertilizers. In order to assure the safety and efficacy of organic fertilizers, restrictions and subsidies for their production and distribution may be necessary.

Encourage the use of locally accessible sources of animal dung, particularly poultry manure, as a reasonably priced substitute for inorganic fertilizers. This can entail offering instruction and assistance for small-scale chicken farming. Increase funding for the production of organic fertilizers and the technologies used to apply them. This could result in new, enhanced organic fertilizers that are more successful and efficient at enhancing crop production and soil fertility. Encourage and support farmer cooperatives and associations to promote the use of organic fertilizers and improve crop production. This could include providing access to credit and market information and facilitating collective bargaining for better prices and market opportunities.

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INTERVAL VALUED PYTHAGOREAN FUZZY AHP-INTERVAL VALUED PYTHAGOREAN FUZZY TOPSIS METHOD FOR SOLAR POWER PLANT SITE DECISION

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ABSTRACT

Solar energy systems yield significant results in terms of environmental, economic, and energy security. They are utilized in residential energy production, industrial applications, agriculture, and the transportation sector. As a renewable energy source, solar energy systems contribute to achieving the Sustainable Development Goals. The utilization of solar energy reduces reliance on fossil fuels, thereby enhancing the sustainability of energy systems. This research aims to evaluate Turkey's investment priorities for efficient, reliable, economic, continuous, and environmentally friendly electricity production, leveraging its notable renewable energy resource potential. In this context, existing literature has been utilized, and the Turkish Ministry of Energy and Natural Resources' (ETKB) 2015-2019 Strategic Plan has been taken as a fundamental reference. The proper establishment of solar energy systems is supported by the systematic analysis of the right criteria and alternatives, allowing for the selection of sustainable energy solutions. The decision regarding the suitable site for newly planned renewable energy plants is based on a multidisciplinary approach and complex process management. In modern decision-making processes, various methods have been developed to cope with uncertainty and complexity. IVPF-AHP (Interval Valued Pythagorean Analytic Hierarchy Process) and IVPF-TOPSIS (Interval Valued Pythagorean Technique for Order of Preference by Similarity to Ideal Solution) methods stand out as approaches used together to manage such uncertainties more effectively. This method has been applied to three regions planned for solar energy investment. The application was conducted with five expert decision-makers in the field. IVPF-AHP and IVPF-TOPSIS methods used to improve decision-making processes in solar energy investments guide the successful selection of locations for solar energy projects. A systematic and rational decision-making process has been carried out for the selection of solar power plant sites. The analysis results provided concrete data to decision makers regarding which option represents an efficient investment. The analytical evaluation results are of great importance in terms of both economic and environmental sustainability.

Keywords: Interval Valued Pythagorean AHP-Interval Valued Pythagorean TOPSIS, Solar Power Plants, Multi-Criteria Decision Making, Turkey.

INTRODUCTION

Renewable energy (RE) is characterized by its capacity for continuous replenishment and sustainable utilization. This encompasses a variety of sources, including solar energy, wind energy, hydroelectric power, tidal energy, biomass, and geothermal energy (Liao and Li, 2025).

The advent of competition in the electricity markets necessitates a thorough reevaluation and strategizing of solar energy technologies. Concerns regarding the implementation of solar energy systems within economic frameworks can be articulated in the context of how competition in the electric power sector will empower consumers, leading to enhanced efficiency and reduced electricity costs. This shift toward competitive structures in power markets encourages innovations that may further optimize the utilization of solar energy, thereby facilitating a transition towards more sustainable energy practices (Akhtar et al., 2021). Solar energy, derived from the sun's rays, is one of the most abundant and widely accessible forms of renewable energy. Photovoltaic cells convert sunlight directly into electricity. The inexhaustible nature of solar energy makes it a viable long-term energy option (Sani and Sule, 2020).

The process of generating electricity through photovoltaic (PV) technology produces minimal greenhouse gas (GHG) emissions or other pollutants, resulting in a relatively low environmental impact. This characteristic plays a crucial role in mitigating global climate change and reducing air and water pollution. Consequently, solar energy has emerged as a highly favorable alternative energy source in recent years (Chao and Nguyen, 2024).

INTERVAL VALUED PYTHAGOREAN FUZZY AHP-INTERVAL VALUED PYTHAGOREAN FUZZY TOPSIS (IVPF-AHP)

Kose et al. (2023) delineates the steps of the Interval Valued Pythagorean Fuzzy AHP-TOPSIS as follows:

Definition 1. Let a set X be a universe. A PFS P is an object having the form:

$$P = \{\langle x, P(\mu_{P(x)}, v_{P(x)})\rangle | x \in X\}, \tag{1}$$

where $\mu_{P(x)}: X \to [0,1]$ shows the degree of membership and $v_{P(x)}: X \to [0,1]$ shows the degree of non-membership of the element $x \in X$ to P, respectively, and, for every $x \in X$, it holds:

$$0 \leq {\mu_{P(x)}}^2 + {v_{P(x)}}^2 \leq 1, \tag{2}$$

For any PFS and $x \in, \pi_{P(x)} = \sqrt{1 - \mu_{P(x)}^2 - v_{P(x)}^2}$ defines the degree of indeterminacy of x to P.

Definition 2. An IVPFS A can be defined in X as follows:

$$A = \{\langle x[\mu_{A_L}(x), \mu_{A_U}(x)], [v_{A_L}(x), v_{A_U}(x)]\rangle; x \in X\}, \tag{3}$$

where

$$0 \leq \mu_{A_L}(x), \mu_{A_U}(x), v_{A_L}(x), v_{A_U}(x) \leq 1, and\ \left(\mu_{A_L}(x)\right)^2 + \left(v_{A_L}(x)\right)^2 \leq 1\ \ x \in X, \tag{4}$$

Similar to PFSs, the degree of indeterminacy is calculated.

$$\pi_A(x) = \left[\pi_{A_L}(x), \pi_{A_U}(x)\right] = \left[\sqrt{1-\left(\mu_{A_U}(x)\right)^2-\left(v_{A_U}(x)\right)^2}, \sqrt{1-\left(\mu_{A_L}(x)\right)^2-\left(v_{A_L}(x)\right)^2}\right], \tag{5}$$

Definition 3. The score ($\mathbb{S}^\circ$) and normalized score ($\mathbb{S}^*$) functions for IVPF number $\alpha = \left(\left[\mu_{A_L}, \mu_{A_U}\right], \left[v_{A_L}, v_{A_u}\right]\right)$ are given below:

$$\mathbb{S}^\circ(\alpha) = \frac{(\mu_{A_L})^2+\left(\mu_{A_U}\right)^2-(v_{A_L})^2-(v_{A_u})^2}{2}, \tag{6}$$

$$\mathbb{S}^*(\alpha) = \frac{1}{2}(\mathbb{S}^\circ(\alpha)+1), \tag{7}$$

Definition 4. The distance measure between two IVPF numbers $\alpha = \left(\left[\mu_{A_L}, \mu_{A_U}\right], \left[v_{A_L}, v_{A_u}\right]\right)$ and $b = \left(\left[\mu_{B_L}, \mu_{B_U}\right], \left[v_{B_L}, v_{B_u}\right]\right)$ can be defined as follows:

$$d(\alpha, b) = \frac{1}{4}\begin{pmatrix} \left|\mu_{A_L}^2-\mu_{B_L}^2\right|+\left|\mu_{A_U}^2-\mu_{B_U}^2\right|+\left|v_{A_L}^2-v_{B_L}^2\right| \\ +\left|v_{A_U}^2-v_{B_U}^2\right|+\left|\pi_{A_L}^2-\pi_{B_L}^2\right|+\left|\pi_{A_U}^2-\pi_{B_U}^2\right| \end{pmatrix}, \tag{8}$$

After determining the fuzzy set, AHP determines the weights of criteria by performing pair-wise comparison between criteria. Definition of IVPF is used to present the real case of problems as it involves the uncertainty in linguistic judgments. However, crisp AHP cannot compute this situation. The application steps of IVPF-AHP are given below:

Compromised pairwise comparison matrix $R = (r_{ik})_{m\times m}$ with respect to criteria is constructed using the linguistic terms and their abbreviations given in Table 1.

Table 1. Weighting scale for the IVPF-AHP.

Linguistic Terms	IVPF Numbers			
	μ_L	μ_U	v_L	v_U
Extremely Low Importance—ELI	0.00	0.00	0.90	1.00
Very Low Importance—VLI	0.10	0.20	0.80	0.90
Low Importance—LI	0.20	0.35	0.65	0.80
Below Average Importance—BAI	0.35	0.45	0.55	0.65
Equal Importance—EI	0.45	0.55	0.45	0.55
Above Average Importance—AAI	0.55	0.65	0.35	0.45
High Importance—HI	0.65	0.80	0.20	0.35
Very High Importance—VHI	0.80	0.90	0.10	0.20

Extremely High Importance—EHI	0.90	1.00	0.00	0.00

The differences matrix $D = (d_{ik})_{m\times m}$ is determined between lower and upper values of the membership and non-membership functions based on Equations (9) and (10):

$$d_{ik_L} = \mu^2_{ik_L} - \vartheta^2_{ik_U}, \tag{9}$$

$$d_{ik_U} = \mu^2_{ik_U} - \vartheta^2_{ik_L}, \tag{10}$$

where m is the number of criteria.

The interval multiplicative matrix $S = (s_{ik})_{m\times m}$ is determined based on Equations (11) and (12):

$$s_{ik_L} = \sqrt{1000^{d_{ik_L}}}, \tag{11}$$

$$s_{ik_U} = \sqrt{1000^{d_{ik_L}}}, \tag{12}$$

The determinacy value $\tau = (\tau_{ik})_{m\times m}$ of the r_{ik} is calculated based on Equation (13):

$$\tau_{ik} = 1 - (\mu^2_{ik_U} - \mu^2_{ik_L}) - (v^2_{ik_U} - v^2_{ik_L}), \tag{13}$$

The determinacy degrees with $S = (s_{ik})_{m\times m}$ matrix for obtaining the matrix of weights, $T = (t_{ik})_{m\times m}$ is multiplied according to Equation (14):

$$t_{ik} = \left(\frac{s_{ik_L} + s_{ik_U}}{2}\right)\tau_{ik}, \tag{14}$$

The normalized priority weights w_i is calculated based on Equation (15):

$$w_i = \left(\frac{\sum_{k=1}^{m} t_{ik}}{\sum_{i=1}^{m}\sum_{k=1}^{m} t_{ik}}\right)\tau_{ik}, \tag{15}$$

Each power plant site may have different characteristics and occurrence of one or more risks. Therefore, the evaluation of each defined risk factor for every site is performed by decision-makers. The TOPSIS methodology is applied under IVPFSs to prioritize power plant sites. TOPSIS methodology considers both positive-ideal and negative-ideal solutions in decision-making. TOPSIS outshines with computational simplicity and flexibility to integrate with different areas. The application steps of IVPF-TOPSIS are given in the following steps.

IVPF number-based decision matrix $R = (C_j(x_i))_{m\times n}$ is computed using the linguistic terms given in Table 2, where n is the number of alternatives. $C_j = (j = 1,2,\dots,m)_{m\times n}$ and $x_i(i = 1,2,\dots,m)_{m\times n}$ represent values of criteria and alternatives.

Step 2.3.2: The positive ideal points (A^+) and negative ideal points (A^-) are defined based on normalized score function:

$$A^+ = \left(\left[\left\{\left(\max_i \mu_{ij_L}(\widetilde{P}^*), \max_i \mu_{ij_U}(\widetilde{P}^*)\right)\right\}\right], \left[\left\{\left(\min_i v_{ij_L}(\widetilde{P}^*), \min_i V_{ij_U}(\widetilde{P}^*)\right)\right\}\right]\right)_{1xn}, \tag{16}$$

$$A^- = \left(\left[\left\{\left(\min_i \mu_{ij_L}(\widetilde{P}^*), \min_i \mu_{ij_U}(\widetilde{P}^*)\right)\right\}\right], \left[\left\{\left(\max_i v_{ij_L}(\widetilde{P}^*), \max_i V_{ij_U}(\widetilde{P}^*)\right)\right\}\right]\right)_{1xn}, \tag{17}$$

Step 2.3.3: The weighted distances from A^+ and A^- are computed with respect to criteria weights for alternatives:

$$D(A_i, A^+) = \sum_{j=1}^{n} w_j[d(A_i, A^+)], \quad (18)$$

$$D(A_i, A^-) = \sum_{j=1}^{n} w_j[d(A_i, A^-)], \quad (19)$$

Step 2.3.4. The closeness coefficient is calculated for each alternative as follows:

$$F_i = \frac{D(A_i, A^-)}{D(A_i, A^+) + D(A_i, A^-)}, \quad (20)$$

Step 2.3.5. The alternatives are ranked in the descending order. In this study, we determine the riskiest task with the highest F_i value.

Table 2. Linguistic terms to evaluate power plant sites.

Linguistic Terms	IVPF Number			
	μ_L	μ_U	v_L	v_U
No relation—NO	0	0	1	1
Extremely Low—EL	0.03	0.16	0.74	0.87
Very Low—VL	0.12	0.25	0.5	0.78
Low—L	0.21	0.34	0.56	0.69
Medium Low—ML	0.3	0.43	0.47	0.6
Middle—M	0.38	0.51	0.38	0.51
Medium High—MH	0.47	0.6	0.3	0.43
High—H	0.56	0.69	0.21	0.34
Very High—VH	0.65	0.78	0.12	0.25
Extremely High—EH	0.74	0.87	0.03	0.16

RESULTS AND DISCUSSION

The average annual sunshine duration of 2,640 hours indicates a consistent and substantial availability of solar energy, suitable for photovoltaic (PV) deployment and solar thermal applications. The average total irradiation intensity, recorded at 1,311 kWh/m²/year, further substantiates Turkey's capabilities in solar energy generation. This intensification enables enhanced energy output from solar installations compared to similar periods and regions.

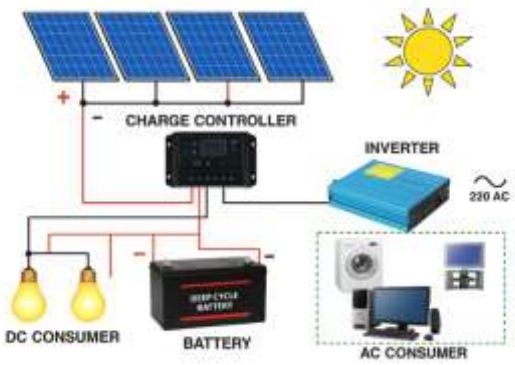

Figure 1. Solar Power System Diagram

Turkey's southern geographic regions are uniquely positioned in the solar belt, providing vast and unencumbered spaces suitable for deploying solar infrastructure, thereby facilitating large-scale solar projects. The expansive land available for solar energy installations offers Turkey a considerable advantage, particularly in comparison with other countries recognized as sector leaders in solar technology. The pronounced difference in solar irradiance values underscores the untapped potential that Turkey possesses. These factors favor the prospect of enhancing solar energy technology adoption, which can yield significant economic and environmental benefits.

Figure 2. Power plant

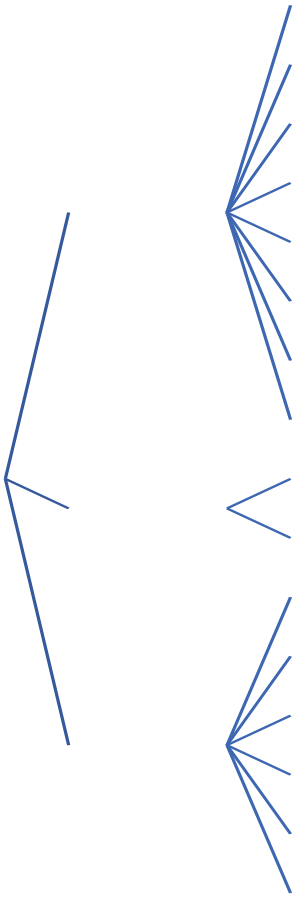

Figure 3. Power plant site decision hierarchy

In this context, the insights of five experts are taken into account. These experts possess substantial experience in the field. Their evaluations are gathered through meetings.
Based on the study, alternatives have been systematically prioritized by integrating the enhancements of the integrated theory with the combined compromise solution method. This dual approach enables a more nuanced evaluation of options, considering both subjective perceptions of risk and objective trade-offs. Furthermore, to demonstrate the practicality of the proposed model, an illustrative example has been provided, showcasing its effectiveness in real-world applications.

The cost of investment in a power plant emerges as a crucial factor that must be addressed in the process. This expense significantly influences customers' choices regarding the location of their power plants. By offering these costs at a more competitive rate, it becomes feasible to engage a broader customer audience. The findings suggest that by employing this combined methodology, decision-makers can achieve a more balanced consideration of various alternatives, resulting in improved outcomes. Future research may focus on expanding this framework to include additional decision-making contexts. Thereby enhancing its flexibility and robustness across diverse scenarios.

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LEGAL ISSUES FACED BY FARMERS AND AWARENESS OF AGRICULTURAL LAW IN THE TRAKYA REGION

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ABSTRACT

This study aims to determine farmers' level of legal knowledge, the legal issues they encounter, and their needs for legal support in the provinces of Tekirdağ, Edirne, and Kırklareli. The research was conducted using a face-to-face survey method with 270 farmers. The average age of the farmers was 50.3 years, and their education level was predominantly (44.8%) at the primary school level.

According to the findings, 79.7% of the farmers expressed a desire to gain more knowledge about agricultural law, while 84.4% believed that legal training programs for farmers would be beneficial. Furthermore, 39.4% of farmers stated difficulty in keeping track of legal regulations concerning their agricultural enterprises. Regarding the most significant legal issues encountered, 47.0% of farmers identified problems related to the marketing and trade of agricultural products, 45.9% cited inheritance or transfer procedures, and 38.9% reported legal barriers related to grants, incentives, and subsidies.

The results of the study reveal that awareness of agricultural law in the region is limited, and that farmers require both informational and advisory support in legal processes. It is therefore recommended that educational programs prioritize key topics such as the legal framework of support programs, inheritance law, land ownership, and marketing legislation.

Keywords: Farmer training, rural development, agricultural legislation, legal literacy

INTRODUCTION

The agricultural sector is a strategic area that constitutes the historical foundations of Turkey's social and economic structure. The agricultural revolution, which began with humanity's cultivation of land, gave rise to sedentary life and the concept of private property, thereby creating the need for the protection and management of agricultural lands (Uyumaz & İlhan, 2018). The complex land ownership structure inherited from the Ottoman Empire, coupled with the fact that a large majority of the population was engaged in agriculture in the early years of the Republic, has consistently positioned the policies and legal framework regulating this sector at the forefront of national priorities (Ortak, 2023). Given the profound importance of agriculture for both society and the nation, the establishment of special regulations distinct from general law for matters such as the use, management, and transfer of agricultural lands has become a necessity (Uyumaz & İlhan, 2018).

Agricultural law, which regulates agricultural production, land use, support policies, and the rights and obligations of farmers, is of critical importance for the sustainability of the sector. This field, which diverges from general law with its unique institutions and complex structure, encompasses not only land ownership but also a wide range of topics, including the transfer of agricultural enterprises, inheritance, land consolidation, agricultural lease contracts, the legal

status of animals, and financial collateral mechanisms. For instance, the Soil Protection and Land Use Law No. 5403 seeks to prevent the loss of productivity through the fragmentation of agricultural lands by inheritance (Uyumaz & İlhan, 2018), while the legal right of pre-emption granted to adjacent landowners aims to protect land tenure integrity (Canarslan, 2020). This legal framework is further detailed with specific regulations such as the pledge of farm animals (Seyhan, 2021) or agricultural lease contracts (Akkışla, 2025), requiring farmers to possess specialized legal knowledge and awareness beyond a general understanding of the law.

A review of the literature reveals that studies on agricultural law are generally conducted from a legislative or policy perspective. While topics such as inheritance (Uyumaz & İlhan, 2018), the right of pre-emption (Canarslan, 2020), agricultural insurance (Yücer & Altıntaş, 2023), or historical reforms (Ortak, 2023) are addressed, studies examining how these legal regulations are perceived by farmers—the primary stakeholders—along with their levels of legal awareness and the difficulties they face in rights-seeking processes, are limited. However, although agricultural legislation in Turkey covers a broad spectrum, the mere existence of these regulations is insufficient to solve the problems alone. The effectiveness of legislation in practice is directly linked to the level of knowledge and awareness of its target audience, the farmers. The lack of legal knowledge at this juncture prevents farmers from effectively exercising their rights, which leads not only to individual losses of rights but also to the deepening of existing problems and a decline in overall agricultural productivity.

Therefore, studies that reveal farmers' knowledge levels regarding agricultural law, the legal issues they encounter, and their expectations for solutions to these problems are of great importance for policymakers and practitioners. The purpose of this study is to identify the main legal issues faced by farmers in the Trakya Region, the methods they use to cope with these problems, and their levels of consciousness and awareness regarding agricultural law. In this context, the study aims to identify the concrete legal problems experienced by farmers in areas such as ownership, inheritance, marketing, subsidies, and contracts, as well as the obstacles they encounter in following legal regulations, accessing professional legal support mechanisms, and exercising their rights.

MATERIAL AND METHOD

Material

The research data were collected through face-to-face surveys with a total of 270 farmers operating in the provinces of Tekirdağ, Edirne, and Kırklareli. The questionnaire used in the study consisted of both closed-ended and open-ended questions, designed to capture the socio-demographic characteristics of the farmers, their agricultural practices, their level of awareness regarding agricultural law, the legal challenges they encounter, and their needs for training and advisory services.

Method

The target population of the research comprised 68,078 agricultural enterprises registered in the Farmer Registration System (FRS), including 27,637 in Edirne, 16,038 in Kırklareli, and 24,403 in Tekirdağ. Agricultural enterprises with a land size of less than 1.5 hectares or more than 110 hectares were excluded from the study.

The sample size was determined using the finite population formula for proportions (Newbold, 1995; Miran, 2002). In the formula, a 90% confidence level, a 5% margin of error, and p=q=0.5 were applied to obtain the maximum possible sample size.

$$n = \frac{N.p.q}{(N-1).\sigma_p^2 + p.q} \quad (1)$$

As a result of the sampling, surveys were conducted with 270 farmers. The collected data were analyzed using descriptive statistics (percentages, frequencies, and means). Data processing and tabulation were carried out with the assistance of the SPSS (Statistical Package for the Social Sciences) software.

RESULTS AND DISCUSSION

In this section, the data collected within the scope of the research are analyzed to present the main findings regarding the demographic and enterprise profiles of farmers in the Trakya Region, as well as the legal issues they face and their awareness of agricultural law.

Socio-Demographic and Enterprise Characteristics of Farmers

All 270 farmers participating in the study were male. This finding suggests that the role of agricultural enterprise manager or the individual representing the family in legal processes in the region is predominantly assumed by men. An examination of the age distribution reveals that the sample comprises an experienced and aging population engaged in agricultural production. It is noteworthy that more than half of the farmers (51.85%) are aged 51 and above (Table 1). Consistent with this age profile, a very large majority of the farmers (80.74%) have 16 or more years of agricultural experience, indicating that the sample represents an established group of farmers well-versed in production practices.

A considerable proportion of the farmers (44.81%) are primary school graduates. When combined with middle school graduates, this proportion rises to 61.85%, while the share of those with a high school education or higher remains relatively limited. This distribution suggests that educational attainment may constitute a limiting factor in comprehending complex legal texts and following legal procedures. The majority of households consist of 3–4 members (57.41%), but the number of individuals actively participating in agricultural activities is generally limited to one (42.23%) or two (42.59%).

Table 1. Socio-demographic characteristics of farmers

		Freq.	%			Freq.	%
Age	20-35	41	15,19	Farming Experience (Years)	1-15	52	19,26
	36-50	89	32,96		16-30	82	30,37
	51-70	103	38,15		31-50	114	42,22
	71 and above	37	13,70		51 and above	22	8,15
Education Level	Primary school	121	44,81	Off-Farm Income Source	Retirement pension	103	38,15
	Middle school	46	17,04		No off-farm income	83	30,74
	High school	62	22,96		Self-employed	43	15,93
	Vocational school	18	6,67		Private sector	31	11,48
	Bachelor's	16	5,93		Worker	6	2,22
	No formal education	7	2,59		Public sector	4	1,48
Household size	1-2 members	35	12,96	Family Members in Farming	1	114	42,23
	3-4 members	155	57,41		2	115	42,59
	5-6 members	73	27,04		3	31	11,48
	7 or more members	7	2,59		4	10	3,70

This finding indicates that the regional production model is largely based on small-scale family farming, managed by a limited labor force. With respect to off-farm income, 38.15% of farmers receive a retirement pension, while 30.74% derive their income solely from agriculture. This situation highlights the vulnerability of this group in terms of economic sustainability.

Production Activities and Structural Characteristics of Enterprises

The average landholding size of the enterprises is 25 hectares (approximately 250 decares). When the production pattern is examined (Table 2), it is observed that agricultural activities are predominantly concentrated on field crop farming. A large proportion of farmers cultivate wheat (89.63%) and sunflower (88.52%). These two crops constitute the main production pattern of the region, while other crops such as barley (40.00%) and maize (27.78%) also occupy a significant share. In contrast, the commercial production of vegetables and fruits remains quite limited.

Table 2. Commercially Produced Crops

Field Crops			Vegetables			Fruits		
	Freq.	%*		Freq.	%*		Freq.	%*
Wheat	242	89,63	Tomato	13	4,81	Watermelon	10	3,70
Sunflower	239	88,52	Pepper	8	2,96	Melon	8	2,96
Barley	108	40,00	Cucumber	7	2,59	Almond	3	1,11
Maize	75	27,78	Eggplant	6	2,22	Cherry	3	1,11
Paddy (rice)	34	12,59	Other	8	2,96	Walnut	2	0,74
Canola	28	10,37				Apple	2	0,74
Fodder beet	15	5,56				Peach	1	0,37
Forage pea	6	2,22						
Common Vetch	4	1,48						
Alfalfa	3	1,11						
Ryegrass	1	0,37						

*Percentages are based on the total number of respondents (N=270). As multiple responses were allowed, the total may exceed 100%

Additionally, 35.93% of the enterprises are engaged in commercial livestock activities (Table 3). This distribution indicates that livestock farming provides an additional source of income and contributes to activity diversification for producers in the region. Among these enterprises, 32.98% are engaged solely in fattening, 27.84% solely in dairy farming, while 39.18% carry out both activities simultaneously.

Table 3. Distribution of Enterprises by Type of Livestock Activity (n=97)

	Frequency	%
Fattening	32	32,98
Dairy Farming	27	27,84
Both (Fattening and Dairy)	38	39,18
Total	97	100,00

Legal Issues Encountered in Farming Activities

When examining the legal issues encountered by farmers, regulations related to the marketing and trade of agricultural products rank first (47.04%) (Table 4). This finding reveals that beyond production, legal uncertainties in the process of bringing products to market constitute a fundamental challenge for farmers. The second most cited issue is "inheritance or land transfer procedures" (45.93%). This outcome confirms that inheritance law—frequently emphasized in previous studies as a factor leading to the fragmentation of agricultural land—remains a persistent structural problem for farmers in the region. The third issue is legal barriers to accessing grants, incentives, and subsidies (38.89%), indicating that farmers face bureaucratic or legislative obstacles in benefiting from government support programs. Additionally, land consolidation (31.85%) and issues concerning land ownership and use (25.19%) were also identified by farmers as significant problem areas.

Table 4. Legal Issues Encountered in Farming Activities

	Frequency	%*
Legal regulations on marketing and trade of agricultural products	127	47,04
Issues related to inheritance or land transfer procedures	124	45,93
Legal barriers to accessing grants, incentives, and subsidies	105	38,89
Land consolidation	86	31,85
Issues concerning land ownership and use	68	25,19
Difficulties in complying with environmental regulations	55	20,37
Did not experience any problems	7	2,59

*Multiple responses were permitted

Despite the prevalence of these issues, 83.33% of farmers stated that they did not experience any difficulty in dealing with them. This finding can be interpreted in two ways: first, farmers may have become accustomed to legal problems and developed confidence in resolving them through their own traditional methods. Second, and more plausibly, farmers may perceive the concepts of "legal issue" and "experiencing difficulty" differently; that is, while they acknowledge the existence of problems, they may not view recourse to formal legal channels as a viable option for resolution, and therefore do not perceive them as "difficulties."

For the farmers who did report difficulties in dealing with legal issues, the most common obstacle was inheritance and property processes (40.00%) (Table 5). Within this category, inheritance sharing, transfer procedures, land boundaries, and land consolidation processes were particularly prominent. The second most cited difficulty was systemic barriers (20.00%), with farmers identifying the high costs of litigation and attorney fees, as well as the excessive length of legal proceedings, as fundamental deterrents. This highlights how decisive financial and bureaucratic obstacles are in limiting access to justice. The third-ranking difficulty was lack of knowledge and inability to access support mechanisms (15.56%). Farmers' statements such as "I don't know where to apply" or "I can't find a contact person/expert" reveal a lack of even the most basic knowledge of legal procedures, as well as the inadequacy of institutional support mechanisms. The findings indicate that the difficulties experienced by farmers reflect an intertwined structure, arising from the complexity of the problems themselves, the burdens imposed by the legal system, and deficiencies in the farmers' informational infrastructure.

Table 5. Difficulties Encountered in Dealing with Legal Issues (n=45)

Category of Difficulty	Freq.	%*
Inheritance and Property Processes (Inheritance sharing, transfer procedures, land boundaries, land consolidation)	18	40,00
Systemic Barriers (High lawyer/litigation fees, lengthy legal processes)	9	20,00
Lack of Knowledge and Access to Support Mechanisms (Not knowing where to apply, unable to find a contact person/expert)	7	15,56
Product marketing, grant procedures	5	11,11
Other (Non-compliance in practice, individual disputes)	6	13,33
Total	45	100,00

*Percentages are based on the 45 farmers who stated they experienced difficulty.

Perception and Awareness of Agricultural Law

To understand farmers' approaches to legal issues and processes, their perceptions of agricultural law and their current levels of knowledge in this field were examined. The findings indicate that farmers largely conceptualize law through the concrete problems they experience and their need for protection, whereas their overall level of awareness remains quite limited.
Open-ended responses to the question “What is agricultural law?” reveal that farmers’ perception of law is predominantly practical and experience-oriented (Table 6). The most dominant theme was “Farmer Rights and Protection” (28.16%). This finding suggests that farmers primarily view agricultural law as a protective mechanism that safeguards themselves and their rights. From this perspective, the fundamental function of law for farmers is perceived as “protection.” The second most common theme, “Land, Property, and Inheritance Law” (21.48%), shows that farmers define law mainly through the areas where they encounter the most problems. This indicates that farmers conceptualize law not as an abstract construct, but as the legal counterpart of the concrete problems they face in daily life. The category “Rules and Supports Established by the State” (14.44%) reveals that law is perceived as a framework that not only imposes obligations but also provides advantages, thereby regulating the relationship between farmers and the state.

Notably, 17.78% of farmers stated that they had no direct knowledge or opinion on the subject. The high frequency of responses such as “I don’t know” or “I have no idea” confirms that agricultural law remains a foreign and distant concept for a significant share of farmers. This also reinforces the earlier finding that the perception of “not experiencing difficulty” may, in fact, stem from a lack of knowledge.

Table 6. Distribution of Responses to the Question "What is Agricultural Law?"

	Freq.	%
Farmer Rights and Protection	76	28,16
Land, Property, and Inheritance Law	58	21,48
Rules and Supports Established by the State	39	14,44
Lack of Knowledge / No Opinion	48	17,78
General Regulation of Agricultural Activities	32	11,85
Justice and Equity	10	3,70
Other (Commercial regulations, court, etc.)	7	2,59
Total	270	100,00

The findings regarding farmers' current knowledge levels of agricultural law, their demand for new information and training, and their use of institutional support mechanisms are presented in Table 7. A large majority (79.66%) of farmers expressed a desire to gain more knowledge about agricultural law, while 84.44% believed that legal training would be beneficial. This demonstrates both a high demand for information and a strong openness to education.

However, 48.89% of farmers stated that they do not possess a general level of awareness regarding agricultural law, while only 17.78% reported that they do. Half of the farmers (50.37%) openly declared that they have difficulty understanding basic legal terms. Additionally, 39.44% reported difficulty in keeping track of legal regulations concerning their enterprises. Furthermore, 52.22% of farmers indicated that they do not collaborate with farmer unions or associations—the institutions expected to be the closest and most accessible in resolving legal problems. This suggests that these institutions may either be inadequate in providing legal support, or that farmers are unaware of such support channels or choose not to make use of them.

Table 7. Farmers' Awareness of Agricultural Law and Their Views on Support Mechanisms

	Yes (%)	No (%)	Don't Know / Undecided (%)
Demand for Information and Training			
Would you like to gain more knowledge about agricultural law?	79,66	7,75	12,59
Do you think legal training for farmers would be beneficial?	84,44	7,78	7,78
Current Awareness and Knowledge Level			
Do you have a general level of awareness of agricultural law?	17,78	48,89	33,33
Do you have difficulty understanding basic legal terms related to agricultural law?	50,37	25,19	24,44
Do you have difficulty keeping track of legal regulations concerning your enterprise?	39,44	43,22	17,34
Use of Institutional Support Mechanisms			
Do you collaborate with farmer unions/associations to solve legal problems?	42,59	52,22	5,19

Legal Processes, Barriers to Access to Justice, and Solution Expectations

In this section, farmers' experiences with legal processes, the main barriers they encounter, and their expectations for overcoming these barriers are examined. The findings indicate that farmers face both structural and individual obstacles in accessing the justice system and hold clear expectations regarding both the issues and the actors involved in generating solutions.

A total of 41.85% of farmers stated that they face difficulties in managing the costs of legal processes (Table 8). This relatively balanced distribution suggests a significant differentiation within the farming population in terms of access to legal and financial resources. Despite these challenges, 47.78% of farmers reported being able to resolve their problems effectively, while only 16.30% receive regular professional legal consultancy. This highlights that professional support remains extremely limited in the resolution of legal problems.

Table 8. Farmers' Experiences and Perceptions Regarding Legal Processes

	Yes (%)	No (%)	Don't Know / Undecided (%)
Do you have difficulty getting support for legal issues concerning your enterprise?	40,74	41,85	17,41
Are you able to effectively resolve the legal issues you encounter in your enterprise?	47,78	24,07	28,15
Do you regularly receive legal consultancy for your enterprise?	16,30	81,48	2,22
Do you have difficulty managing the costs of legal processes affecting your enterprise?	41,85	42,59	15,56

When asked about the most significant problems encountered in legal processes, it was observed that the barriers are concentrated on structural deficiencies in the functioning of the system, rather than on individual capacity limitations (Table 9). A majority of farmers (67.04%) identified "the lengthy duration of legal processes" as the most significant problem. This finding indicates that the slow pace of the justice system acts as a major deterrent for farmers. The second most cited problem was "distrust in the system" (35.93%). This perception of distrust, combined with the lengthy duration of proceedings, helps explain the underlying psychological factors driving farmers' reluctance to engage with the legal system. Barriers such as "not knowing where to apply" (20.37%) and "inability to afford the costs" (16.67%)—which are related to knowledge and financial constraints—rank behind these two fundamental systemic issues.

Table 9. The Most Significant Problems Encountered in Legal Processes

	Freq.	%*
Lengthy duration of the legal process	181	67,04
Distrust in the system	97	35,93
Not knowing where to apply	55	20,37
Inability to afford the costs (Financial barriers)	45	16,67
Inability to find a lawyer	21	7,78
Other (Expert witness issues, lack of attention, etc.)	11	4,07

*Multiple responses were permitted.

The responses to the question, "Which topics should be emphasized more to increase legal awareness?" show that farmers' training demands closely align with the problems they experience (Table 10). The most frequently requested topic was "Inheritance, Property, and Land Law" (32.22%). This finding confirms that the structural problems identified as the most fundamental legal issues in this study are also perceived by farmers as their most urgent need for information.

The second most cited topic, "General Farmer Rights and Legal Processes" (26.67%), indicates that beyond specific issues, farmers need basic knowledge of the general legal framework that protects them and the mechanisms available for exercising their rights. Following these, "State Supports and Policies" (18.15%) reflects farmers' desire to understand the legal framework of their financial relations with the state.

These results clearly demonstrate that the content of any legal training program designed for farmers should primarily be structured around these three core areas.

Table 10. Priority Topics for Legal Awareness Training

	Freq.	%
1. Inheritance, Property, and Land Law (Inheritance sharing, inheritance lawsuits, land consolidation, land laws, title deeds, and boundaries, etc.)	87	32,22
2. General Farmer Rights and Legal Processes (Farmer rights, protection of rights, functioning of legal processes, information on legal rights)	72	26,67
3. State Supports and Policies (Subsidies, grant procedures, state policies, updates on new developments)	49	18,15
4. Law Specific to Agricultural Activities (Livestock and agricultural law, product marketing, environmental and water laws, commercial law)	40	14,81
5. Other (Need for more expert witnesses, seminars and meetings, clearer explanations)	22	8,15
Total	270	100,00

Finally, the actors expected to play a role in this information and support process have been clearly identified (Table 11). Farmers' expectations for legal support are concentrated on two main actors: "Legal Professionals" (40.74%) and "Agricultural Institutions and Experts" (34.08%). This indicates that farmers place their trust both in specialized legal knowledge (lawyers) and in institutional structures that are the closest and most accessible to them (such as chambers of agriculture and provincial/district directorates of agriculture). Particularly noteworthy are the responses categorized under "Mixed Support Models" (11.85%), such as "lawyer and agricultural engineer," which emphasize the farmers' demand for an interdisciplinary support mechanism capable of addressing both the legal and technical dimensions of their problems.

Table 11. Expected Actors for Legal Support

	Freq.	%
1. Legal Professionals (Specialized lawyers, law firms)	110	40,74
2. Agricultural Institutions and Experts (Provincial/district directorates of agriculture, chambers of agriculture, agricultural engineers, cooperatives, farmer unions)	92	34,08
3. State and Public Institutions (Ministry of Agriculture and Forestry, municipalities)	36	13,33
4. Mixed Support Models (Lawyers and agricultural engineers, village heads and cooperatives, state and lawyers etc.)	32	11,85
Total	270	100,00

CONCLUSIONS

This study, conducted through surveys with 270 farmers in the provinces of Tekirdağ, Edirne, and Kırklareli, examined farmers' knowledge levels of agricultural law, the legal issues they encounter, and their needs for legal support. The research results reveal that farmers' awareness of agricultural law in the region is limited, and there is an urgent need for information, support, and systemic improvements in legal processes.

The research further demonstrates that a large majority of farmers wish to gain more knowledge about agricultural law and believe that legal training programs would be beneficial. This indicates that policies aimed at enhancing legal literacy are of critical importance, not only for protecting farmers' individual rights but also for ensuring the sustainability of agricultural production. In this context, it is essential to convey priority topics—such as inheritance law, land ownership, land consolidation, marketing legislation, and the legal framework for state supports—to farmers through clear, practice-oriented content. Delivering these trainings on a regular and continuous basis via local agricultural directorates, chambers of agriculture, and farmer unions, and making use of farmers' existing information networks, will significantly contribute to strengthening their legal awareness.

In addition, the establishment of specialized legal consultancy centers in the field of agricultural law, with services provided free of charge or at a low cost, would enable farmers to resolve their legal problems more quickly and effectively. It is also recommended to disseminate practical, step-by-step guidance materials—such as guidebooks, informational brochures, and online platforms prepared in simple and accessible language—on topics including agricultural support procedures, inheritance processes, and marketing regulations.

Efforts to increase legal awareness should not rely solely on lawyers but should also be carried out through institutions that farmers already interact with—such as chambers of agriculture, agricultural directorates, and cooperatives—by integrating technical expertise with legal knowledge. Such an approach would strengthen the effectiveness of awareness-raising efforts. Consequently, by establishing a more reliable, transparent, and accessible legal system, farmers’ trust in law will be reinforced, thereby contributing both to the protection of individual rights and to the achievement of regional agricultural development goals.

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MOLECULAR MARKER-BASED APPROACHES IN DETERMINING ALMOND GENETIC DIVERSITY: A REVIEW OF RECENT STUDIES

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ABSTRACT

Almond (*Prunus dulcis* Mill.) is one of the most important nut crops cultivated worldwide due to its high nutritional value and economic significance. Across different production regions, almond exhibits broad genetic diversity, the assessment of which is crucial for breeding programs aimed at improving stress tolerance, fruit quality, and yield. This review provides a comprehensive evaluation of recent molecular characterization studies that employed various molecular marker systems to assess the genetic diversity of almond genotypes. Among these, simple sequence repeat (SSR) markers have emerged as the most widely used and reliable tools due to their high polymorphism, codominant inheritance, reproducibility, and strong discriminatory power. Evidences from these studies indicate that genotypes typically cluster according to their geographical origin and genetic background, thereby contributing to the development of targeted breeding strategies. Furthermore, molecular data serve as a valuable resource for the conservation of genetic materials and for the identification of promising genotypes that can be exploited in hybridization programs. This review underscores the significance of integrating molecular approaches into almond breeding programs to facilitate the development of new cultivars and ensure the sustainable management of genetic resources. Ultimately, such integration will enhance the planning and effectiveness of future breeding efforts.

Keywords: *Almond, Genetic Diversity, Molecular Characterization, Molecular Markers, SSR Markers*

INTRODUCTION

Almond (*Prunus dulcis* (Miller) D.A. Webb) belongs to the genus *Amygdalus* within the subfamily *Prunoideae* of the family Rosaceae (Özbek, 1978). It is recognized as a nut crop of high nutritional value (Topçuoğlu & Yılmaz-Ersan, 2021). Native to Central and Western Asia, *P. dulcis* is widely distributed worldwide and constitutes an important hard-shelled fruit species cultivated across many regions of Türkiye (Dokuzoğuz & Gülcan, 1973; Küden & Küden, 2000). Owing to its geographical position, Türkiye is both a center of origin for many fruit species and part of the natural distribution range of almond (Özbek, 1978). In Türkiye, almond occurs naturally in almost all regions except the high plateaus of the Black Sea Region (Küden et al., 2000). According to FAO statistics for 2023, global almond production was 3,513,970.31 tons (Anonymous, 2025a).

Advances in DNA technologies have provided powerful tools for plant breeding, analysis of genetic structures, and elucidation of evolutionary relationships. With the adoption of molecular markers such as isoenzymes, RFLP, RAPD, and SSR, research emphasis has shifted from phenotype- to genotype-based analyses (Aka Kaçar, 2004). Since the 1990s,

molecular markers have become integral to agricultural production, particularly in plant breeding. These tools enable the rapid acquisition of detailed information on the genetic attributes and inheritance patterns of economically important crops. Such information shortens breeding timelines in perennial fruit species and yields substantial savings in land and labor. Moreover, molecular marker–derived data support the development of higher-quality, higher-yielding cultivars by researchers working in fruit production and breeding (Aksu & Şahin Çevik, 2015). Because morphological, physiological, and cytological traits may be insufficient for robust plant systematics, there is growing reliance on molecular approaches to better characterize genetic variation (Yılmaz, 2021). Genotypes produced through breeding are new individuals and therefore constitute valuable plant material (Gök et al., 2025). Consequently, the identification, conservation, and molecular characterization of newly developed almond genotypes—and the incorporation of superior genotypes into subsequent breeding programs—are of paramount importance (Gök et al., 2020).

The objective of this review is to systematically examine molecular characterization studies in almond that employ molecular markers and to provide a comprehensive synthesis of the current state of knowledge. By comparing different marker systems, we aim to identify the most effective approaches for the evaluation of almond genetic resources.

MOLECULAR MARKERS

Molecular markers are essential tools for elucidating the genetic basis of phenotypic diversity and quantifying genotypic polymorphism. Over the past five decades, marker technology has progressed from a handful of isoenzyme markers to an almost limitless array of DNA-based systems (Arús, 2017). Because morphological traits are influenced by the environment, various fruit species are now characterized using molecular markers. A molecular marker is defined as a DNA sequence or protein located on a chromosome or organelle (or encoding such regions) that reveals polymorphism among genetically distinct individuals. A marker may be a protein, a gene, a gene fragment, or a sequence in a non-coding region (Kaçar et al., 2006). DNA-based markers are stable, detectable in all tissues, largely unaffected by environmental conditions, and generally follow simple Mendelian inheritance as codominant or dominant loci (Williams et al., 1990).

APPLICATIONS OF MOLECULAR MARKER TECHNOLOGY

Molecular markers are widely applied in plant genetics for the improvement of qualitative and quantitative traits, selection, genetic and linkage mapping, cultivar identification and protection, and the estimation of genetic distances among genotypes (Bilgin & Korkut, 2005).

ESSENTIAL CHARACTERISTICS OF MOLECULAR MARKERS

Marker systems differ in ease of use, reproducibility, level of polymorphism, and information content. A key distinction is whether markers are dominant (e.g., RAPD, AFLP, ISSR) or codominant (e.g., SSR, SNP, SSCP, CAPs). Codominant markers permit discrimination between homozygous and heterozygous individuals because both parental alleles can be detected; they are therefore considered more informative than dominant markers, which cannot distinguish heterozygous from homozygous dominant genotypes (Kaçar et al., 2006).

MOLECULAR MARKER-BASED STUDIES ON THE GENETIC DIVERSITY OF ALMOND

Molecular markers are highly effective for assessing genetic divergence at species, genus, and family levels (Federici et al., 1998). Numerous studies in Türkiye and worldwide have addressed the identification and characterization of almond genotypes. Since the advent of PCR-based markers, the use of molecular tools in almond has increased steadily. The principal marker systems employed include isoenzymes, RFLP, RAPD, SSR, AFLP, and sequence-based markers.

Isoenzymes were the first molecular markers used in almond studies. Although codominant and reproducible, they are constrained by the limited number of loci detectable with traditional staining methods and by low variation at many loci (Company et al., 2012). RFLPs have been used to construct genetic maps, identify cultivars, and characterize almond germplasm (Viruel et al., 1995; Company et al., 2012). RAPD markers—PCR amplifications using arbitrary primers—have been useful for characterizing genetic resources (Bartolozzi et al., 1998; Martins et al., 2003; Shiran et al., 2007; Company et al., 2012), but their dominant nature and limited reproducibility restrict their utility for cultivar identification and mapping. SSR (microsatellite) markers, developed for almond from *Prunus* genomic sequence resources (Testolin et al., 2004; Company et al., 2012), have proven particularly effective for inferring genetic relationships, cultivar identification, and mapping due to their high polymorphism, codominance, and cross-transferability among related species (Company et al., 2012). Microsatellites consist of tandem repeats of 1–6 bp units along chromosomes. Polymorphism arises from differences in repeat number; each distinct band represents a different allele. Flanking sequences are locus-specific and allow the design of SSR primers to amplify the target locus by PCR. As a codominant system, SSRs enable discrimination between homozygous and heterozygous alleles. PCR amplification followed by electrophoretic separation provides locus-specific insights into genomic variation (Şimşek et al., 2018).

Bartolozzi et al. (1998) analyzed genetic relationships among 17 almond genotypes and one peach genotype using 37 RAPD markers and, despite the obligate outcrossing nature of almond, reported limited genetic diversity; RAPD data grouped accessions into three clusters with similarity indices ≥0.75. Aka Kaçar et al. (2003) assessed genetic diversity in eight cultivars and 21 almond hybrids (crosses between ‘Gülcan 1’ and ‘Ferragnes’) using 12 SSR primers; 10 of the 12 SSRs were polymorphic (3–5 alleles per primer), nine primers conformed to expected Mendelian segregation, and some hybrids were identified as outcrosses. Küden et al. (2004) evaluated 14 local genotypes and ‘Texas’ using 11 RAPD primers; genotypes 47-1, ‘Hacı Alibey’, and ‘Texas’ were most similar, whereas 101-9 was most divergent. Bayazit and Küden (2007; 2011) performed RAPD-based molecular identification of 69 wild almond accessions from various regions of Türkiye together with cultivated almonds (‘Nonpareil’, ‘Cristomorto’) and selected local genotypes [‘Hacı Alibey’ (48-5), ‘Gülcan-1’ (101-23), and 101-13]. Wild almonds from Konya-Ereğli, Nevşehir-Göreme, and Niğde were similar morphologically and molecularly, while those from Gaziantep and Şanlıurfa-Birecik formed two groups. In the resulting dendrogram, cultivated almonds (*P. dulcis* L.) clustered with wild types selected from Central Anatolia. Shiran et al. (2007) examined genetic relationships among Iranian almond cultivars, major foreign cultivars, and three related species using 42 RAPD and 18 SSR markers; eight unidentified ‘Shahrodi’ accessions and wild almonds (*P. communis*, *P. orientalis*, *P. scoparia*) were included. Of 729 RAPD bands, 664 were polymorphic; SSR loci yielded 3–10 alleles per locus (mean 6.64). Both systems effectively discriminated genotypes: cultivated almonds clustered with *P. communis*, while *P. orientalis* and *P. scoparia* were

separated from the remaining *P. dulcis*. Sorkheh et al. (2007) analyzed 45 cultivars from Iran, Europe, and the USA with AFLPs (19 primer combinations), detecting 781 polymorphic bands out of 813 (96.23%). Genetic similarity ranged from 0.50 to 0.96, marker indices from 51.37 to 78.79, and PIC from 0.56 to 0.86; clustering clearly separated genotypes and wild species by origin and lineage, revealing substantial diversity. Martí et al. (2009) investigated 63 local and international cultivars from Spain using 19 SSR markers, obtaining on average 17 alleles per primer (88–260 bp). Observed heterozygosity was 0.72, higher than reported for other *Prunus*datasets. Dendrograms grouped cultivars by geographic origin, indicating distinct evolutionary trajectories of almond ecotypes; SSRs proved effective for cultivar identification, relationship inference, and pedigree analysis. Hamzaoui et al. (2013) evaluated 68 Moroccan local genotypes and 32 foreign cultivars from nine countries using 16 SSRs, detecting 234 alleles (89–252 bp); alleles per locus ranged from 4 to 24 (mean 14.63), with mean *H*o = 0.62 and *H*e = 0.79. All SSRs were highly polymorphic and discriminative; UPGMA clustering grouped accessions by geographic origin, with Moroccan materials forming a distinct cluster. To address self-incompatibility and limited market interest in local hard-shelled types, Açar et al. (2013) initiated crosses between self-incompatible local cultivars (‘Gülcan 1’, ‘Gülcan 2’, ‘Akbadem’, ‘Nurlu’) and self-compatible foreign cultivars (‘Lauranne’, ‘Guara’, ‘Moncayo’) to develop late-flowering, self-compatible almonds. The 2009 crosses yielded 6,116 seeds and 4,082 F1 plants (66.7% germination). S-allele screening by PCR identified 1,743 self-compatible F1s. Regarding ‘Guara’—widely cultivated in Spain and suspected to be related to the Italian cultivar ‘Tuono’—Dicenta et al. (2015) compared ‘Guara’, ‘Tuono’, and presumptive clones ‘Supernova’ and ‘Mazzetto’ using 12 SSRs at INRA and 23 additional SSRs from multiple reference collections (CEBAS-CSIC, INRA, IRTA, University of Bari). Across 35 SSRs, ‘Guara’ and ‘Tuono’ shared identical DNA fingerprints, demonstrating that they are the same cultivar. Rigoldi et al. (2015) assessed genetic diversity in almond from Sardinia and Apulia using 11 SSRs. Sardinian germplasm exhibited high polymorphism (mean 14.5 alleles per locus; mean heterozygosity 0.71). Model-based clustering resolved two genetic groups; most Sardinian accessions, all commercial cultivars, and most Apulian accessions fell into the first cluster, with Sardinian accessions largely distinct from commercial materials. Yılmaz (2017) selected late-flowering genotypes suited for fresh (“çağla”) and nut use from seedling populations in Araban and Yavuzeli (Gaziantep) and characterized them with SSRs, retaining selections for future breeding. Zeinalabedini et al. (2019) analyzed 158 *Prunus scoparia* genotypes—an economically and ecologically valuable wild species with traits such as self-fertility and tolerance to drought and fungal diseases—using 36 SSRs. High genetic diversity was detected, and genotypes clustered by geographic origin, underscoring *P. scoparia* as a valuable gene pool for almond improvement. Dikmetaş (2020) determined sweet/ bitter kernel alleles in local cultivars (‘Nurlu’, ‘Hacı Alibey’ (48-5), 48-1, ‘Akbadem’, 17-4, ‘Gülcan-2’ (101-13), ‘Dokuzoğuz-2’ (120-1), ‘Gülcan-1’ (101-23)) using an SNP marker: six cultivars were genotypically homozygous sweet (Sk/Sk) despite sweet phenotypes in all, while two were heterozygous sweet (Sk/sk), suggesting a preference for homozygotes in future breeding to avoid recessive bitter progeny. Serdengeçti (2020) examined genetic relationships among four peach cultivars (‘Küden’, ‘Tanrıver’, ‘Balcalı’, ‘İlayda’) and various commercially important *Prunus* taxa (cherry, nectarine, plum, almond, apricot, peach) using 24 SSRs. Twenty primers amplified 126 bands, all polymorphic. The highest band numbers (11) were produced by BBCT017, BBCT004, BBCT001; the lowest (2) by MA040 (mean 6.3). PIC values ranged up to 0.95 (BBCT025, BBCT005), with a minimum of 0.49 (BBCT018). Dendrograms grouped ‘Küden’ with ‘Fantasia’ nectarine, ‘Rio Oso Gem’ and ‘Üstün’ peach; ‘Balcalı’ clustered with ‘Extreme Candy’ nectarine, ‘Extreme July’, and ‘Transvalia’. The study emphasized increasing SSR primer numbers to refine relationship inference. Hasanbegović et al. (2021) characterized 60 almond genotypes from Bar (Montenegro) and Šibenik (Croatia) using ten SSRs—nine

derived from *Prunus persica* (UDP and BPPCT loci) and one from *P. armeniaca* (PacA33)—and detected high polymorphism, revealing genetic differentiation between regional populations. Esgandaripirmorad et al. (2022) evaluated 22 cultivars from eight countries with 28 SSRs, detecting 265 alleles (mean 9.46 per locus), an effective allele number of 5.85, *H*o = 0.75, and *H*e = 0.76. Locus CPDCT042 showed the highest allele number and heterozygosity; 21 loci produced cultivar-specific alleles in 17 cultivars. UPGMA, PCoA, and STRUCTURE clustered accessions into three groups, with Turkish cultivars forming a distinct cluster; the greatest genetic distance was between 'Nurlu' and 'Cristomorto', and the closest between 'Nonpareil' and 'Sonora'. Results support fingerprinting and breeding. Mahmood et al. (2023) analyzed 12 Iraqi genotypes using RAPD and ISSR markers. Both systems produced polymorphic bands (means: RAPD 5.8; ISSR 7.6). PIC values ranged 0.64–0.84 (RAPD) and 0.52–0.91 (ISSR). Jaccard similarities spanned 0.34–0.77 (RAPD) and 0.35–0.79 (ISSR), with four clusters recovered in each analysis, indicating substantial within- and among-group diversity—valuable for breeding and conservation. Uzun et al. (2024) examined natural Turkish genotypes, foreign commercial cultivars, and intra-specific hybrids using ten ISSR primers, yielding 99 bands, 84 polymorphic. UPGMA separated genotypes into two main groups (similarity 0.67–0.97); 98 of 141 hybrids clustered in the first group. Five primers exhibited 100% polymorphism, and seven exhibited ≥80% polymorphism, providing actionable information for breeding. Gök (2024) investigated genetic relationships among selected genotypes from Nevşehir, Niğde, and Mersin-Mut and several *Prunus* species, rootstocks, and cultivars using 18 SSRs. The highest band number per primer was 11 (MA014), with some primers producing one band (mean 3.38); mean polymorphic bands per primer were 2.5. In total, 61 bands (45 polymorphic) were obtained, with mean polymorphism of 33.33%; several primers (1, 2, 4, 14, 16, 18) showed 100% polymorphism. Dendrograms revealed two major clusters: 'Rootpac-R' separated on the first main branch; all others formed the second branch, which split into two sub-branches—one including 'Pabuç' and 'Gülcan-2', and the other comprising the remaining genotypes. Results provide genetic insights to better plan almond breeding. Yangöz & Güney (2025) assessed nine genotypes from Yozgat together with seven local/foreign cultivars using 18 SSRs, detecting 132 alleles (mean effective alleles 4.90), *H*o = 0.73, *H*e = 0.78, and a maximum PIC of 0.83, indicating high discriminative power. UPGMA and PCoA resolved two main groups and four subgroups, consistent with STRUCTURE, highlighting the richness and breeding value of regional germplasm. Kırca (2025) analyzed pomological, colorimetric, and genetic traits of 38 naturally occurring genotypes from Tavas (Denizli). Cluster analysis grouped genotypes into two main clusters; genetic distances ranged from 1.21 to 10.05. PCA showed that the first five components explained 68.84% of total variation; the largest contributors to PC1 were b* (0.433), fruit length (0.389), and kernel length (0.375), whereas chroma (0.429) and kernel width (0.200) contributed most to PC2. The population exhibited substantial genetic diversity; genotypes 1, 5, 9, and 17 were identified as valuable breeding resources. Studies employing diverse molecular marker systems to assess almond genetic diversity provide valuable data for the management of genetic resources. A review of the literature indicates that, among the molecular markers applied in almond genetic diversity studies, SSRs are the most widely used, followed by RAPD and ISSR markers. The predominance of SSR markers can be attributed to their superior features, including high levels of polymorphism, codominant inheritance, and reproducibility. Owing to these advantages, SSRs stand out as the most reliable and widely adopted system. Although dominant markers such as RAPD and ISSR are relatively simple and rapid to apply, they are limited in terms of reproducibility and discriminatory power. By contrast, AFLP and SNP markers provide more detailed information and have proven particularly effective in discriminating among genotypes. Overall, molecular analyses have consistently demonstrated that almond genotypes cluster according to their geographical origin.

CONCLUSIONS

This review examined studies conducted in Türkiye and worldwide that utilized molecular markers to identify almond genotypes, assess their genetic diversity, and elucidate relationships among them. Various molecular marker systems, including SSR, RAPD, AFLP, ISSR, and SNPs, have been employed. Among these, SSR markers have proven to be particularly powerful for genotype identification, pedigree analysis, and the determination of genetic relationships with other *Prunus* species and cultivars. Due to their high polymorphism, codominant inheritance, and reliability, SSRs have become the most widely preferred markers in almond breeding studies.

The results consistently demonstrate that both Türkiye and other regions of the world harbor a rich genetic diversity of local almond genotypes. These valuable genetic resources represent an important gene pool for almond breeding programs. Dendrograms derived from molecular analyses classify almond genotypes according to their geographical origin, clearly revealing similarities and differences among populations from different regions. Such analyses provide detailed and reliable insights into almond germplasm, thereby offering critical information to support the design and implementation of more targeted and effective breeding strategies in the future.

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THE EFFECTS OF GLOBAL WARMING ON THE WATER QUALITY OF LAKES AND RIVERS

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ABSTRACT

Global warming has emerged as a critical driver of change in freshwater ecosystems, exerting multifaceted influences on the physical, chemical, and biological characteristics of freshwater systems. Rising air temperatures cause to increase in surface water temperatures, which reduces the solubility of dissolved oxygen and intensifies thermal stratification, promoting hypoxic or anoxic conditions in deeper layers.. Enhanced evaporation rates and altered precipitation patterns exacerbate seasonal and annual fluctuations in water levels, concentrating contaminants and reducing the self-purification capacity of lotic systems. Concurrently, elevated runoff during extreme rainfall events transports greater loads of nutrients and associated agrochemicals into water bodies, fueling harmful algal blooms and promoting toxin production by cyanobacteria. Intensified drought periods further increase pollutant concentrations and facilitate the proliferation of invasive or opportunistic species, weakening local biodiversity and destabilizing trophic interactions. These changes collectively threaten aquatic ecosystem services, compromise drinking-water quality, and increase treatment costs, while also posing direct risks to human and animal health through exposure to algal toxins and waterborne pathogens.

This review synthesizes recent findings on the mechanisms by which climate warming is changing freshwater quality and identifies key knowledge gaps. Finally, it outlines adaptive management strategies such as enhanced monitoring networks, watershed-scale nutrient management, restoration of riparian buffers, and controlled flow regimes to mitigate adverse outcomes and enhance the resilience of inland water resources in a warming world.

Keywords: Global warming, lakes, rivers, water quality

INTRODUCTION

Freshwater ecosystems provide drinking water, facilitate food production, and provide the foundation for energy production and biodiversity. Anthropogenic climate change is transforming the hydrology and water quality of lakes and rivers by altering key climatic drivers such as temperature, changes in precipitation patterns, evaporation, snow and glacier melt, and sea level. Intensification of the hydrological cycle (e.g., higher atmospheric humidity and more intense precipitation per degree of warming), earlier snowmelt, and increased terrestrial evaporation are observed with high confidence, along with regionally heterogeneous drying/wetting patterns that shape water quality trajectories through both chronic stresses and extreme events (IPCC WGII). This review integrates mechanistic understanding with recent global syntheses to map how warming alters water temperature regimes, stratification and mixing, oxygen budgets, nutrients, pollutants, and biota, leading to measurable deterioration of water quality indicators and ecosystem services. We highlight the cross-scale interactions between climate forcing, watershed land use, and management (Garrabou et al., 2009; Pershing et. al., 2018; Woolway et al., 2022).

2. Mechanistic Pathways: From Warming to Water Quality

2.1. Thermal Forcing and Lake Stratification

The latest alterations in the global carbon balance and the associated climate tendencies in the thermal and ice dynamics of inland water bodies raise new essential inquiries regarding energy and substance transfer within aquatic systems. Rising air temperatures warm lake surfaces, increase stability, and lengthen stratified periods. Stronger and longer stratification suppresses vertical mixing, reduces oxygen replenishment of bottom waters, and reinforces nutrient and redox stratification. Phenological shifts in onset and breakdown of stratification alter habitat availability and the timing of biogeochemical processes, with cascading effects on primary production and food webs (e.g., trophic mismatches) (Woolway et. al., 2022). Observations and modeling demonstrate earlier stratification onset, prolonged duration, and tendencies toward more frequent and intense lake heatwaves, with clear anthropogenic attribution. Subsurface (metalimnetic/hypolimnetic) heatwaves are also being documented, extending thermal stress below the surface (Woolway et. al., 2021: Kirillin, Shatwell, 2016).

2.2. River Thermal Regimes and Hydroclimatic Extremes

Hydroclimatological dynamics shape river discharge (i.e., streamflow). While numerous interacting factors affect the condition of river ecosystems—such as light availability, thermal regime, nutrient supply, spatial connectivity, biodiversity, and human management—discharge remains a fundamental driver in defining the structural framework of river habitats (Laize et al., 2014). In rivers, warming elevates water temperatures, while droughts and heatwaves reduce flows and dilution capacity; floods increase non-point source loads and resuspension of sediments. A synthesis of global case studies shows that river water quality often worsens during droughts and heatwaves due to higher concentrations of nutrients, organic matter (increased BOD/COD), salinity, and pathogens; floods degrade quality via sediment and pollutant pulses. Multidecadal trends in many basins reflect the combined effects of climate change and intensifying extremes (Vliet et.al., 2023)

2.3. Oxygen Dynamics and Biogeochemical Feedbacks

Warmer water bodies bind less dissolved oxygen (DO), while increased microbial activity, respiration, mineralization, and sediment oxygen demand further reduce DO. In stratified lakes, bottomwater (hypolimnetic) DO declines are common. In many systems, browning (higher dissolved organic carbon (DOC) increases light attenuation and hypolimnetic respiration, increasing oxygen loss, compressing coldwater habitats, and altering ecosystem metabolism (Jane et. al., 2024).

2.4. Nutrients, Internal Loading, and Redox Chemistry

When water oxygen levels decrease or disappear completely, sediments release phosphorus through reductive dissolution of iron oxides, increasing ammonium accumulation; denitrification and anammox alter nitrogen balances. Therefore, stronger and longer stratification with hypolimnetic anoxia increases internal phosphorus load, leading to the risk of eutrophication and harmful algal blooms (HABs) during mixing events and subsequent growing seasons, even when external inputs are reduced (NSTC, 2003; Carpenter, 2005).

2.5. Contaminant Mobilization and Salinity Intrusion

Hydroclimatic extremes regulate pollutant pathways: drought concentrates salts and contaminants; floods transport pesticides, pathogens, pharmaceuticals, and microplastics from catchments; sea-level rise promotes salinity intrusion into tidally influenced rivers, threatening drinking-water intakes. The thawing of permafrost and watershed browning mobilize metals and organic carbon, altering acidity and ionic composition in Arctic and mountainous systems (Byl et, al., 2025).

3. Lakes: Warming, Deoxygenation, and Eutrophication

3.1. Lake Heatwaves and Attributed Change

Lake heatwaves extended intervals of anomalously warm surface water are becoming more frequent, longer, and more intense. Recorded ecological impacts include mass fish mortalities, shifts in plankton phenology and metabolism, and altered food-web structure. Detection–attribution studies link severe lake heatwaves to anthropogenic warming. Subsurface heatwaves in the metalimnion and hypolimnion are increasingly reported, extending thermal stress into deeper aquatic zones (Woolway et al., 2025).

3.2. Widespread Deoxygenation and Habitat Squeeze

Sustained monitoring reveals broad DO decrease in many temperate lakes, driven by warming and stratification effects. Simultaneously, increased terrestrial inputs (browning) intensify hypolimnetic oxygen demand. The net effect is habitat squeeze for cold-water taxa such as salmonids and coregonids as cool, oxygenated waters contract. In many lakes, browning-induced oxygen loss outpaces any surface-cooling benefits for habitat, raising biodiversity risks (Jane et. al., 2024).

3.3. Harmful Algal Blooms (HABs) and Toxin Risks

Climate change interacts with nutrient enrichment to increase HAB frequency, duration, and toxicity. Modeling for large reservoirs projects substantial increases in cyanobacterial biomass under plausible warming and stratification scenarios—even with constant nutrient inputs—highlighting the synergism of warming and eutrophication. Treatment plants face challenges related to toxins, taste and odor, and disinfection by-products (Chapra et al., 2017). Climate change and nutrient dynamics influence each other both directly and indirectly. Rising temperatures clearly alter the habitats suitable for harmful algal blooms (HABs). Additionally, climate shifts can drive the expansion of harmful algal species by modifying precipitation patterns—leading in some regions to more frequent droughts, and in others to increased intensity or frequency of storms. Such episodic storm events and broader climate variability impact freshwater flow timing, water residence periods, the scale and timing of nutrient inputs, and the subsequent biological responses (Heisler et al., 2008).

4. Rivers: Flow Regimes, Extremes, and Quality

4.1. Droughts and Heatwaves

During droughts and heat waves, low flows increase residence times, temperatures, and concentrations of nutrients, salts, and pathogens. As dilution decreases, point source contributions increase. Biological oxygen demand increases while DO decreases, stressing the biota and creating treatment challenges for utilities. Observational syntheses indicate that water quality deteriorates under such extremes in the majority of cases (Vilet et al., 2023).

4.2. Salinity Intrusion and Heat Stress in Regulated Rivers

In tidal rivers and deltas, sea level rise and drought are advancing salt fronts, threatening water intakes and altering ionic composition. Active reservoir releases and temporary barriers are increasingly being used to control salinity intrusion, demonstrating the interactions of climate risk across water supply and estuary management (Byl et, al., 2025).

5. Water Quality Constituents and Processes

5.1. Temperature and Dissolved Oxygen

Direct thermal effects reduce oxygen solubility. Indirect effects (higher respiration, nitrification and denitrification rates, and sediment oxygen demand) further deplete DO. Under low oxygen levels or anoxia, reduced compounds (NH4+, Fe2+, Mn2+, H2S) accumulate, and emissions of methane (CH4) and nitrous oxide (N2O) may increase. (Naqvi et. al., 2010).

5.2. Nutrients (Nitrogen and Phosphorus)

Rising temperatures stimulate cyanobacterial blooms and nitrogen-fixing capacity in some species; redox-driven sediment processes increase phosphorus release under oxygen-depleted conditions, while storm events introduce significant external nutrient inputs. Internal nutrient recycling maintains eutrophic conditions even after external inputs have diminished, highlighting the need for long-term, multifaceted restoration approaches (Nürnberg 2024).

5.3. Contaminants and Pathogens

Extreme events mobilize legacy metals, pesticides, and emerging contaminants (pharmaceuticals, PFAS, microplastics), while warm, nutrient-rich waters favor pathogenic bacteria such as Vibrio and E. coli. Permafrost thaw enhances delivery of DOC and metals to high-latitude rivers and lakes, modifying acidity and redox state with visible ecosystem consequences. The distribution of pathogens and the incidence of waterborne disease outbreaks are closely linked to environmental and climatic conditions. Global climate change will affect the hydrological cycle in many different ways that will influence waterborne pathogen survival, infectivity and distribution (Nicholas, 2014).

6. Ecological and Health Consequences

Thermal habitat loss and hypoxia reduce niche availability for coldwater and stenothermic species, restructure plankton communities, and increase fish mortality risks during heatwaves and turnover events. Browning and deoxygenation compress vertical habitat, leading to mismatches in predator-prey dynamics (Jane et al., 2024).

HAB toxins (microcystins, anatoxins, cylindrical spermopsin) pose a threat to drinking water safety and recreation; storm-related increases in microbial indicators and pathogens complicate treatment. Warmer waters extend seasonal ranges for some pathogens, requiring more intensive monitoring and risk communication (Elidrissi et al., 2024).

CONCLUSION

Global warming is degrading the water quality of lakes and rivers through thermal, hydrological, biogeochemical, and microbiological pathways. Lakes are experiencing increasingly severe and prolonged stratification, heat waves, and bottom-water anoxic conditions, which increase nutrient loading and the risk of HABs, threatening the health of organisms. Rivers are experiencing quality degradation reflecting changes in pollutant dilution, mobilization, and transformation during droughts, heat waves, and floods. These dynamics threaten ecosystems, human health, and economies; however, targeted adaptation (load reduction, nature-based solutions, and dynamic operations) and robust monitoring and forecasting can significantly reduce these risks. Aligning climate change mitigation with water quality management is crucial for maintaining freshwater security throughout the 21st century.

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CHLORINE CONTAMINATION IN DILUTE NITRIC ACID PRODUCTION: SOURCES, EFFECTS, AND PREVENTION METHODS

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ABSTRACT

Despite its widespread use in the fertilizer and chemical industries, dilute nitric acid (NA) can cause significant operational and material integrity problems due to chloride contamination. Chloride ions, in particular, can lead to pitting and stress corrosion cracking (SCC) in stainless steel equipment, thereby reducing the service life of industrial facilities

In this study, potential sources of chloride contamination in dilute nitric acid production were identified, and the measures taken to control them were evaluated. The sources of chloride include:

Feed water: residues in inadequately treated water,

Raw material purity: ammonia and other auxiliary chemicals,

Cleaning agents and sealing materials used in process equipment,

Intermediate species: e.g., nitrosyl chloride (NOCl)

Analyses conducted by TSE Ankara Chemical Laboratory initially measured chloride levels at approximately 74 ppm, with historical data ranging from 70 to 300 ppm. These levels are significantly above the literature-recommended safe limit of <5–10 ppm, posing a serious corrosion risk.

As part of process improvements, measures such as modernization of water treatment systems, replacement of equipment materials, and control of NOCl formation were implemented. Additionally, controlled purge operations in critical circuits were employed to prevent chloride accumulation. Field analyses conducted after these improvements showed that chloride levels remained below 10 ppm over a 30-day monitoring period, even with the adjacent KNO_3 production unit in operation, staying well within safe limits.

These findings are consistent with approaches recommended in the literature (purge operations, water treatment, intermediate species control), confirming that purge is the most effective strategy to control chloride contamination in large-scale industrial facilities.

Keywords; Nitric Acid, Chlorine Contamination, NOCl, Corrosion, Product Purity.

INTRODUCTION

Nitric acid is a strong mineral acid primarily used in the production of nitrate fertilizers and various chemical products. It constitutes a critical input in modern agricultural systems, significantly enhancing crop productivity (Thiemann et al., 2021). The world's population reaching 8 billion would not have been possible without the development of the fertilizer industry (Randive et al., 2021). Nitric acid, due to its high nitrogen content (15.5–34.5%) and rapid availability to plants, serves as an essential feedstock for nitrogen-based fertilizers alongside ammonia. Currently, approximately 80% of global nitric acid production is dedicated to fertilizer manufacturing (Smil, 2004).

Industrially, nitric acid (HNO_3) is a key inorganic compound widely used in the production of explosives, dyes, organic chemicals, plastics, and numerous other products. Highly concentrated nitric acid (95–99%) is preferred in intensive chemical processes, whereas dilute nitric acid (20–68%) is commonly employed in applications where material compatibility and controlled reactivity are critical.

One of the most significant operational challenges in nitric acid production plants is corrosion, which can reduce equipment lifespan and lead to unplanned shutdowns. Process design strategies must therefore include green hydrogen production via electrolysis or solar-powered thermochemical cycles, as well as the supply of highly pure nitrogen with oxygen impurities below 10 ppm to prevent catalyst deactivation in the subsequent Haber–Bosch process (Budama et al., 2022; Moghaddam and Krewer, 2020).

Chlorine contamination, particularly problematic in the health and food sectors, is a major factor accelerating corrosion in production facilities. Chlorine reacts with organic compounds (e.g., humic and fulvic acids) and inorganic species (e.g., ammonia) accumulated in wash tanks, thereby trapping free chlorine (Simpson and Mitch, 2021). High chloride ion concentrations can damage stainless steel and alloys used in nitric acid plants, leading to pitting and stress corrosion cracking, which severely reduces equipment lifespan. Therefore, chlorine contamination must be closely monitored and minimized to ensure operational reliability and extend equipment service life.

MATERIAL AND METHOD

This review evaluates chloride contamination in dilute nitric acid production by focusing on:

1.Sources (feed air, cooling water, raw materials, corrosion products),
2.Removal methods (purge, gas-phase treatment, ion-exchange resins, chemical additives),
3.Reaction mechanisms ($Cl^- \rightarrow HCl$, Cl_2 formation, NO_2 oxidation),
4.Industrial practice (purge as the primary method in large-scale facilities).

Key references include Wilkinson (1961), Spence (2011), Kurley et al. (2019), Roux et al. (2019), Li et al. (2022), Wang et al. (2020).

Sources:

Chloride ions originate mainly from marine aerosols, cooling water, low-purity raw materials, and corrosion-derived leaching.

Removal Methods:

-Purge operations limit chloride accumulation by continuously withdrawing a fraction of low-concentration acid streams.

-Gas-phase oxidation converts Cl^- ions into Cl_2 through NO_2-mediated oxidation, facilitating their removal in the purge gas stream.

-Ion-exchange resins are applied in specialized production lines but are economically unfeasible for large-scale plants.

-Chemical additives ($NaNO_3$, KNO_3) mitigate corrosion but do not remove chloride from the system.

Reaction Mechanisms:

1.Oxidation of chloride ions to chlorine gas:

$$2Cl^- \rightarrow Cl_2 + 2e^-$$

2.Overall reaction in nitric acid production involving NO_2 and water:

$$2Cl^- + 4NO_2 + 2H_2O \rightarrow 2HNO_3 + Cl_2 + 2NO$$

These mechanisms describe the oxidation of chloride ions and their volatilization into the gas phase.

Industrial Application in Our Facility:

In our nitric acid production facility, we regularly implement purge operations. Controlled side-stream withdrawal from critical circuits ensures that chloride concentrations remain below critical thresholds, effectively minimizing corrosion risk and extending equipment life.

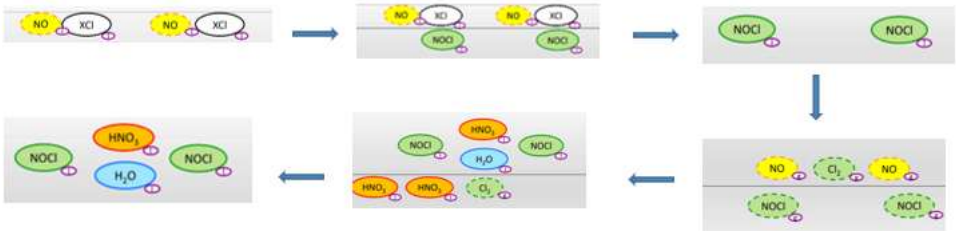


Figure 2. Most accepted mechanism Nitrosyl chloride (NOCl) : N=O-Cl

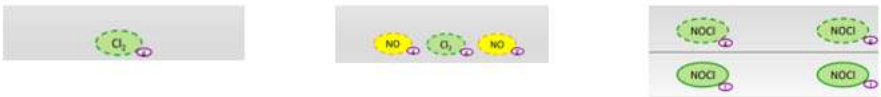


Figure 2. Most accepted mechanism Nitrosyl chloride (NOCl) : N=O-Cl

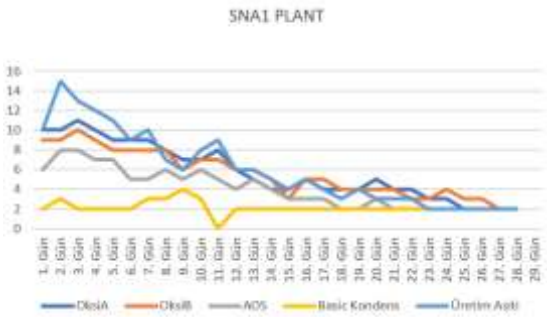
RESULTS AND DISCUSSION

Field analyses were conducted over a period of 30 consecutive working days at 09:00 AM. Samples were collected from multiple critical points within the nitric acid production system, including OksiA, OksiB, AOS, Basic Condensate, and Production Acid streams. The results demonstrated a consistent and gradual decrease in chloride concentrations over time, indicating the effectiveness of the purge operations and other process improvements implemented in our facility for controlling chloride contamination.

To assess potential cross-contamination, comparative sampling was performed while the adjacent KNO_3 production facility was both operational and non-operational. The data showed that even when the KNO_3 unit was active, chloride levels in our nitric acid circuits remained below critical thresholds, confirming that the purge strategy effectively mitigates contamination risks. These findings are consistent with literature reports emphasizing the importance of controlled side-stream withdrawal and purge operations in maintaining low chloride concentrations and minimizing corrosion in large-scale nitric acid plants (Wilkinson, 1961; Roux et al., 2019; Zhang et al., 2018).

The observed reduction in chloride levels also aligns with previous studies on the role of water treatment, equipment material selection, and intermediate species control (e.g., NOCl) in limiting chloride accumulation and volatilization (Kurley et al., 2019; Wang et al., 2020). Collectively, these results demonstrate that the combination of purge operations and process optimization provides an effective strategy to enhance equipment lifespan and operational reliability in industrial nitric acid production.

Table 1. Chloride Analysis in Nitric Acid (Measurements Taken While the Potassium Nitrate Facility Was Non-Operational and Purge Operations Were Applied)



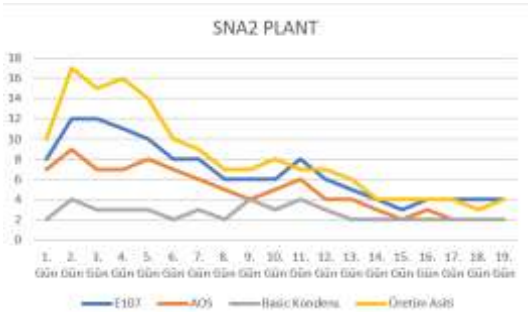
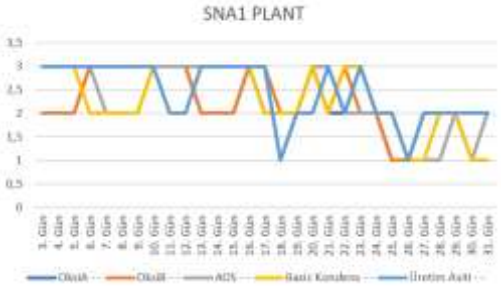
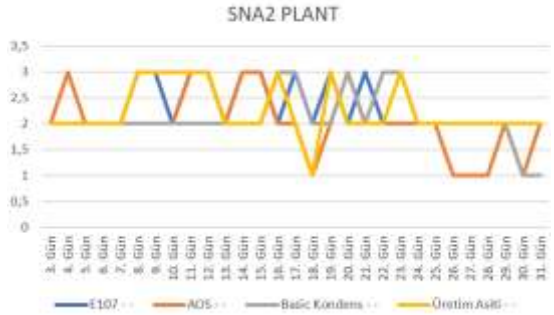


Table 2. Chloride Analysis in Nitric Acid (Measurements Taken While the Potassium Nitrate Facility Operational and Purge Operations Were Applied)





CONCLUSIONS

This study confirms that chlorine contamination in dilute nitric acid production is a multifactorial issue. Initial analyses conducted at the TSE laboratory indicated chlorine levels of approximately 74 ppm, with historical data showing values reaching up to 300 ppm, posing significant corrosion risks. Literature reports emphasize that high chloride concentrations in nitric acid plants can cause pitting and stress corrosion cracking in stainless steel and alloys (Spence, 2011; Li et al., 2022; Kurley et al., 2019).

Targeted process improvements implemented in our facility included the modernization of water treatment systems, raw material control, equipment upgrades, and suppression of NOCl formation. As a result, field data demonstrated that chloride levels dropped below 10 ppm. Furthermore, even when the adjacent KNO_3 production unit was operational, chloride concentrations remained below critical thresholds, confirming the effective mitigation of cross-contamination risks.

These findings are consistent with literature recommendations indicating that purge operations, water treatment, and intermediate species control are effective strategies to minimize corrosion in nitric acid production plants (Wilkinson, 1961; Roux et al., 2019; Zhang et al., 2018; Wang et al., 2020). However, to ensure long-term operational reliability, continuous monitoring and further process refinements are necessary.

In conclusion, this study demonstrates that an integrated approach can be successfully applied in dilute nitric acid production facilities to minimize corrosion and extend equipment lifespan.

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SHORT-TERM EFFECTS OF DIFFERENT ORGANIC FERTILIZERS ON SOIL CHEMICAL PROPERTIES AND PLANT GROWTH IN MELON AND WATERMELON CULTIVATION

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ABSTRACT

The integration of organic fertilizers into horticultural systems has gained increasing importance as a sustainable strategy to enhance soil fertility and crop productivity under challenging soil conditions. This study aimed to evaluate the effects of various organic fertilizers including manures from small ruminants, cattle, poultry, and vermicompost on the chemical properties of a sandy-calcareous soil and the growth performance of melon (*Cucumis melo L.*) and watermelon (*Citrullus lanatus Thunb.*). The study focused on how recommended fertilizer doses influence soil chemical quality and plant growth. The results indicated that organic fertilization altered soil electrical conductivity (EC) and available phosphorus (P) levels. Poultry and cattle manures led to notable improvements in soil nutrient content. Additionally, plant growth parameters such as stem diameter and fruit weight were positively affected by organic fertilizers, particularly poultry manure. Crop-specific responses were observed: melon plots exhibited higher soil EC and available P, while watermelon plants showed superior performance in stem diameter, fruit flesh thickness, and soluble solid content. In contrast, melon plants recorded higher chlorophyll levels. Overall, poultry manure emerged as the most effective input for enhancing plant growth, while vermicompost and cattle manure also contributed depending on the crop type. These results suggest that organic fertilizers, especially poultry manure, are valuable tools for improving soil fertility and crop productivity in calcareous soils.

Keywords: Calcareous soils, Fruit quality, Mediterranean climate, Organic amendments

INTRODUCTION

Sustainable agricultural systems are an emerging model that promotes the integrated use of organic materials as alternatives to chemical inputs are being developed to ensure more environmentally friendly and efficient food production. In this context, organic fertilizers that enhance soil fertility have come to the forefront. The core component of organic fertilizers, organic matter, serves as a primary source of energy and nutrients for soil-dwelling microorganisms. However, in Turkey and many other agricultural countries, inadequately matured animal manures characterized by high C/N ratios and the presence of root rot pathogens or weed seeds have long been used in crop production. These manures often fail to provide the expected agronomic benefits for both soil and plants (Karaman 2012).

In recent years, there has been a growing demand particularly in intensively cultivated horticultural and greenhouse production areas for thermally treated or fermented organic fertilizers. This trend reflects efforts to promote sustainable agriculture and to optimize the combined use of organic and chemical fertilizers. Nevertheless, these processed fertilizers are significantly more expensive than traditionally matured manures, due to additional processing, packaging, registration, and storage costs. Furthermore, their claimed benefits for practical use often rely more on manufacturer assertions than on robust scientific evidence. Therefore, it is essential to evaluate their effects on soil fertility and plant growth in order to better understand their potential contribution to sustainable agricultural systems and to promote their effective and economical use.

Melon (*Cucumis melo* L.) and watermelon (*Citrullus lanatus* Thunb.), among the most widely produced and consumed vegetables globally and in Turkey, are annual crops adapted to warm and temperate climates. While both crops are cultivated in open fields and greenhouses, only about 3% of melon and 2% of watermelon production is conducted under protected cultivation (Anonymous 2001; Taşkaya and Keskin 2004). These crops are valued not only for their edible parts but also for their seeds and rinds, which are utilized across the food, pharmaceutical, and cosmetic industries, making them strategic export commodities. Deep, well-drained, sandy-loam or loamy-sand soils with high water-holding capacity are ideal for watermelon cultivation, while poorly drained soils with shallow groundwater levels (below 1 meter) are unsuitable (Tuna and Özer 2005). Like many other cultivated plants, melons and watermelons respond positively to organic fertilization. Accordingly, the application of solid and/or organic fertilizers in the cultivation of these crops is considered important for improving both yield and quality, as well as enhancing soil health (Bayram et al. 2020).

In this study, the effects of processed organic fertilizers including sheep, cattle, and poultry manures, as well as vermicompost on the chemical properties of calcareous sandy soils and on plant growth in melon and watermelon cultivation were investigated. Additionally, the study aimed to assess how the optimum application rates recommended by organic fertilizer manufacturers for plant growth influence soil fertility. By comparing the effects of these commonly used organic fertilizers on both soil chemical parameters and plant growth indicators, this study identified the strengths and weaknesses of each fertilizer type. The findings offer valuable insights into the practical use of organic fertilizers in sustainable farming systems.

MATERIAL AND METHODS

Site Description

This study was conducted as a field trial at the Finike campus of Akdeniz University (36°18′31″N, 30°08′48″E; altitude: 40 m). According to meteorological data collected during the trial period, the site had an average daily temperature of 19.3 °C, an average daily sunshine duration of 9.10 hours, an average daily open surface evaporation of 125.58 mm, an average daily rainfall of 1.4 mm, an average daily wind speed of 1.6 m/sec, and an average relative humidity of 66.03%.

Soil samples collected to represent the trial area were analyzed prior to the experiment. The soil was classified as sandy loam in texture. It had a neutral pH and was classified as non-saline. The soil also contained a high level of lime and was rated as sufficient in terms of humus content. Total nitrogen (N) and available phosphorus (P) levels were determined to be adequate, while the cation exchange capacity (CEC) was classified as low.

Organic Fertilizers and Fertilization Practices

In this trial, various heat-treated organic fertilizers (processed at 65–70 °C) were applied, including small ruminant manure (goat and sheep manure; T1), cattle manure (T5), poultry manure (T4), and vermicompost (T2) (Table 1). Additionally, control (T3) plots were established, which received only chemical fertilizers (detailed below) without any organic fertilizer input. The application rates of the organic fertilizers were determined based on the manufacturers' recommendations and supported by findings from previous studies (Maltaş 2023; Maltaş et al. 2024). This approach aims to develop practical organic fertilization strategies suitable for field conditions, specifically for melon and watermelon cultivation, while improving soil chemical properties and supporting plant growth.

Table 1. Some properties of organic fertilizers used in the experiment

Parameter	Organic fertilizer			
	Vermicompost	Chicken manure	Cow manure	Goat-sheep manure
Organic matter, %	45	62	35	70
EC (dS/m)	6.0	2.6	11.2	7.5
Organic C, %	26.1	35.96	20.3	40.6
Humic/fulvic acid, %	41.5	36	15	28
Total N, %	3.0	2.2	1	1.5
Total P, %	1.5	1.76	1.5	1.5
Total K, %	1.0	1.62	-	2
Humidity max., %	20	20	20	20
pH	6-8	6.2	7-9	8-10
C/N	9/1	16/1	20/1	27/1
C/P	17/1	20/1	13/1	27/1

The application rates were as follows: 4 Mg ha^{-1} for vermicompost, 8 Mg ha^{-1} for cattle manure, 6 Mg ha^{-1} for goat manure, and 4 Mg ha^{-1} for poultry manure. In addition to these inputs, chemical fertilizers certified for use in organic farming were applied through a drip irrigation system to support plant nutrition throughout the experimental period. These chemical inputs were selected based on the initial soil analysis and estimates of nutrient removal by the crops (Zengin 2023).

Previous studies have demonstrated that the use of organic mulching materials (such as straw, sawdust, and compost) can improve microclimatic conditions and enhance the

effectiveness of organic fertilizers (Gao et al. 2019; Teasdale and Mohler 2000). Mulching has been shown to promote soil biological activity, thereby improving nutrient uptake efficiency by plants and contributing to sustainable farming practices (Li et al. 2019). Therefore, in this study, a mulch was applied to the experimental plots to maximize the effectiveness of organic fertilizers.

Implementation of the Experiment

In this study, melon and watermelon cultivation was carried out using four types of heat-treated organic fertilizers. The experimental setup was established according to a randomized block design with three replications. The organic fertilizers were applied uniformly to the plots based on their respective application rates (in dry weight).

Melon (*Cucumis melo L.*) and watermelon (*Citrullus lanatus Thunb.*) seedlings were procured from a commercial nursery. Melon seedlings (Anka F1) were transplanted at a spacing of 2.0 m between rows and 0.3 m within rows, while watermelon seedlings (Peykan F1) were planted at 2.0 m between rows and 0.6 m within rows. Both crops were cultivated over a vegetation period of approximately 90–100 days.

Throughout the growing period, all essential cultural practices such as weed control, hoeing, and plant protection treatments were carried out. In addition, irrigation and fertigation were performed using a drip irrigation system. The daily irrigation requirements of the plants were calculated based on climatic parameters (including rainfall, temperature, and relative humidity), soil texture and moisture, and plant needs (e.g., signs of wilting). These water requirements were integrated with the optimized fertilization strategy to ensure proper crop management.

Sampling and Analysis

Soil samples were air-dried and sieved through a 2 mm mesh prior to analysis. To characterize the initial soil conditions, samples were collected from the 0–30 cm soil depth using an auger before the experiment began. These samples were analyzed for texture, pH, electrical conductivity (EC), lime content, organic matter, total nitrogen (N), available phosphorus (P), and cation exchange capacity (CEC). Additionally, to evaluate the effects of organic fertilizer applications on soil chemical properties, samples collected at harvest were analyzed again for organic matter, total nitrogen, available phosphorus, and CEC.

The methods used for analyzing the above-mentioned soil parameters were as follows: soil texture was determined using the hydrometer method (Bouyoucos 1955); pH and EC were measured in a 1:2.5 soil-to-water suspension (Jackson 1967; Anonymous 1982); lime content was determined using the calcimeter method (Çağlar 1949); organic matter was measured using the modified Walkley-Black method (Black 1965); total nitrogen was determined using the Kjeldahl method (Kacar 1995); available phosphorus was analyzed according to the Olsen method (Olsen and Sommers 1982); and CEC was determined using the ammonium acetate method (Kacar 1995).

At the end of the experiment, pomological and physiological traits were assessed in plant and fruit samples (Figure 1 and Figure 2). For each treatment replication, measurements and evaluations were performed on three randomly selected plants per plot. The results were expressed as the average of these three plants.

Figure 1. Views of selected physiological and pomological traits in watermelon plants (A: Soluble solids content, B: Chlorophyll content, C: Fruit flesh thickness, D: Stem base diameter, E: Fruit juice pH, F: Fruit weight)

Figure 2. Views of selected physiological and pomological traits in watermelon plants (A, C: Harvested fruits; B: Fruit juice pH)

The evaluated pomological characteristics included: stem base diameter (cm; measured with a caliper), fruit fresh weight (g; measured with a precision balance), and yield (kg da^{-1}; calculated). The physiological parameters included: soluble solids content (SSC), fruit juice pH, total chlorophyll content (measured with a chlorophyll meter), and rind thickness (mm; measured with a caliper).

Statistical Analyses

The numerical data obtained in this study were statistically analyzed using the SPSS 23.0 software package. The significance levels of soil chemical properties and plant pomological and physiological parameters were determined through analysis of variance (ANOVA) at the 5% significance level. Parameters showing statistically significant differences were further evaluated using Duncan's multiple range test for mean separation. In addition, Pearson correlation analysis was performed to determine the relationships among the measured parameters (SPSS 2008).

RESULTS AND DISCUSSION

Soil Chemical Properties

At the end of the growing season, the effects of different organic fertilizer treatments on soil cation exchange capacity (CEC), organic matter (OM) content, total nitrogen (N%), and available phosphorus (P, mg/kg) were evaluated for watermelon cultivation (Table 2). According to the statistical analysis, changes in CEC, total N, OM (%), and available P values in watermelon plots were found to be statistically non-significant.

Table 2. Effects of applications on CEC, OM, total N and available P contents in watermelon soil

Treatments	CEC (meq/100)	% OM	% N	P (mg/kg)
T1	26.54	2.26	0.16	22.17
T2	29.33	2	0.15	19.47
T3	31.04	2.1	0.16	13.36
T4	28.11	2.17	0.18	18.52
T5	30.58	1.92	0.18	38.54
ANOVA (%5)	NS[1]	NS	NS	NS

[1] NS; not significant.

In contrast, in melon cultivation plots, although changes in CEC, total N, and OM (%) were also statistically non-significant, the variation in available phosphorus content was statistically significant at the $P < 0.05$ level (Table 3). Among the treatments, T4 (48.60 mg/kg) resulted in the highest increase in available phosphorus content compared to the other applications.

Table 3. Effects of applications on CEC, OM, total N and available P contents in melon soil

Treatments	CEC (meq/100)	% OM	% N	P (mg/kg)
T1	32.82	2.29	0.15	13.31 b[1]
T2	30.99	2.26	0.16	23.72 ab
T3	30.10	1.85	0.14	9.36 b
T4	29.32	1.97	0.13	48.60 a
T5	29.68	2.00	0.17	40.04 ab
ANOVA (%5)	NS	NS	NS[3]	2.966 **[2]

[1] Differences between values not indicated by the same letter are significant at the 5% level. [2] **; significant at the 1% level. [3] NS; not significant.

Previous studies have indicated that increasing doses of poultry manure significantly contribute to the enhancement of soil nitrogen content (Okur et al. 2008), while farmyard manure has been found to have a more pronounced effect on available phosphorus levels in soils (Agbenin and Goladi 1997). In the present study, the available phosphorus level in soils under melon cultivation (T4: 48.60 mg/kg) was approximately 20% higher than that in watermelon soils.

Göksu and Kuzucu (2017), in their study investigating the effects of different vermicompost doses on yield and quality parameters in watermelon, reported that vermicompost and chemical fertilizer applications increased the soil ion exchange capacity. However, in the current study, organic fertilizer treatments did not result in statistically significant changes in CEC for either melon or watermelon soils.

Similarly, Kılıç and Sönmez (2019), in their study on the effects of increasing doses of various organic materials (vermicompost, poultry manure, farmyard manure, leonardite) on soil fertility parameters in lettuce cultivation, found that the effects on soil organic matter content were statistically significant. In contrast, the findings of the current study showed that different organic matter applications had no statistically significant effect on soil organic matter levels in either melon or watermelon cultivation.

Plant Growth Properties

At the end of the cultivation period, among the yield and quality parameters obtained from the watermelon plant, leaf chlorophyll content (SPAD), fruit flesh thickness, fruit juice pH, and soluble solid content were found to be statistically non-significant (Table 4). However, collar diameter was found to be statistically significant at the $P < 0.01$ level, with higher values observed in T1 and T4 (23.41 mm and 23.23 mm, respectively) compared to other treatments. Fruit weight was also found to be statistically significant at the $P < 0.05$ level, with higher fruit weights recorded in T2, T4, and T5 (7.38 kg, 8.13 kg, and 7.52 kg, respectively).

Table 4. Effects of treatments on SPAD, root collar diameter (RCD), fruit weight (FW), fruit flesh thickness (FFT), fruit juice pH (FJpH) and soluble solid content (SSC) in watermelon

Treatments	SPAD	RCA (mm)	FW (kg)	FFT (cm)	FJpH	SSC
T1	37.13	23.41 a[1]	6.99 ab	16.13	5.34	8.38
T2	38.99	17.74 c	7.38 a	15.53	5.60	9.26
T3	34.48	15.95 c	5.50 b	17.55	5.70	8.43
T4	34.71	23.23 a	8.13 a	14.29	5.49	8.70
T5	31.01	20.08 b	7.52 a	10.31	5.46	8.71
ANOVA (%5)	NS[4]	21.282 ***[3]	3.575**[2]	NS	NS	NS

[1] Differences between values not indicated by the same letter are significant at the 5% level. [2] **; significant at the 1% level. [3] ***; significant at the 0.1% level. [4] NS; not significant.

Regarding the melon plant, leaf chlorophyll content (SPAD), fruit flesh thickness, fruit juice pH, and soluble solid content were also found to be statistically non-significant (Table 5). However, root collar diameter was found to be statistically significant at the $P < 0.05$ level, with higher values observed in T1, T3, and T4 (14.67 mm, 14.83 mm, and 14.85 mm, respectively). Fruit weight was found to be statistically significant at the $P < 0.01$ level, with the highest fruit weight observed in T1 (2.02 kg).

Table 5. Effects of treatments on SPAD, root collar diameter (RCD), fruit weight (FW), fruit flesh thickness (FFT), fruit juice pH (FJpH) and soluble solid content (SSC) in melon

Treatments	SPAD	RCA (mm)	FW (kg)	FFT (cm)	FJpH	SSC
T1	42.53	14.67 ab[1]	2.02 a	9.78	5.93	4.80
T2	36.74	13.22 b	1.20 b	9.12	5.79	4.13
T3	48.32	14.83 ab	1.42 b	11.65	5.60	4.13
T4	44.78	16.85 ab	0.95 b	9.98	5.85	4.37
T5	40.28	16.15 a	1.45 b	10.97	5.86	3.97
ANOVA (%5)	NS	1.701 **[2]	5.448 ***[3]	NS[4]	NS	NS

[1] Differences between values not indicated by the same letter are significant at the 5% level. [2] **; significant at the 1% level. [3] ***; significant at the 0.1% level. [4] NS; not significant.

Üçok et al. (2019), in their study investigating the effects of different organic fertilizer applications on yield, quality, and plant nutrient contents in lettuce (*Lactuca sativa* L. var. crispa), reported no significant differences in chlorophyll content among the treatments. Similarly, in our study, although the differences between applications were not statistically significant for both watermelon and melon plants, the chlorophyll value in melon was found to be approximately 20% higher than in watermelon.

Özen (2018) investigated the effects of different doses of mushroom compost, leonardite, and vermicompost on lettuce yield and quality, and reported that increasing doses of organic material applications resulted in increased collar diameter. In a study conducted on watermelon (*Citrullus lanatus* (Thunb.) Matsum & Nakai), it was reported that different doses of vermicompost (0, 300, and 600 kg/da) did not have statistically significant effects on fruit flesh thickness, fruit juice pH, and soluble solid content (Göksu and Kuzucu 2017). Similarly, in our study, although the differences between treatments were not statistically significant in both watermelon and melon, the collar diameter, fruit flesh thickness, and soluble solid content in watermelon were found to be 40%, 40%, and 100% higher, respectively, compared to melon. In addition, the fruit weight of watermelon was found to be six times higher than that of melon. One of the main reasons for this large difference is the physiologically larger fruit size of watermelon compared to melon.

Correlations Between Soil Properties and Plant Growth

Based on the numerical data obtained from the trial, relationships between soil chemical properties and plant growth parameters were examined. Accordingly, in melon soils, a negative correlation was observed between fruit flesh thickness and organic matter content (r = -0.539*), while a positive correlation was found between root collar diameter and organic matter content (r = 0.728**). These correlations suggest that soil organic matter may play a dual role in influencing plant morphological traits. While the positive correlation between root collar diameter and organic matter content implies a supportive role of soil organic matter in promoting vegetative growth and stem development, the negative correlation between fruit flesh thickness and organic matter content might indicate a potential trade-off between vegetative and reproductive growth processes under certain soil conditions. This could be due to nutrient partitioning or changes in water-holding capacity and aeration associated with increased organic matter. However, these relationships are likely to be influenced by plant species, growth stage, and environmental factors.

CONCLUSION

This study aimed to evaluate the effects of various organic fertilizers including manures from small ruminants, cattle, poultry, and vermicompost on the chemical properties of a sandy-calcareous soil and the growth performance of melon (*Cucumis melo* L.) and watermelon (*Citrullus lanatus* Thunb.). The study focused on how recommended fertilizer doses influence soil chemical quality and plant growth. Poultry and cattle manures stood out in improving soil chemical properties in watermelon cultivation areas, whereas vermicompost and poultry manure significantly enhanced plant growth parameters. For melon cultivation, cattle manure was most effective for soil chemical improvements, while small ruminant, poultry, and cattle manures showed prominent positive effects on plant development. Overall, poultry manure emerged as the most effective input for enhancing plant growth, while vermicompost and cattle manure also contributed depending on the crop type. These results suggest that organic fertilizers, especially poultry manure, are valuable tools for improving soil fertility and crop productivity in calcareous soils. Nonetheless, expanding these findings through comprehensive studies involving different crops, soil types, cultivation locations, and production systems (e.g., greenhouse or organic farming) will be essential to deepen understanding and broaden the applicability of these organic fertilization strategies. These results underscore the critical role of optimized organic fertilization in promoting sustainable agricultural productivity, supporting both soil health and crop performance in Mediterranean agroecosystems.

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IDENTIFICATION OF SNP MARKERS ASSOCIATED WITH GENES CONTROLLING POTASSIUM AND SODIUM CONCENTRATION IN WALNUT FRUIT BY GWAS METHOD

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ABSTRACT

Owing to its beneficial impact on human health and its abundance of essential nutrients, the walnut is considered one of the most nutritionally valuable tree nuts. This study's objective was to find SNPs related to potassium (K) and sodium (Na) accumulations in walnut fruits collected in 2021 and 2022 from a population of 154 different walnut genotypes, using the GWAS method. It was observed that K concentrations ranged from 131.75 mg/100g to 850.00 mg/100g, while Na concentrations varied between 2.00 mg/100g and 84.50 mg/100g. The genotypes exhibited a statistically significant impact on both nutrient elements, according to variance analysis (p-value ≤ 0.01). The concentrations of K and Na exhibited high heritability estimates, both recorded at 0.99. 13.611 clean SNPs and the MLM (PCA+K) approach were used GWAS analysis and a total of 17 significant associations were identified (16 for K and 1 for Na). Of the 16 significant SNPs identified for K, 14 were found in both years. Additionally, the SNP identified for Na was consistent for both years. For the investigation of potential candidate genes, 17 SNP sequences were aligned with the *Juglans regia* genome, and potential candidate genes were detected. It was discovered that thirteen of these SNPs match onto particular chromosomes in the walnut genome. For the SNPs related to K accumulation, 118 candidate genes in all were found. For the SNP marker related to Na accumulation, 18 candidate genes were detected. The findings obtained from our study suggest that the SNPs related to K and Na accumulation in walnut fruit, along with the candidate genes related to these SNPs, could contribute to marker-assisted selection applications in future walnut breeding efforts and plant breeding studies using genetic engineering approaches.

Keywords Walnut, GWAS, SNP, potassium, sodium, plant-based nutrition

INTRODUCTION

Juglans regia L. (2n = 32, nearly 606 Mb per 1C genome), known as walnut and *J. regia* L. is a monoecious, dichogamous, typically cultivated in temperate regions, pollinated by wind, possessing kernels with very high nutritional value, and a long-lived tree species belonging to the Juglandaceae family (Zhu et al., 2015; Akca et al., 2015; Delaviz et al., 2017). The walnut is widely grown around the world and has significant commercial value because of its high nutritious content and high-quality wood (Ozcan et al., 2010; Gupta et al., 2019). In walnut production, the leading countries worldwide are China, The United States, Iran, Turkey, Ukraine, France, and India, respectively (Taha and Al-wadaan, 2011; Orman et al., 2020).

Walnut stands out as a medically intriguing and promising natural food, owing to its highly valuable nutritional composition that benefits human health (Taha and Al-wadaan, 2011; Liu et al., 2020). Diverse components of the walnut, including its kernel, blossoms, leaves, green husk, and oil, exhibit distinct pharmacological qualities and are utilized in the management of diabetes, rheumatic pain, dementia and Alzheimer's, malaria, and dermatological conditions (Delaviz et al., 2017; Gupta et al., 2019).

Previous studies have determined the macro-nutrient elements in walnuts as potassium (K), phosphorus (P), calcium (Ca), magnesium (Mg), and sodium (Na) (Muradoglu et al., 2010). The recommended daily intake is about 3.5 grams for K and 3.0 grams for Na. K is essential for preserving acid-base equilibrium, sending neural signals, and managing cell membrane functions, while Na is necessary for regulating and maintaining acid-base balance, ensuring cell permeability, and facilitating the absorption of bile salts (Soetan et al., 2010; Zoroddu et al., 2019). Macronutrients are also essential for plant growth, development, and the maintenance of vital activities. K plays a role in maintaining tissue turgor pressure, regulating the opening and closing of stomata, and controlling osmotic pressure in plant cells, while Na performs key functions such as regulating turgor pressure and cell expansion (Fageria, 2001; Raddatz et al., 2020).

Recently, plant-based nutrition has become popular worldwide due to its economic affordability, and health benefits (Suliburska et al., 2012; Sharma et al., 2023). The macro- and micro-nutrient content of commonly consumed plants can be rapidly and precisely improved through genetic biofortification. This process involves identifying genomic regions that affect nutrient content, developing SNP markers via genotyping-by-sequencing (GBS), mapping quantitative trait loci (QTL), and creating DNA-based molecular markers using Diversity Array Technology (DArT) (Orman and Ok, 2016; Gupta et al., 2020; Koc et al., 2022; Sharma et al., 2023).

The "Genome-Wide Association Studies (GWAS)" technique is used for mapping quantitative characteristics based on linkage disequilibrium (LD) in genetically diverse individuals of the same species and for elucidating the relationship between DNA markers and phenotypic traits (Uffelmann et al., 2021). There are GWAS studies on shell color and characteristics, phenology, and the accumulation of nutrient elements such as iron (Fe), manganese (Mn), calcium (Ca), and phosphorus (P) in walnuts (Arab et al., 2019; Orman et al., 2020; Bernard et al., 2020; Akpunar et al., 2024; Doganay et al., 2025). The aim of this study is to determine the K and Na concentrations in walnut kernels obtained from 154 different genotypes and to identify the genes associated with these concentrations using the GWAS method. This study is significant as it is the first association mapping study to identify both the K and Na concentrations in walnut fruit and the genes controlling these concentrations using the GWAS method.

MATERIALS AND METHODS

Plant materials

A walnut population comprised of 154 distinct walnut genotypes was cultivated in the Genetic Resources Parcels of the Yalova Atatürk Central Horticultural Research Institute (ABKMAE). Information about these varieties is presented in **Table S1.**

Determination of potassium (K) and sodium (Na) concentrations in walnut kernels

The concentrations of K and Na in 154 different walnut genotypes were measured using the method developed by Kacar and İnal (1972) (Kacar and Inal, 1972). To obtain more reliable and consistent results, all analysis procedures were performed in triplicate for each element from each genotype.

Variance analysis

To determine the statistical significance of the effects of replication (R), genotype (G), year (Y), and Y x G interactions on K and Na concentrations in 154 walnut genotypes, a two-way analysis of variance (ANOVA) was conducted using the SPSS (Statistical Package for the Social Sciences) software program (version 12.0J; SPSS Inc., Chicago, IL, USA). Year and genotype interactions were viewed as constant effects in the analysis of variance, whereas K and Na accumulation values were viewed as response variables and replications as random factors. The variance analysis was carried out at significance levels of 5% and 1%. Additionally, heritability (H^2) values were calculated to evaluate the effect of the genetic component on the concentrations of both dietary elements (Lamichhanea et al., 2021; Sary et al., 2022).

Genotypic data

The raw SNP dataset produced by Orman et al. (2020) using the DArT genotyping approach provided the genotypic data used in this investigation (Orman et al., 2020). To improve data quality, SNPs with a call rate below 80% were removed from the raw SNP dataset containing 16,774 SNPs. The genotypic data used in this study consisted of the remaining 13,611 clean SNPs.

GWAS analysis

A GWAS study was performed using TASSEL 5.2.93, a program that uses evolutionary trait connections and linkage disequilibrium, as explained by Bradbury et al. (2007), to find SNPs related to K and Na concentrations (Bradbury et al., 2007). In this analysis, the relationships between SNP markers and phenotypes were evaluated using mixed linear model [MLM (PCA + K)] approaches (Upadyayula et al., 2006). The association between SNP markers and phenotypic traits was revealed with the help of Manhattan and quantile-quantile (Q-Q) plots generated for both elements and both years. To detect false positive SNPs, the False Discovery Rate (FDR) threshold was determined (Benjamini and Hochberg, 1995), and SNPs that exceeded the FDR threshold and showed significance at the $p < 0.01$ level were considered statistically significant for the accumulation of both elements.

Putative Candidate Gene analysis

To determine the genomic locations and functional importance of SNP markers associated with K and Na concentrations in walnut fruit, the SNP sequences obtained from the GWAS analysis were aligned with the *J. regia* genome with accession number PRJNA350852, thereby identifying candidate genes. Information on these potential genes is listed in Table S2, Table S3 and Table S4.

RESULTS AND DISCUSSION

Determination of potassium (K) and sodium (Na) concentrations in walnut kernels

The K and Na concentrations measured for both years in 154 different walnut genotypes evaluated for K and Na concentrations are shown in Table 1.

Table 1. K and Na accumulations in walnut genotypes over two years: minimum, maximum, and mean values.

Trait	2021			2022		
	Min	Max	Mean	Min	Max	Mean
K (mg/100g)	257.50	822.50	411.80	131.75	850.00	414.57
Na (mg/100g)	2.50	80.00	18.70	2.00	84.50	18.61

The lowest K concentration for both years was determined to belong to genotype 104/114 (genotype number 11, Table S1), while the highest K concentration was found in genotype BAYIR-1 (genotype number 107, Table S1).

While the variation between K concentration values in this study was found to be 6.45-fold, previous studies reported smaller ranges, such as 1.39-fold (Polat et al., 2015), 1.45-fold (Simsek, 2016), and 1.23- and 1.38-fold (Rabadán et al., 2018). These results imply that the range of K concentrations seen in this investigation is greater than those documented in previous research. The wider genetic diversity of the walnut genotypes employed in this study could be the cause of this increased variation (Polat et al., 2015; Simsek, 2016; Rabadán et al., 2018).

These results suggest that genotype 104/114 could be employed in test cross studies to locate genotypes that are rich in K, whereas the BAYIR-1 genotype may be evaluated as a parent in hybridization and biofortification-oriented walnut breeding programs targeting the development of K-rich genotypes.

Genotype 144/84 had the lowest Na concentration in both years, while genotype GIR/14 (Table S1) had the highest Na concentration in both years.

In the present study, the variation between the minimum and maximum Na concentration values was determined to be 42.25-fold, whereas in the aforementioned studies, this variation was 3.23-fold (Muradoglu et al., 2010), 2.83-fold (Yılmaz and Akca, 2017), and 1.93-fold (Ozyigit et al., 2019). The differences in Na concentrations observed in this study display a broader range compared to the findings in previous literature. This may be attributed to the greater genetic diversity of the walnut genotypes used in the current research (Muradoglu et al., 2010; Yılmaz and Akca, 2017; Ozyigit et al., 2019).

Based on the results obtained from this study, the GIR/14 genotype can be utilized in biofortification studies, hybridization programs, and sustainable agriculture practices to create efficient, nutrient-rich, and healthy food sources.

The two-year (2021–2022) data from the K and Na concentration analyses in walnut fruits were used to build a histogram. It was found that the frequency distribution of both elements for the 154 genotypes displayed a normal distribution (Figure 1a, b, c, and d). This indicates that the identified phenotypic traits are likely controlled by numerous genes, may be impacted by environmental factors, are both measurable and continuous, and that most individuals in the population have average values, while individuals with extreme values are fewer. This also indicates that the population is suitable for GWAS studies due to the presence of both extreme and average value individuals.

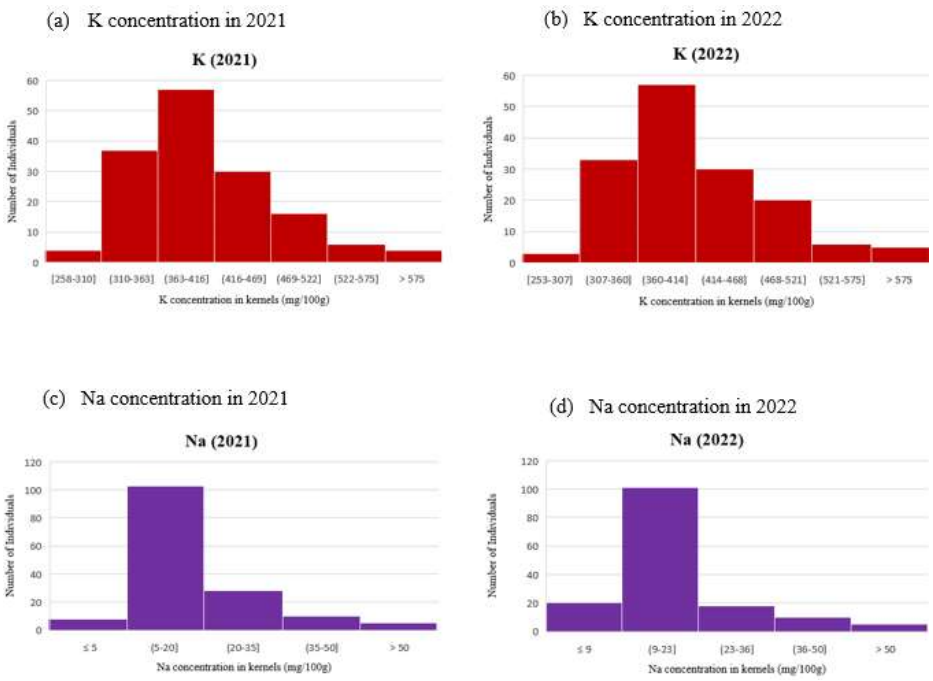

Fig. 1. Histogram graphs depict the distribution of K and Na contents for two years.

Variance analysis

After analyzing the findings of analysis of variance, it was found that R did not create statistically significant differences in K and Na concentrations (dependent variables). G showed a statistically significant effect at $p \leq 0.01$ level for both dependent variables. However, only K concentration was found to be statistically significantly affected by Y and the Y x G interaction at $p \leq 0.01$.

When the variance analysis results of previous studies were examined, it was found that potassium (K) concentrations showed a significant difference only for crop year (CY) alone, while the genotype (G) × crop year (CY) and genotype interactions in consecutive crop years did not exhibit statistically significant differences at the $p \leq 0.05$ level (Rabadán et al., 2019). However, significant differences were detected at the $p < 0.01$ level for genotype × crop year (G × CY), crop year (CY), and genotype (G) interactions for K concentration (Rabadán et al., 2018). These results indicate that the variability in K content in walnut fruits arises from both genetic factors and environmental influences. In previous studies conducted to determine sodium (Na) concentrations, analysis of variance revealed statistically significant differences among genotypes at the $p < 0.01$ level (Ozcan et al., 2010) and at the $p \leq 0.05$ significance level (Simsek, 2016). In the literature, no other variance analysis studies have been found that evaluate the effects of genotype (G), year (Y), and G × Y interactions on Na concentration in walnut kernels.

These results show that while K accumulation in walnut fruits is regulated by both genetic and environmental variables, Na accumulation is impacted only by genetic factors. Since the walnut fruits were harvested from the same location but grown in two different years, it is considered that soil properties such as pH, total salt (%), and organic matter, as well as climatic conditions such as the number of rainy days and total monthly precipitation, may have changed between the two years. This result provides important insights, particularly for plant breeding, fertilization strategies, and biofortification studies, as it highlights the necessity of focusing not only on genetic composition but also on environmental management strategies.

In the present study, the heritability value for K and Na concentrations was determined to be 0.99. These high values suggest that the influence of genotypic factors on K and Na accumulation in walnut kernels is substantially greater than that of environmental factors. In contrast, previous studies conducted on different plant species have reported lower heritability

values; for instance, Amoah et al. (2023) reported heritability estimates of 0.20 for K and 0.46 for Na concentrations in peanuts (Amoah et al., 2023). A number of variables, such as variations in plant species, genetic variability among genotypes, and environmental circumstances, could be responsible for the disparities between the results of this study and those of other investigations.

The study's conclusions offer significant new information and a useful foundation for genotype selection in walnut breeding initiatives aimed at enhancing nutritional value.

Table 2. Variance analysis results of two-year K and Na accumulations obtained from walnut genotypes.

K accumulation						
Variations Source	df	MS	F	P ≤ 0.05	P ≤ 0.01	H^2
Replication	2	64.98	0.44 ns	3.07	4.79	0.99
Year (Y)	1	1814.96	12.45 **	3.92	6.85	
Genotype (G)	153	40368.40	277.02 **	1.25	1.38	
Y x G	153	297.22	2.04 **	1.25	1.38	
Error	614	145.72				
General	923					
Na accumulation						
Variations Source	df	MS	F	P ≤ 0.05	P ≤ 0.01	H^2
Replication	2	15.11	2.59 ns	3.07	4.79	0.99
Year (Y)	1	3.89	0.66 ns	3.92	6.85	
Genotype (G)	153	1096.91	187.96 **	1.25	1.38	
Y x G	153	1.45	0.25 ns	1.25	1.38	
Error	614	5.83				
General	923					

df=degree of freedom; MS=mean sum of square; H^2=Heritability
ns =Not significant
* =Significant at p ≤0.05
**=Significant at p ≤0.01

GWAS analysis

In the GWAS analysis, to eliminate false positive results among the identified SNPs, FDR threshold was applied: K-2021 and K-2022 (FDR = 3.33), Na-2021 and Na-2022 (FDR = 3.50). Manhattan plots were used to show SNPs that exceeded these levels (Fig. 2). Additionally, Q-Q plots were created to illustrate the distribution of P-values demonstrating the relationship between significant SNP markers and K and Na concentrations (Fig. 3). A total of 17 significant SNPs (P <0.01) exceeding the FDR threshold was identified as associated. Out of these SNPs, 16 were related to K accumulation (Table 3) while one SNP was related to Na accumulation (Table 4).

Table 3. List of SNP markers related to K accumulations in walnut kernels.

Trait-Year (FDR)	SNP ID	Marker P	-log(P)	Marker R^2
K-2021 (3.33)	M664	2.33E-06	5.63	0.16
	M2987	2.98E-06	5.52	0.15
	M5697	2.98E-06	5.52	0.15
	M7893	2.98E-06	5.52	0.15
	M15813	2.18E-05	4.66	0.12
	M16456	4.22E-05	4.37	0.11
	M14109	6.37E-05	4.19	0.11
	M14820	1.13E-04	3.94	0.10
	M5560	1.24E-04	3.90	0.10
	M13781	1.86E-04	3.73	0.10
	M12921	1.96E-04	3.70	0.10
	M15542	2.07E-04	3.68	0.09
	M13511	2.39E-04	3.62	0.09
	M1994	3.35E-04	3.47	0.08
	M2343	3.94E-04	3.40	0.08
	M4326	4.29E-04	3.36	0.08
K-2022 (3.33)	M664	1.49E-06	5.82	0.16
	M2987	8.85E-07	6.05	0.17
	M5697	8.85E-07	6.05	0.17
	M7893	8.85E-07	6.05	0.17
	M15813	1.95E-05	4.71	0.12
	M16456	4.84E-05	4.31	0.11
	M14109	3.39E-05	4.47	0.12
	M14820	2.20E-04	3.65	0.09
	M5560	1.37E-04	3.86	0.10
	M13781	1.23E-04	3.90	0.10
	M12921	1.30E-04	3.88	0.10
	M15542	2.39E-04	3.62	0.09
	M13511	1.89E-04	3.72	0.09
	M2343	4.42E-04	3.35	0.08

Table 4. List of SNP markers related to Na accumulations in walnut kernels.

Trait-Year (FDR)	SNP ID	Marker P	-log(P)	Marker R^2
Na-2021 (3.50)	M1547	2.26E-04	3.64	0.09
Na-2022 (3.50)	M1547	2.10E-04	3.67	0.09

Research conducted for the present study did not find any other studies in the literature that identified SNP markers related to nutrient accumulation in walnut kernels. However, there are research that has found SNP markers related to various traits in walnuts. When these studies are examined, 98 SNP markers associated with seven traits of walnut fruit, 55 significant SNP markers related to nut, kernel, and shell traits, and 259 significant SNP markers identified in different walnut genotypes in response to drought stress have been reported (Arab et al., 2019; Orman et al., 2020; Arab et al., 2022). These findings in the literature demonstrate the importance of the GWAS method in identifying SNP markers associated with specific traits. The identification of SNP markers linked to desirable traits can contribute to plant breeding efforts.

In this study, the R^2 values of the identified SNPs were determined to range from 0.08 to 0.17, while in other studies in the literature, R^2 values were observed to range from 0.08 to 0.19 and from 0.01 to 0.35 (Orman et al., 2020; Arab et al., 2022). These results are consistent with the literature. Based on these findings, markers with high R^2 values can be used in marker-assisted selection (MAS) and breeding programs to select individuals targeting these markers, thereby enabling faster genetic gain.

Future genetic research on walnuts can leverage the SNP markers shown in this work to be related to K and Na concentrations in walnut fruit as genetic resources.

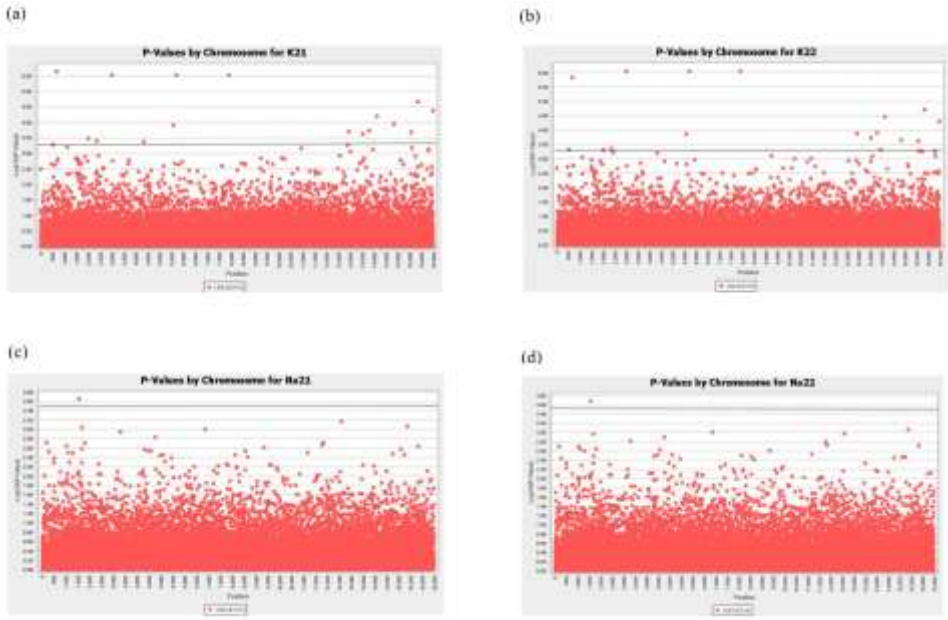

Fig. 2. Manhattan plots of both macronutrients: (a) K-2021, (b) K-2022, (c) Na-2021, (d) Na-2022.

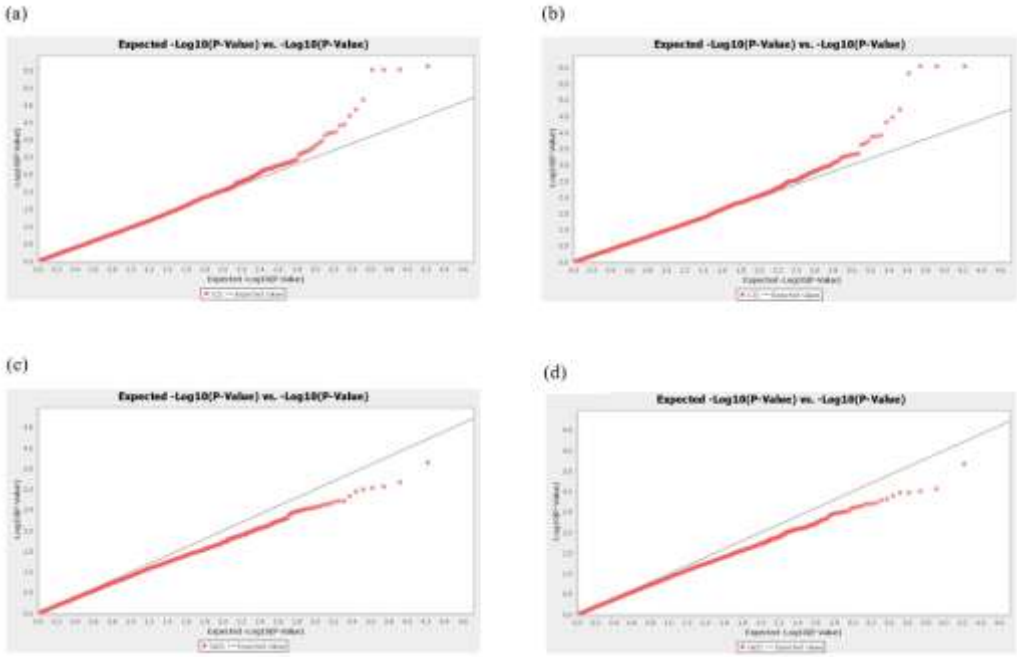

Fig. 3. Q-Q plots of both macronutrients: (a) K-2021, (b) K-2022, (c) Na-2021, (d) Na-2022.

Putative Candidate Gene analysis

The 17 SNP markers associated with K and Na accumulation, identified through the GWAS analysis, were aligned with the *Juglans regia* L. genome and 13 of these SNPs were localized to particular chromosomes in the walnut genome. For 11 SNPs associated with both K-2021 and K-2022 accumulation, 105 candidate genes were identified (Table S1), while 13 candidate genes were detected for the SNP associated only with K-2021 accumulation (Table S2). For the SNP associated with both Na-2021 and Na-2022 accumulation, 18 candidate genes were identified (Table S3).

The serine/threonine-protein kinase PBL9 identified for SNP664 is a member of the PBS1-like kinase family, which has been characterized in *Arabidopsis thaliana* and plays important roles in immune responses and signal transduction (Huang et al., 2021). The gene phospholipid-transporting ATPase 4, located near SNP7893, encodes a protein belonging to the phospholipid-transporting ATPase family and has been identified in *Arabidopsis thaliana*. This gene plays a critical role in maintaining membrane stability and cellular functions by regulating the incorporation of phospholipids into the cell membrane and intercellular transport (Jensen et al., 2017). The candidate genes identified in this study as being associated with K concentration have not previously been reported in the literature to be associated with K concentration in walnut.

These findings may help us better understand the functions of genes associated with K concentration in plant cells. Additionally, investigating genes that do not directly affect K content but may have the potential to influence it indirectly can provide a basis for future research. Considering the importance of the K nutrient for human health and nutrition, the data obtained from the research can offer supportive information for improving the quality of plant-based food sources. Understanding the functions of genes related to nutrient accumulation can also contribute to the cultivation of nutrient-rich plants through genetic engineering approaches, particularly in plant breeding efforts.

CONCLUSION

This study represents the first research in which SNP markers associated with K and Na concentrations were identified using the GWAS method among 154 different walnut genotypes. Genetic variation in K and Na concentrations was detected, and the obtained data hold significant potential for future genetic research on walnuts. Investigating potential genes associated with SNP markers related to nutrient accumulation in the walnut and determining their traits may contribute to plant breeding efforts through genetic engineering approaches. Additionally, enhancing K and Na concentrations in edible plants through biofortification studies may also contribute to the improvement of human nutrition.

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PROPOLIS AGAINST *BOTRYTIS CINEREA*: A REVIEW OF STUDIES COMBINING IN VITRO AND IN VIVO TESTS

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ABSTRACT

Botrytis cinerea is a necrotrophic fungal pathogen that is responsible for substantial losses in a wide range of plant species. The detrimental impacts of chemical fungicides on human health and the environment have given increase the interest in alternative control strategies. In this review, the antifungal effects of different propolis extracts on this pathogen were evaluated based on studies that combined *in vitro* and *in vivo* tests. The findings of the in vitro experiments demonstrated that propolis extracts have the capacity to inhibit various developmental stages of this pathogenic fungus such as mycelial growth, sporulation, and conidial germination. However, the effectiveness of this inhibition was changed with concentration of the extract and the method of preparation. In this context, it has been demonstrated that extracts prepared based on the use of ethanol at high concentration and sonication method have a stronger antifungal effect. *In vivo* test demonstrated that the treatment of propolis to the fruits of plants including strawberries, grapes, blueberries and tomatoes led to a reduction in both the disease incidence and infection area by the pathogen, although this effect exhibited the variability with plant species, the application method and dosage. Consequently, while propolis reveals considerable antifungal potential against *B. cinerea*, additional researches are required on the standardization of extracts, the optimization of doses, the determination of application timing and *in vivo* tests on different plant species to increase its applicability in agricultural practices.

Keywords: *Botrytis cinerea*, propolis extract, antifungal effect, *in vitro* and *in vivo* tests

INTRODUCTION

Propolis is a complex natural compound that is formed by honeybees (*Apis mellifera*) by combining the natural substances they collect from plant buds, resins and leaf sap with their own enzymatic secretions. The word 'propolis' is derived from the Greek word’s 'pro', meaning 'front or entrance', and 'polis', meaning 'city-colony', which indicates that it was used in its hive defense. Propolis is characterised by its flexibility, softness and stickiness at high temperatures, while it becomes hard and brittle when cooled. Due to these properties, it is used by honeybees to close cracks and holes and strengthen the structure of beeswax combs in order to keep the temperature and humidity inside the hive constant throughout the year (Sforcin, 2007).

From antiquity to the present, propolis has been utilized as an antiseptic and disinfectant for oral and skin illnesses in ancient Greece, primarily in traditional medicine, and by the Egyptians during mummification procedures (Havsteen, 1983; Burdock, 1998). This particular bee product, which has a wide range of uses, has been shown to contain over 300 chemical compounds (Huang et al., 2014). The chemical composition of raw propolis typically consists of 50% plant resins, 30% waxes, 10% essential and aromatic oils, 5% pollen, and 5% other organic substances (Bankova, 2005). However, the main chemical groups in propolis resin are

phenolic acids and their esters, terpenes, flavonoids, aromatic aldehydes and alcohols, stilbenes, fatty acids, and β-steroids (Watanabe et al., 2011; Ahangari et al., 2018; Przybylek and Karpinski, 2019). Despite the chemical diversity of propolis, comprehensive research has revealed the existence of at least six main types (Ghallab et al., 2024).

Figure 1. The main types of propolis (Ghallab et al., 2024).

Following the demonstration of the anticancer, antioxidant, anti-inflammatory, antibiotic, antifungal and antihepatotoxic properties of propolis by Ghisalberti (1979), there has been an increase in the number of modern controlled experimental studies on the chemical structure and biological activities of this substance. *Botrytis cinerea*, the causal agent of gray mold disease in several plant species and a major cause of yield losses both in the field and post-harvest (Williamson et al., 2007; Dean et al., 2012), took a place among the fungal pathogens against which propolis was tested. The ability of this fungal pathogen for development of resistance exhibited by this fungal pathogen to fungicides has rendered the use of alternative compounds to control it, such as propolis, increasingly significant.

Some of the studies have been conducted *in vitro* to investigate the impact of propolis on *B. cinerea* (Sapieha-Waszkiewicz et al., 2011; Curifuta et al., 2012; Yang et al., 2016; Ezazi and Davari, 2018; Wuaden et al., 2018; Ouahab et al. 2023; Erdoğan and Güzel, 2025). However, the results of *in vivo* tests involving plant parts are more significant in determining the practical applicability of this compound. In this review, the effect of propolis against this pathogen was discussed in the light of the findings obtained from both *in vitro* and *in vivo* stages, with emphasis placed on the highest effects observed in the studies. Within this context, *in vitro* studies are incorporated into the text according to their chronological sequence, while *in vivo* studies are presented in tabular form. Articles belonging to the research conducted until today were obtained by using many databases in Tekirdağ Namık Kemal University library.

STUDIES ON THE EFFECTS OF DIFFERENT PROPOLIS EXTRACTS AND APPLICATIONS *ON Botrytis cinerea*

In the study conducted by Darweesh (2010), samples of propolis collected from Baghdad, Iraq, were prepared using 96% ethyl alcohol and subsequently added to Potato Dextrose Agar (PDA) medium at doses of 1.25, 2.5, and 5 mg/ml. The researcher then has determined the pathogen's colony diameter and mycelial growth rate seven days after placing a mycelial disk of the pathogen in the center of each petri dish. In the study, the same concentrations were also added to Potato Dextrose Broth (PDB) medium and mycelial dry weights were measured after 14 days of incubation at 25°C. *In vitro* tests of the study revealed that a concentration of 5

mg/mL of propolis extract led to a 100% reduction in both the colony growth and the mycelial dry weight in PDA and PDB media. This dose was applied to eggplant leaves and cucumber fruit, and its efficacy against the pathogen *in vivo* was tested (Table 1). In addition, the chemical composition of propolis was analysed using infrared spectroscopy, and the presence of flavonoids was determined.

In a study conducted by Moura et al. (2016), extracts of two different types of propolis (green and brown) prepared with ethanol were added to PDA medium, which were present in 9 cm Petri dishes at doses of 0.5% and 2.5%, and the mycelial disk of the pathogen was placed on it. Petri dishes were incubated at 25°C and the colony diameter of the pathogen was measured at 48, 72 and 96 hours after inoculation. Following a thorough evaluation of the study's findings, it was determined that both 0.5% and 2.5% doses of green propolis exhibited a higher antifungal effect against the pathogen in comparison to brown propolis. Findings from the study indicated that a 2.5% dose of green propolis led to a 33.67%, 43.90%, and 43.23% inhibition in colony development at 48, 72, and 96 hours after inoculation, respectively; these values were higher in comparison to the inhibition rates of brown propolis at same dose. In the study, both propolis were tested using strawberry fruit *in vivo* at both doses (Table 1).

Table 1. Some studies conducted under *in vivo* conditions against *Botrytis cinerea*

Treatment	Scope	Result / Effectiveness	Author
Application of propolis ethanol extract (5 mg/ml) to eggplant leaves and cucumber fruits at different times before and after inoculation [dropping of conidia suspension (1×10^6 conidia/mL) into wounds opened on leaves and fruits] of the pathogen	Determination of the effect of application time (both 24, 48 hours before and after inoculation) on the growth of *B. cinerea*	The treatments made 24 and 48 hours before inoculation significantly slowed down disease development.	Darweesh (2010)
Spraying of a conidia suspension of the pathogen at a concentration of 1×10^5 conidia/mL after one minute immersion of strawberry fruits in solutions of green and brown propolis at 0.5% and 2.5% doses	Control of pathogen development in the cultivar (cv) Camarosa with different propolis doses	No difference was observed between the two propolis extracts in terms reducing disease incidence, with an overall inhibition rate of 12.5% being determined. The 0.5% dose of green propolis inhibited the disease severity at the highest rate (23.08%).	Moura et al. (2016)
After covering cherry tomatoes with pullulan film containing propolis ethanol extract (5% and 10%), the inoculation of the pathogen conidia suspension (10µl) to the wound	Determining the protection of the application by measuring the pathogen population on tomatoes	There was no reduction in the population of coated pathogens after 14 or 21 days.	Pobiega et al. (2020)
Inoculation of pathogen (10 µL) into wounds opened with a pipette tip after immersing grapes	Evaluation of the effect of different propolis types	In the study, after 15 days, Portuguese propolis (~16.3%) was	Diallo (2021)

berries in propolis solutions (15 g/L) for 2 minutes (min)	(Portuguese, Brazilian red) on the decay area caused by *B. cinerea*	found to be effective in red grapes and Brazilian red propolis in white grapes (~21.5%) were found to be effective, even if at low rates.	

Treatment	**Scope**	**Result / Effectiveness**	**Author**
Immersion of strawberry fruits in propolis extract (20-, 40- and 60-mL/L) for 30 da, keeping at 25ºC for 24 hours and inoculation of conidia suspension of the pathogen	Monitoring the rate of disease incidence at different times (24, 48, 72 hours), determining the PAL (phenylalanine ammonia-lyase) enzyme activity and total phenolic substance amount, which play a role in resistance	No significant decrease in disease incidence was observed at any time point at any of the three doses, and an increase was observed at higher doses. Although the total phenol content of the fruit decreased, PAL enzyme activity increased by only 7.61% after 72 hours.	Stefanski et al. (2024)
Inoculation of 5µl of conidia suspension of the pathogen into the fruits using a sterile needle; for volatile compounds, the placement of the inoculated fruits on sterile papers with 100 µl of extract; for non-volatile compounds, treating the extracts (5 µl) to the wounds on the fruit arranged on the grid one hour after inoculation.	Evaluation of the effect of volatile and non-volatile compounds in extracts on disease incidence	PFvg1 extract exhibited strong antifungal activity (~60-80%) for both compounds. PFvg2 followed it.	Sadallah et al. (2025)

Pobiega et al. (2020) determined the minimum inhibitory concentration (MIC) value for *B. cinerea* as 2 mg/mL and the minimum fungicidal concentration (MFC) value as 8 mg/mL as a result of serial dilution tests prepared with propolis ethanol extract (EEP). In the conducted study, additionally, pullulan films containing 5% and 10% EEP were prepared, and 6 mm diameter discs were cut from these films and placed on Sabouraud agar media spread with conidia suspension. Cellulose discs coated with EEP at the same concentration have been used as the control group. The experimental findings revealed that no inhibition zones were observed in any of the treatment groups. The *in vivo* tests of the study were carried out on cherry tomato fruits (Table 1).

In a study performed by Diallo (2021), the antifungal effects of three distinct propolis ethanol extracts (Portuguese propolis, Brazilian green propolis, and Brazilian red propolis) on B. cinerea G3 isolate were investigated. For the present study, the extracts were prepared at doses of 0.5 g/L, 1.5 g/L, 5 g/L, and 15 g/L, and were compared with a commercial fungicide and a fungicide containing the active ingredient tebuconazole. Both agar disk diffusion and well diffusion methods were used in the *in vitro* experiments. In the disk diffusion assay, square dishes containing 50 mL of PDA were prepared, and a conidial suspension with a density of 1×10^6 conidia/mL was spread evenly onto the medium using a sterile swab. Subsequently, a total of nine filter paper disks were placed on the surfaces, and 100 µL of the extract was

dropped onto each disk. In the well diffusion method, the same volume (100 µL) of solution was applied to each well (nine wells punched with the back of a sterile Pasteur pipette on the agar) and the experiments were conducted separately for different extracts. The dishes were then subjected to an incubation process at a temperature of 25°C for a period of five days. Following the conduction of the experiments, it was reported that none of the propolis species examined in both methods demonstrated antifungal efficacy at the applied doses. *In vivo* tests were conducted utilising extracts at a concentration of 15 g/L and grape berries. (Tablo 1).

Stefanski et al. (2024) examined the effects of an ethanol extract of brown propolis on mycelial development, sporulation and conidia germination of *B. cinerea* under *in vitro* and in *vivo* conditions. In the *in vitro* experiments, propolis extract was added to PDA medium at doses of 20, 40, and 60 mL/L. Petri dishes with a 5 mm diameter *B. cinerea* mycelium disk placed in the center were then incubated at 25°C for 6 days. Colony growth was measured daily, and conidia counts were performed at the conclusion of the sixth day. In the study, it was indicated that mycelial growth rates were significantly reduced and that sporulation was absent at doses of 40 and 60 mL/L. *In vivo* studies on strawberry fruits demonstrated that the three doses were ineffective in reducing disease incidence (Table 1.).

Sadallah et al. (2025) evaluated the antifungal activity of propolis samples [PFvg1 (Friuli Venezia Giulia), PFvg2 (Friuli Venezia Giulia), PU3 (Umbria), PV4 (Veneto), PT5 (Tuscany)] obtained from different geographical coordinates of various Italian regions against *B. cinerea* isolate obtained from grape berries, both *in vitro* and *in vivo* (Table 1). In the study, samples of propolis were prepared by extraction with a range of solutions of ethanol (30%, 60% and 90% v/v). *In vitro* tests were carried out in which the conidia suspension of the pathogen (1×105 conidia/mL) was spread on PDA medium, 80 µL of extract was applied to the wells (5 mm in diameter) on the medium, and the inhibition zones were measured after 72 hours. The findings of this test demonstrated that the widest inhibition zone was observed in the use of PU3 propolis extract, which was prepared with 90% ethanol. The researchers also had ascertained the EC_{50} value for volatile and non-volatile compounds of each extract based on conidia germination using increasing doses (0, 3.2, 6.5, 12.5, 25, 50, 100, 150 and 200 ppm) of propolis extracts obtained by sonication with 90% ethanol for 24 hours. For tests involving volatile compounds, filter papers were placed on conidia suspensions spread on PDA medium, and the relevant dose of propolis extract was applied to each. For non-volatile metabolites, volatile compounds (100 µl) were added one hour after the pathogen spread onto the medium. Petri dishes were subjected to an incubation process at a temperature of 20°C for a duration of 48 hours. The outcomes of these experimental tests had indicated that PFvg2 (EC_{50}: 1.36 mg/ml) was identified as the most effective extract in regard to volatile compounds, while PU3 (EC_{50}: 2.24 mg/ml) was determined to be the most effective extract in relation to non-volatile compounds. In *in vivo* tests, the EC_{50} value of each extract was used as the dose.

CONCLUSION

The *in vivo* studies referenced in this review demonstrate the significant antifungal potential of propolis extracts against *B. cinerea*. However, the extent of this efficacy is dependent on numerous factors, including the chemical composition of the propolis, the extraction method, the type of solvent utilised, the dose, the timing of application and the target plant tissue. The extant literature reveals that research studies have examined the efficacy of propolis extracts on the plants at post-harvest stage, with promising results obtained. The efficacy of propolis under *in vivo* conditions has been proven by significant reductions in disease incidence and lesion development, particularly in high-value crops with perishable properties, including strawberries, grapes, blueberries, cucumbers and tomatoes. The fact that propolis is not only used in the context of agricultural products but also in human health, with

its antimicrobial, antioxidant and anti-inflammatory properties, renders it a versatile and safe product. In this context, the utilisation of propolis, a substance that has been demonstrated to be effective in both human health and in the inhibition of plant pathogens, particularly in the control of fungal diseases in the post-harvest period in fruits, will not only result in a reduction of product loss but also contribute to food safety by eliminating the risk of chemical residues. In conclusion, propolis extracts, with their naturality, environmentally friendly composition, and multiple biological activities, have the potential to become a significant component of sustainable agricultural practices in the future to control postharvest diseases. However, further studies on various fruits and vegetables are needed to integrate this potential into agricultural practice.

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BIOCONTROL ACTIVITY OF Trichoderma harzianum AGAINST *Fusarium culmorum* CAUSING ROOT AND CROWN ROT IN WHEAT UNDER SALT STRESS CONDITIONS

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ABSTRACT

Fusarium culmorum is a destructive soil-borne fungal pathogen responsible for root and crown rot in wheat (*Triticum aestivum*), leading to significant yield losses globally in the areas where wheat was cultivated. Biological control agents such as *Trichoderma harzianum* have shown promising antagonistic effects against *F. culmorum* under optimal conditions; however, understanding their performance under abiotic stress is also crucial for practical applications. This study aimed to evaluate the antagonistic activity of *T. harzianum* against a highly virulent *F. culmorum* isolate under increasing salinity. In the first stage, Potato Dextrose Agar (PDA) medium was amended with 50, 100, and 150 mM NaCl, and dual culture assays were conducted. In these tests, *T. harzianum* inhibited the mycelial growth of *F. culmorum* by 40.62%, 40.00%, and 38.75% at 50 mM, 100 mM and 150 mM salt concentrations, respectively, compared to 65% inhibition under non-saline control. In the second stage, wheat seeds co-inoculated with both the pathogen and antagonist were placed on PDA medium containing 50 and 100 mM NaCl. Under these conditions, *T. harzianum* suppressed *F. culmorum* colony development around the seeds by 70.64% at 50 mM and 45.16 % at 100 mM NaCl. These results indicate that *T. harzianum* retains significant biocontrol potential under moderate salt stress. It was demonstrated that high salt levels reduced antagonistic activity in dual culture tests, while protective activity increased around the seed at salt concentrations that did not inhibit plant growth. The study presents promising results regarding the potential use of *T. harzianum* as a biocontrol agent against *F. culmorum* in wheat cultivation in saline regions.

Keywords: *Trichoderma harzianum*, salt stress, *Fusarium culmorum*, antagonistic effect, seed colonization

INTRODUCTION

Approximately 7% of the world's land, including 20% of agricultural land, is affected by salinity problems. Consequently, it is estimated that all agricultural lands worldwide will face challenges related to salinity by the year 2050 (Shahid et al., 2018; Singh et al., 2019; Otlewska et al., 2020). The increase in salt concentrations is capable of inducing alterations in both plant life and microbial ecosystems. Recent studies have demonstrated that the application of varying salt levels to cultivated plants or the cultivation of the plants under salinity stress can enhance the flavour and aroma of the crop. However, it has been reported that such conditions can facilitate the growth of fungal pathogens (Dikilitaş ve Karakaş, 2014). Plants and microorganisms both exhibit distinct responses to salt stress. It has been demonstrated that plants exposed to salt stress become more susceptible to fungal pathogens such as *Alternaria solani*, *Fusarium oxysporum*, *Macrophomina phaseolina*, *Phytophthora* spp. and *Verticillium dahliae*, due to their reduced resistance (Swiecki and MacDonald 1991; Nachmias et al. 1993; Triky-Dotan et al. 2005; Goudarzi et al. 2011).

Wheat (*Triticum aestivum* L.) is considered to be one of the most significant crops cultivated in arid and semiarid regions (Shiferaw et al., 2013). Limiting factors for wheat production include nutrient deficiencies in soil, drought, salinity stress and the presence of the pathogens causing the diseases. *Fusarium culmorum*, a fungal pathogen transmitted through seeds and soil, has been responsible for significant yield losses by causing pre- and post-emergence damping-off in plants (Pasquali et al., 2016). In the case of the pathogen being present in the soil, the recommendation has been for seed treatment with various fungicides. However, due to the various adverse effects of chemicals on the environment and human health, seed treatments with the biological control agent *Trichoderma harzianum* have also been shown to be sufficiently successful against this pathogen (Erdurmuş and Katırcıoğlu, 2008; Kthiri et al., 2020; Özdamar and Özer, 2022; Vitti et al., 2022). Upon the colonisation of plant roots by this antagonist fungus, the increase in the activities of various enzymes, including peroxidase, chitinase, β-1,3-glucanase, and lipoxygenase-pathway hydroperoxide lyase is occurred. This process is of critical importance for the increases in plant growth, the accumulation of compounds that are involved in resistance, and improved nutrient uptake, as well as the development of tolerance to salt stress in plants (Shoresh et al., 2010; Contreras-Cornejo et al., 2016). However, it is known that the development of *T. harzianum* is also affected by different factors (salinity, temperature, heavy metal) (Mohammed ve Haggag, 2006; Poosapati et al., 2014; Rawat et al., 2013; Contreras-Cornejo et al., 2016; Mbarki et al., 2017; Bozdeveci et al., 2019; Abu-Shahab et al., 2022; Rawat et al., 2022; Abdelrhim et al., 2024).

In previous years, studies have been conducted on the effects of *T. harzianum* on various fungal pathogens causing root and crown rot in different plant species under in vitro and in vivo conditions at different salinity levels. These include *Fusarium oxysporum* f. sp. *lycopersici* in tomato (Mohammed and Haggag, 2006), *F. oxysporum* f. sp. *ciceri* in chickpea (Rawat et al., 2013), *Sclerotinia sclerotiorum* in bean (Abdelrhim et al., 2024) and *F. udum* in pigeon pea (Mishra et al., 2024). In the case of wheat, only *F. pseudograminearum* has been investigated in this context (Boamah et al., 2021). To date, no study has examined the effects of *Trichoderma harzianum* application on wheat under combined salinity stress and *F. culmorum* infection, which is prevalent in our country. This work aimed to test the antagonistic activity of *T. harzianum* against *F. culmorum* and its impact on seed colonization by the pathogen, under different levels of salinity stress.

MATERIAL AND METHOD

The pathogenic fungus isolate used in the experiments was *F. culmorum* (S-14), which was isolated from wheat exhibiting symptoms of root and crown rot in the Thrace Region and is known to be pathogenic. The wheat cultivar Flamura 85, which is susceptible to this pathogen, was employed in the study (Köycü and Özer, 2019). The antagonist fungus was *T. harzianum* (TRIC8, Accession number: MH351669), which was isolated from onion-grown soils in Tekirdağ province and exhibited antagonistic properties (Özer et al., 2009) The pathogenic isolate was grown on Potato Sucrose Agar (PSA), while the antagonist fungus was cultured on Potato Dextrose Agar (PDA-Merck) in an incubator (Binder) maintained at 23°C.

In *in vitro* experiments, 0, 50, 100 and 150 mM NaCl concentrations were added to the PDA medium for salt tolerance and then poured into 8.5 cm diameter petri dishes after autoclaving at 121 ℃ for 20 min. Following the solidification of the medium, an agar disc (5 mm diameter) from the pathogen culture (grown in PSA medium) and the culture of the antagonist (grown in PDA medium) were positioned in opposite directions, at a distance of 1 cm from the outer edges of the petri dish (dual culture). Furthermore, in the context of pathogen control (PK), an agar disc containing solely the pathogen was positioned in the center of the petri dish. All petri dishes were incubated in a refrigerated incubator (Binder) at 23℃ for 7 days. Subsequent to the incubation period, the colony radius of the pathogen was measured in

each treatment, and the inhibition rate of the pathogen by *T. harzianum* was calculated using the following formula: [Inhibition rate (%) = (colony radius of the pathogen in pathogen control (PC) dishes– colony radius of the pathogen in dual cultures containing different salt concentrations) / PC × 100]. The experiments were conducted in four replicates, with a petri dish in each replicate.

In order to determine the effect of *T. harzianum* on *F. culmorum* colony development around wheat seeds under salt stress, concentrations of 50 mM and 100 mM were selected, corresponding to low and moderate salt levels, respectively. PDA media containing NaCl at these concentrations were poured into 8.5 cm diameter petri dishes. The seeds were immersed in a 2% sodium hypochlorite (NaOCl) solution for a period of seven minutes, following which they were rinsed three times with sterile distilled water. Thereafter, they were dried on sterile filter paper for a period of 30 minutes. Surface-sterilized seeds were then placed in a suspension mixture containing conidia of *F. culmorum* (1×10^6 conidia/ml) and *T. harzianum* (1×10^7 conidia/ml), which had been supplemented with 0.1% Tween 20, and shaken at 25 °C for one hour. For the pathogen seed control (PSC), only the conidial suspension of the pathogen was used. The treated seeds were then placed in Petri dishes (10 seeds per dish). The experiments were conducted with five replicates, each consisting of one Petri dish. Subsequent to a period of incubation at a temperature of 23 °C for a duration of seven days, the diameter of the colony of *F. culmorum* developing around the seeds was measured. The inhibition rate by *T. harzianum* was calculated using the following formula: [Inhibition rate (%) = (PSC – colony diameter of the pathogen around seeds treated with pathogen + *T. harzianum* under different salt concentrations) / PSC × 100], where PSC refers to the colony diameter of the pathogen around seeds inoculated only with the pathogen.

The homogeneity of variances and the normality of distribution were first assessed using Levene's test and the Shapiro–Wilk test, respectively, in SPSS version 23 (IBM SPSS Statistics for Windows, IBM Corp., Armonk, NY, USA). All experiments met the assumptions of the ANOVA test. The Tukey HSD test was performed using JMP Pro version 17 for Windows (SAS Institute Inc., Cary, NC, USA) to compare treatment means at the 0.05 probability level.

RESULTS AND DISCUSSION

In the present study, the colony radius of *F. culmorum* was determined to be 4.00 cm in pathogen control (PK). In the salt-free medium (0 mM, P+T), its colony radius decreased to 1.40 cm with the presence of *T. harzianum* (dual culture), with the lowest value observed in this treatment (Table 1, Figure 1 A). In this medium, *T. harzianum* demonstrated an inhibition rate of 65% against the growth of the pathogen. However, the inhibition rate of the pathogen by the antagonist fungus at salt concentrations ranging from 50 to 150mM (Figure 1 B-D) varied between 40.62% and 38.75%, and the differences between these levels were determined to be statistically non-significant.

Two doses, which exhibited a similar inhibitory effect on mycelial colony development of *F. culmorum*, were added to the PDA medium. Subsequently, wheat seeds coated with the pathogen and *T. harzianum* were placed on the medium, and the colony development of the pathogen around the seeds was measured. In the conducted tests, the colony diameter of F. culmorum was measured at 2.55 cm in the pathogen seed control (PSC) group. The colony diameter of the pathogen decreased to 0.26 cm in the presence of *T. harzianum* in salt-free medium (0 mM, P+T), with the highest recorded inhibition rate of the pathogen by the antagonist (89.92%) being determined in this medium (Table 2, Figure 2A). The medium supplemented with 50 mM NaCl was found to be the second rank in terms of low colony diameter around the seed (Figure 2B). In this medium, *T. harzianum* inhibited the colony

growth of the pathogen by 70.64%. At a salt concentration of 100 mM (Figure 2C), the inhibition rate decreased to 45.16%. The differences between 50 and 100mM salt concentrations with regard to colony diameter and inhibition rate were detected to be statistically significant.

Table 1. Effect of *Trichoderma harzianum* on mycelial colony growth of *Fusarium culmorum* at different salt concentrations.

Salinity (mM)	**Mycelial colony radius (cm)***	**Inhibition of mycelial colony growth (%)***
PC	4.00±0.00 a	-
0 (P+T)	1.40±0.07 c	65.00±1.77 a
50 (P+T)	2.37±0.05 b	40.62±1.20 b
100 (P+T)	2.40±0.04 b	40.00±1.02 b
150 (P+T)	2.45±0.03b	38.75±0.72 b

*Each value is the mean of 4 replicates. Differences between values indicated with different letters in the same column are significant according to Tukey's HSD test. (For mycelial colony diameter; $F_{4,15}=444.8936$, $P<0.0001$, Inhibition rate of mycelial colony growth; $F_{3.12}=104.234$, $P<0.0001$). PC: Pathogen Control, P: Pathogen, T: *Trichoderma harzianum*

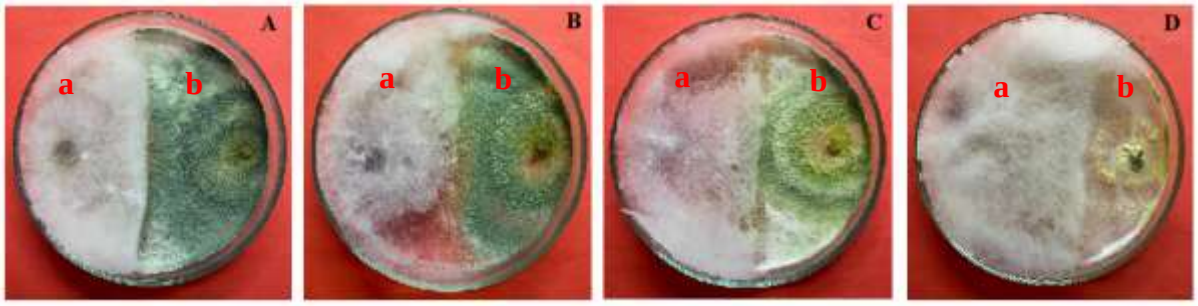

Figure 1. The growth of *Fusarium culmorum* (a) and *Trichoderma harzianum* (b) in dual culture at different salt concentrations A: 0 mM, B: 50 mM C: 100 mM D: 150 mM

Table 2. Effect of *Trichoderma harzianum* on mycelial colony growth of *Fusarium culmorum* around wheat seeds at different salt concentrations.

Salinity (mM)	**Mycelial colony diameter (cm)***	**Inhibition of mycelial colony growth (%)***
PSC	2.55±0.05 a	
0 (P+T)	0.26±0.05 d	89.92±2.00 a
50 (P+T)	0.74±0.04 c	70.64±1.79 b
100 (P+T)	1.40±0.03 b	45.16±1.19 c

Each value is the mean of 5 replicates. Differences between values indicated with different letters in the same column are significant according to Tukey's HSD test. (For mycelial colony diameter; $F_{3,16}= 464.7357$, $P<0.0001$. Inhibition rate of mycelial colony growth; $F_{2,.12}=175.2877$, $P<0.0001$). PSC: Pathogen Seed Control, P: Pathogen, T: *Trichoderma harzianum*

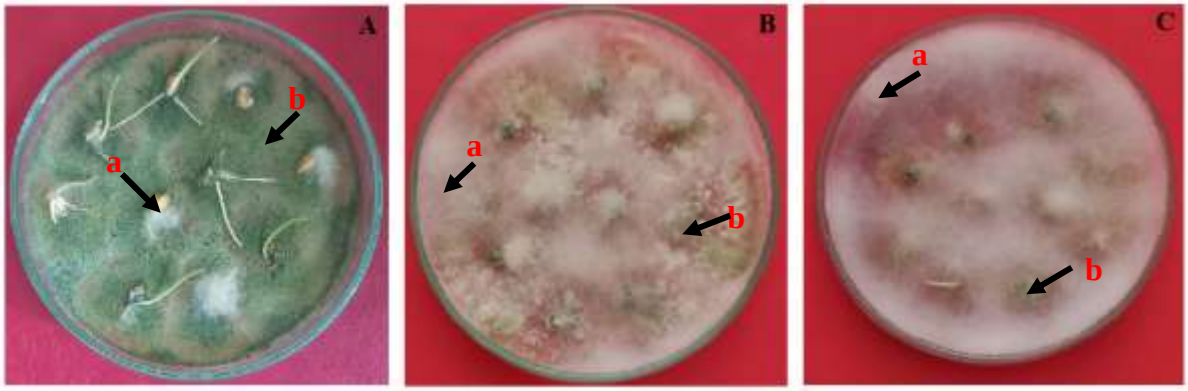

Figure 2. The growth of *Fusarium culmorum* (a) around of the seeds in the presence of *Trichoderma harzianum* (b) at different salt concentrations. A: 0 mM, B: 50 mM, C: 100 mM

It has been established for a long time that there are variations in salt tolerance among different *Fusarium* spp. (Mandeel, 2006; Shoaib et al., 2018; Maharshi et al., 2021; Tiwari et al., 2024). This has resulted in a varying range of consequences regarding the impact of *T. harzianum* on *Fusarium* spp. at differing levels of salinity. In this context, the highest inhibition of colony growth of *F. oxysporum* f. sp. *lycopersici* by *T. harzianum* (40.4%) was at 69 mM salinity concentration (Mohammed and Haggag, 2006); while these values were determined as 70 mM for *F. oxysporum* f. sp. *ciceri* (93.66%) (Rawat et al., 2013), 150 mM for *F. pseudograminearum* (46.42%) (Boomah et al., 2021), and 684.46 mM for *F. udum* (60%) (Mishra et al., 2024). As stated in the study by Rodriguez et al. (2008), some isolates of *F. culmorum* were capable of growth at concentrations of up to 500 mM NaCl. This finding suggests that some isolates of the species may have developed tolerance to high salt concentrations. The inhibitory effect of *T. harzianum* on *F. culmorum* was found to be limited, with levels of 40.62%, 40% and 38.75% observed at 50, 100 and 150 mM NaCl concentrations, respectively, in the present study. This may be attributable to the salt tolerance exhibited by the *F. culmorum* isolate of the current study.

In the present study, *T. harzianum* and *F. culmorum* were applied to seeds, and the level of *T. harzianum*'s ability to prevent the development of the pathogen around the seed was determined. The experiment revealed that a higher inhibition rate (70.64%) as compared to the 40.62% inhibition rate obtained in dual culture at 50 mM salinity, was determined at the same concentration in the seed medium. This finding is consistent with studies conducted on plant-salinity-*T. harzianum*-*Fusarium* spp. interactions (Mohammed ve Haggag, 2006; Boomah et al., 2021; Mishra et al., 2024). In a study conducted by Mohammed and Haggag (2006), it was observed that *T. harzianum* was capable of inhibiting *F. oxysporum* f. sp. *lycopersici* infection in tomato by 56.48% in a 4 mM NaCl concentration. In a similar manner, *F. pseudograminearum* infection in wheat was reduced by 52.78% in 50 mM NaCl concentration (Boomah et al., 2021), and *F. udum* infection in pigeon pea was significantly inhibited at 48 mM NaCl level (Mishra et al., 2024). It has been documented that in the context of pathogen-antagonist interactions occurring in plant parts, fungi compete for nutrients secreted from the plant, with *T. harzianum* prevailing in this competition (Tyśkiewicz et al., 2022). *T. harzianum* has the capacity to saprophytically colonise the seed surface. This growth is hypothesised to be the result of several mechanisms, including competition and mycoparasitism (Brozova, 2004, Güçlü and Özer 2022). It is thought that the *T. harzianum* isolate used in this study provides an advantage over *F. culmorum* in salty conditions in relation to the ability to use the nutrients released during seed germination, thus increasing its capacity to suppress this pathogen.

CONCLUSIONS

The present study demonstrated that the *T. harzianum* isolate used in this work significantly inhibited the mycelial growth of *F. culmorum*, particularly around seeds, under different salinity conditions. The high inhibition rates observed at low and moderate salinity levels indicate that this isolate can remain effective under salt stress. These findings suggest that the applied T. *harzianum* isolate may be a promising biocontrol agent against *F. culmorum*, the causal agent of crown and root rot in wheat, in saline environments. However, further investigations under field conditions and on different crops are required to substantiate these results.

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POLY(4,4"-BIS(2'-FLUORENYLIDENEIMINO)-3,3"-DIMETHYLBIPHENYL): ELECTROSYNTHESIS, CHARACTERIZATION AND APPLICATION IN ORGANIC PHOTOVOLTAIC CELL

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ABSTRACT

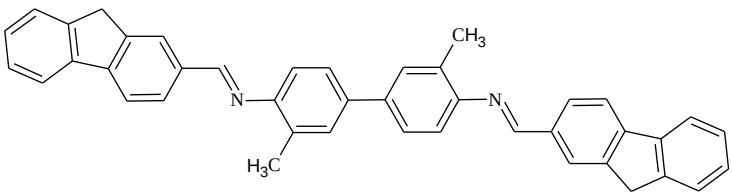
In this study, we report the synthesis and full characterization of a novel o-tolidine-based fluorescent monomer, 4,4″-bis(2′-fluorenylideneimino)-3,3″-dimethylbiphenyl, obtained via Schiff base condensation between o-tolidine and two equivalents of fluorene-2-carbaldehyde. Structural confirmation was achieved through IR and NMR spectroscopies, while optical properties were assessed using UV–Vis and fluorescence spectroscopy, revealing strong absorption in the ultraviolet region and a band gap of 2.58 eV. Electrochemical analysis via cyclic voltammetry showed the monomer has a HOMO energy level of −6.04 eV and a LUMO level of −3.46 eV. Subsequently, the monomer was successfully electropolymerized onto a platinum electrode through cyclic voltammetry, forming a thin, adherent, and electroactive film. The resulting polymeric material displayed enhanced electronic delocalization due to increased π-conjugation. This was confirmed by a significant narrowing of the band gap to 1.80 eV, as well as an upward shift in the HOMO level to −5.41 eV, and in the LUMO level to −3.61 eV.

These results demonstrate that polymerization leads to a reduction in band gap and improved charge transport, consistent with the extension of the conjugated system in the polymer backbone. Considering the electronic properties of the polymer, it is well-suited as a donor material in organic photovoltaic (OPV) applications. Its HOMO/LUMO alignment is favorable for pairing with fullerene-based acceptors such as [6,6]-Phenyl-C_{61}-butyric acid methyl ester (PCBM), which possesses a LUMO level of approximately −4.0 eV. This offset ensures efficient exciton dissociation and electron transfer, satisfying the energy level requirements for high-performance bilayer or bulk heterojunction solar cells. These findings underscore the potential of the synthesized electropolymer as a promising donor material for future development of solution-processed or electrochemically deposited OPV devices.

Keywords: Organic photovoltaic cells; Cyclic voltammetry; HOMO; LUMO; Gap.

INTRODUCTION

Electroactive polymers have gained increasing interest due to their reversible redox properties, their ability to store charge, and their potential applications in sensors, electrochromic devices, supercapacitors, and fuel cells [1–4]. Among these, semiconducting polymers synthesized via electropolymerization on conductive substrates offer the advantage of in situ controlled film growth, enabling precise tuning of thickness and morphology [5,6]. The monomer FLTFL, investigated in this study, exhibits structural features favorable for the formation of a stable, reversible, and electroactive polymer film. To evaluate its electrochemical behavior, a multi-technique approach was adopted: cyclic voltammetry (CV) to monitor oxidation, reduction, and polymerization processes [7], and electrochemical impedance spectroscopy (EIS) to characterize the deposited film [8].



RESULTS AND DISCUSSION

2.1. Oxidation and Reduction Behavior of FLTFL Monomer

Cyclic voltammetry of the FLTFL monomer shows a distinct oxidation wave between +0.6 and +0.9 V, indicating the formation of radical cations that initiate polymerization. A smaller reduction wave appears on the reverse scan, near +0.8V, suggesting a partially reversible redox process, characteristic of an EC mechanism (electrochemical step followed by a chemical reaction). This confirms the monomer's ability to form an electroactive polymer film.

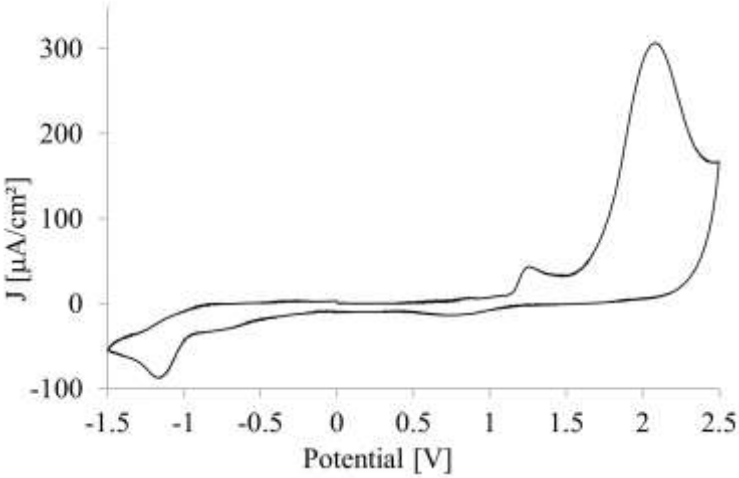


Figure 1. Cyclic voltammogram of the FLTFL monomer.

2.2. Electronic properties of monomer

The tolidine-based azomethines, characterized by their symmetrical structure and dual-point functionalization, provide greater versatility in bandgap modulation and a finer degree of control over frontier orbital energy levels. FLTFL is particularly well-suited for applications such as in bulk heterojunction (BHJ) organic solar cells (Figure 2).

Successive CV scans reveal a progressive increase in oxidation current, indicating continuous growth of the polymer film on the electrode surface. The increasing peak currents and evolving wave shapes are typical of electroactive polymer deposition. The growing current response confirms that the film is adhering and expanding effectively, consistent with a conductive polymer film being formed during each cycle.

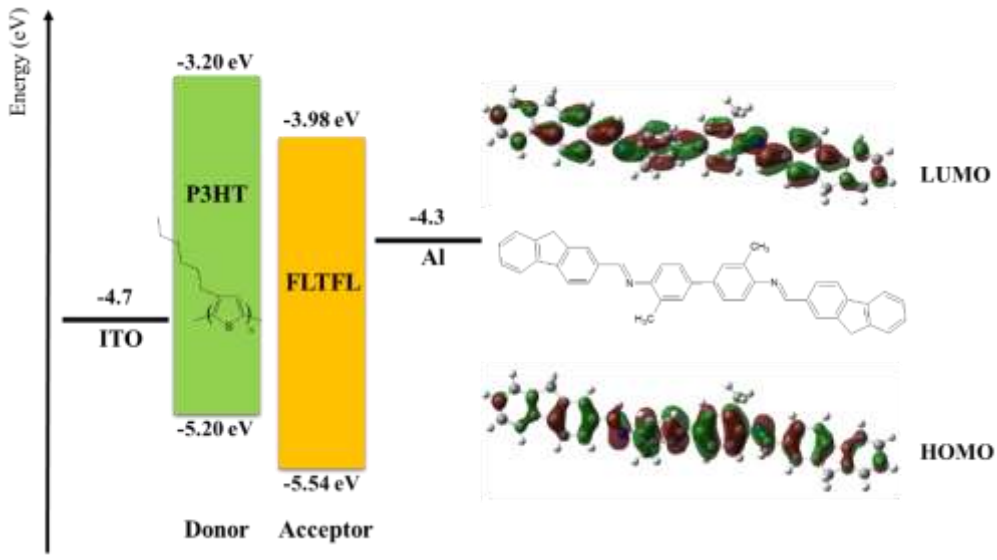


Figure 2. Work functions of electrodes and HOMO and LUMO energy levels of the materials for ITO/poly-3-hexylthiophene/FLTFL/Al device.

2.3. Electropolymerization of FLTFL

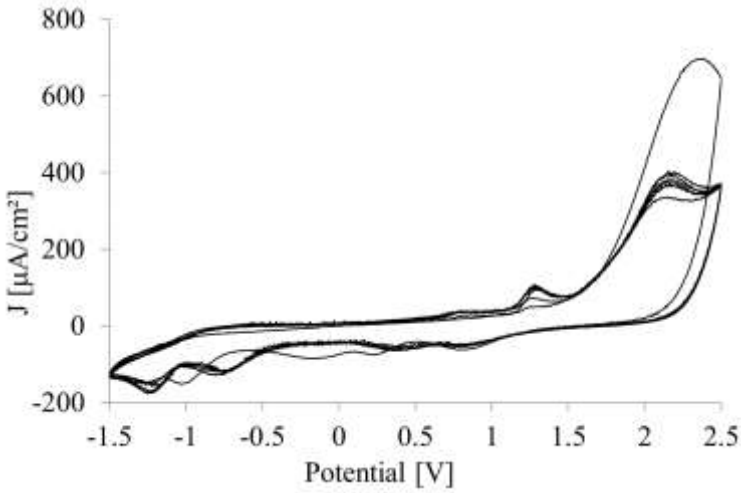


Figure 3. Successive CV scans during FLTFL electropolymerization. The increasing peak current shows continuous growth of the polymer film.

2.4. Electroactivity of Poly(FLTFL)

The redox behavior of the polymer shows a clear oxidation peak and a less intense reduction peak, indicative of partial reversibility. This suggests that the polymer undergoes doping (oxidation) and dedoping (reduction) transitions within a defined potential window, behaving like a typical p-type conducting polymer. The polymer is thus capable of reversible charge exchange, essential for practical electrochemical applications.

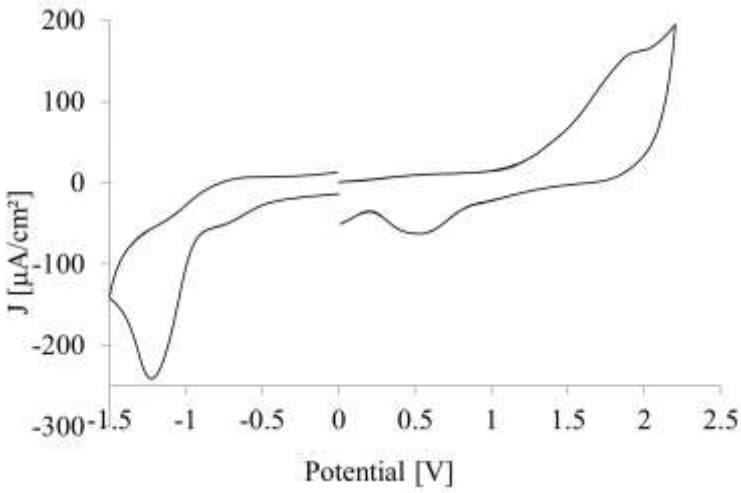


Figure 4. Redox behavior of poly(FLTFL): clear oxidation peak and asymmetric reduction wave, typical of p-dopable materials.

The optoelectronic properties of the polymer derived from the o-tolidine-based fluorinated monomer highlight its strong potential for application in organic photovoltaic (OPV) devices. The significant reduction in band gap after polymerization (from 2.58 eV to 1.80 eV) reflects an enhanced π-electron delocalization, resulting from the extension of the conjugated backbone. This favors light absorption across a broader portion of the solar spectrum. Moreover, the HOMO/LUMO energy levels of the polymer (−5.41 eV / −3.61 eV) exhibit favorable alignment with fullerene-based acceptors, such as [6,6]-Phenyl-C_{61}-butyric acid methyl ester (PCBM), which has a LUMO level around −4.0 eV. This energy offset ensures efficient exciton dissociation and electron transfer, two key factors for optimal solar energy conversion.

Owing to its excellent electrochemical stability, ability to form thin and adherent films via electropolymerization, and intermediate semiconducting properties, the polymer presents itself as a promising donor material for application in bulk heterojunction organic photovoltaic (OPV) cells, high-performance bilayer device architectures, and fully electrodeposited active layers, which allow for simplified and potentially more cost-effective fabrication compared to conventional solution-processed devices. These findings also suggest that the polymer may be suitable for integration into other organic electronic systems, including electrochemical sensors, organic light-emitting diodes (OLEDs), and electrochromic devices, where controlled conductivity and tunable optical responses are essential.

2.5. Electrochemical Impedance Spectroscopy (EIS)

EIS analysis of the polymer-coated electrode displays a semicircular Nyquist plot, indicative of charge transfer control. The data was fitted using a Randles equivalent circuit, yielding the following parameters:

- Rs = 1794.29 Ω·cm² (solution resistance)
- Rct = 11,178.79 Ω·cm² (charge transfer resistance)
- Cdl = 2.18 µF·cm⁻² (double-layer capacitance)

The high Rct reflects the film's low conductivity in its neutral state, while the modest Cdl suggests a compact, low-porosity interface. This behavior is typical of weakly doped semiconducting polymers.

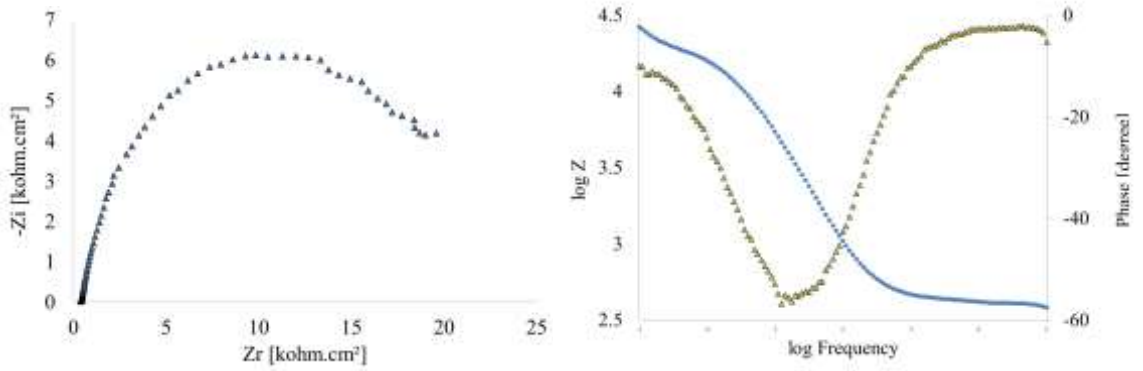


Figure 5. Nyquist and Bode plots (EIS) of poly(FLTFL) on platinum, showing a semicircular arc characteristic of charge transfer control. Fit with Randles model.

Compared to doped polymers like polypyrrole (PPy) or poly(3,4-ethylenedioxythiophene) (PEDOT), which typically show Rct values of 100–500 Ω·cm² and Cdl values around 4–8 μF·cm⁻² [9–11], poly(FLTFL) shows significantly higher resistance and lower capacitance. These results suggest an intermediate behavior: more active than insulating polymers like polythiophene (PTh) [12], yet less conductive than PEDOT. This positions poly(FLTFL) as a promising candidate for applications that require moderate redox activity and good film stability, such as sensors or electrochromic **devices** [13,14].

CONCLUSION

The results demonstrate that FLTFL can be successfully electropolymerized onto a platinum electrode to form a stable and electroactive polymer film. The film exhibits typical p-type redox behavior with significant oxidative activity and minimal reduction response. EIS data confirm a compact and weakly conductive interface in the neutral state, but one that is potentially redox-active under applied potential. These characteristics suggest that poly(FLTFL) is suitable for devices relying on oxidative switching, such as unidirectional redox sensors or asymmetric charge storage systems. Future work could explore doping strategies, co-monomer integration, or alternative potential windows to optimize its performance.

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ASSESSMENT OF RIPARIAN VASCULAR FLORA OF THE RIVER SHKUMBIN NEAR ELBASAN AREA, ALBANIA

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ABSTRACT

The river Shkumbini is one of the most erosive rivers in Albania and it lies in the central part of the country. Our fieldworks were done in the riparian areas from Peqin to Elbasan area, during the last two years. Among vascular plant collected, we identified up to 254 taxa, consisting into 55 plant families, each of them represented by different levels of species richness. The most represented families were: Poaceae (12%), Fabaceae (11%), Lamiaceae (7%), and Brassicaceae (5%), according to their proportional occurrence. The predominant biological forms observed were hemicryptophytes, therophytes, and phanerophytes. Referring to the data collected, the riparian flora of the studied area is predominated by *Populus alba* L. *Salix alba* L., *Salix eleagnos* Scop., *Alnus glutinosa* (L.) Gaertn., *Typha minima* Hoppe, *Carex sp.*, etc. The anthropogenic influence is remarked by the presence of many ruderal species, as well as the presence of grapevine cultivars (*Vitis vinifera L.*), wild cabbage (*Brassica oleracea*), tomato (*Solanum lycopersicum*) and potatoes *Solanum tuberosum* L. as the areas near were agricultural ones.

Key words: Shkumbini river, riparian, flora, taxa, Elbasan

INTRODUCTION

The Shkumbin River basin lies entirely in the central area of Albania, which flow from the eastern slopes of the Valamara and Kamja mountain ranges. The 2445km^2 area of the watershed of the river is characterized by a deep valley in the upper part (Gjeta , 2014).

In the middle course of the river, in that part of the valley that coincides with the Elbasan plain, it begins to widen and the riverbed widens greatly, creating meanders. Shkumbini is one of the most erosive rivers in our country, with an average flow of suspended alluvium of about 187 kg/s (Kabo, 1998; Qirjazi, 2006).

Elbasan region has a **mediterranean climate, hot-summer** with an **average annual temperature** around **15 °C (**Qiriazi, 1998). Under the prevailing climatic and geographic conditions, the riparian floristic diversity of this section is predominantly characterized by woody vegetation, especially shrubs and tree species (Miho *et al*, 2005). Among these, willows (*Salix* sp.), alders (*Alnus* sp.), and poplars (*Populus* sp.) are the most representative taxa, reflecting their ecological adaptation to periodically flooded and moisture-rich habitats typical of riparian ecosystems (Naiman *et al.*, 2005; Tabacchi *et al.*, 2000).

The river waters are widely used in agriculture and industry with numerous irrigation canals built along the river whith two main canals located in our study area that have favored

the development of urban, agricultural and anthropogenic activities are: the Naum-Panxhi and Peqin-Kavajë canals (Pano, 2008).

This study was conducted to establish a general floristic list, with a particular focus on riparian areas of Elbasan (referring to Jagodinë, Murriqan and Shushicë villages) and Peqin (referring to Çengelaj, Paulesh, Broshkë villages) where urban development has had a noticeable impact. During our field trips we identified up to 254 plant taxa, belonging to 55 families, with the highest representation of Poaceae, Compositae, Fabaceae and Lamiaceae families, with respective percentages of 12%, 11%, 11%, 7% .

In relation to the studied areas, we observed a notable association between the floristic elements and their native taxa, indicating that the composition of the riparian vegetation is strongly shaped by the ecological and biogeographical conditions. The percentage of life-forms, follows hemicryptophytes with 22%, therophytes with 26%, and phanerophytes with 29%. The mediterranean elements, including eurimediterranean, submediterranean, and stenomediterranean are clearly distinguished in the chorological spectrum with the highest percentage that of 17%, which is followed by Cosmopolite with 6% and Balkanic 5%.

The study shows a clear predominance of native species, precisely 87%. İn smaller proportions are cultivated and naturalised species with 5% and introduced species with 3%. Along the river, we collected taxa not typically associated with riparian zones such as: *Vitis vinifera L.*, *Brassica oleracea* L., *Lycopersicon esculentum* Mill. and *Solanum tuberosum* L wich shows the anthropogenic influence.

MATERIAL AND METHOD

We collected data in the studied areas from March 2024 to the end of June 2025. The study areas included sites located along the river meanders near the settlements of Karinë, Çengelaj, Paulesh, Broshkë, Jagodinë (Figure 2), Murriqan, and Byshek. Plant samples were collected in growing seasons, date of the collection were recorded for each sample and photographs were taken from different parts of the samples.

Figure 2. Study site along the Shkumbini River near Jagodinë (N $41^0$04' 22.0" / E 20^0 01' 02.5")

The plants were mostly identified directly in the field and the process is followed in the laboratory by using existing valid botanical sources such as: Albanian Flora Vol. I-IV (Paparisto *et al*., 1988; Qosja *et al*., 1992; 1996; Vangjeli *et al*., 2000); European Flora Vol. I-V (Tutin *et al*., 1964; 1968; 1972; 1976; 1980),); for the plant names and other data elaboration was used: Euro+Med 2024+, Chytrý *et al.*, 2024 etc. During fieldwork, the sites were visited and surveyed multiple times to collect additional data, as the areas are subject to ongoing urban disturbance, agricultural activities, and other human influences.

RESULTS AND DISCUSSION

During the study, a total of 254 plant taxa were identified in Elbasan and Peqin area. These taxa belong to 55 plant families, reflecting a high floristic diversity along the river. Some of the recorded plant taxa is presented in the table 1 (Figure 3, 4, 5, 6, 7 and 8).

Table 1. Some of the plant taxa present in the riparian environments of Peqin and Elbasan area

	Plant taxa		**Plant taxa**		**Plant taxa**
1	*Artemisia vulgaris* L	21	*Dorycnium pentaphyllum* Scop	41	*Potentilla reptans* L
2	*Anagallis foemina* Mill	22	*Dactylis glomerata* L	42	*Pteridium aquilinum* (L.) Kuhn in Kersten
3	*Allium cepa* L	23	*Dittrichia viscosa* (L.) Greuter	43	*Sanguisorba officinalis* L
4	*Avena sativa* L	24	*Daucus carota* L	44	*Rubus ulmifolius* Schott
5	*Arenaria serpyllifolia* L	25	*Epilobium hirsutum* L	45	*Ruscus aculeatus* L
6	*Blackstonia perfoliata* (L.) Huds	26	*Eupatorium cannabinum* L	46	*Trifolium repens* L
7	*Bromus arvensis* L	27	*Fraxinus ornus* L	47	*Typha minima* Hoppe
8	*Cercis siliquastrum* L	28	*Helianthus annuus* L	48	*Teucrium chamaedrys* L
9	*Calystegia sepium* (L.) R. Br	29	*Cornus mas* L	49	*Ulmus minor* Mill
10	*Cynoglossum officinale* L	30	*Linaria vulgaris* Mill	50	*Geum urbanum* L
11	*Catapodium rigida* (L.) C. E. Hubb	31	*Leonurus cardiaca* L	51	*Sonchus arvensis* L
12	*Convolvulus arvensis* L	32	*Lolium multiflorum* Lam	52	*Senecio vulgaris* L
13	*Cornus sanguinea* L	33	*Medicago sativa* L	53	*Prunus cocomilia* Ten
14	*Clematis vitalba* L	34	*Melissa officinalis* L	54	*Prunella vulgaris* L
15	*Celtis australis* L	35	*Mentha aquatica* L	55	*Silene vulgaris* (Moench) Garcke
16	*Cirsium arvense* (L.) Scop	36	*Melilotus officinalis* (L.) Lam.	56	*Ranunculus repens* L
17	*Cichorium intybus* L	37	*Oxalis corniculata* L.	57	*Verbascum phlomoides* L
18	*Chenopodium album* L	38	*Plantago media* L	58	*Satureja montana* L
19	*Colutea arborescens* L	39	*Populus nigra* L	59	*Taraxacum* F. H. Wigg
20	*Bellis perennis* L	40	*Poa pratensis* L	60	*Vinca major* L

Figure 3. *Ranunculus repens* L. Figure 4. *Ruscus aculeatus* L. Figure 5. *Oxalis corniculata* L.

Figure 6. *Trifolium repens* L Figure 7. *Arenaria serpyllifolia* L Figure 8.*Vinca major* L

The analysis of the collected data demonstrates that the *Poaceae*, *Compositae*, *Fabaceae*, *Lamiaceae* and *Brassicaceae* are the most prevalent families, highlighting their significant contribution to the floristic composition of the riparian zone (Figure 9). In the graphic below (Figure 9), we grouped under the category 'Others' all families represented by fewer than two taxa in the studied flora, in order to simplify the presentation and highlight the most representative families. The presence of these dominant families indicates that the riparian areas are characterized by high floristic diversity and ecological stability, reflecting vegetation well-adapted to Mediterranean conditions.

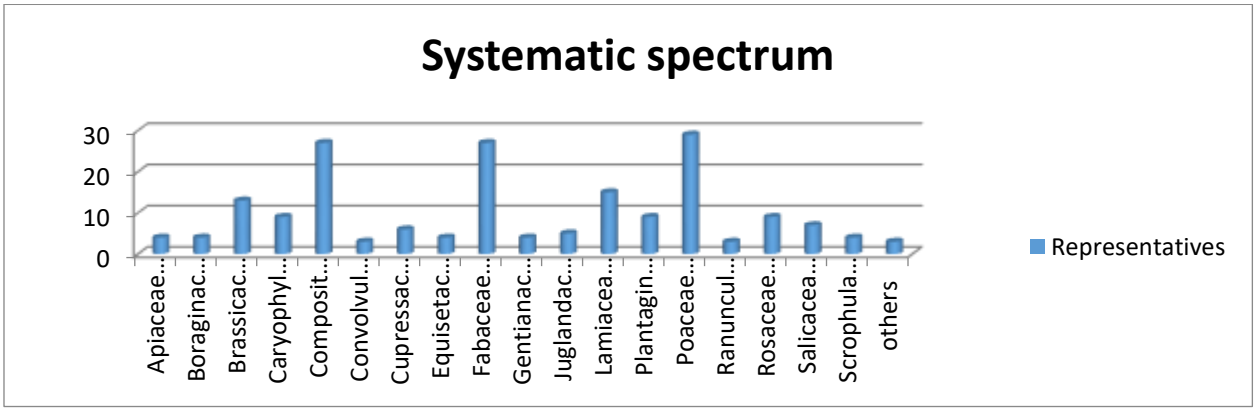

Figure 9.Graphic of systematic spectrum

The chorological spectrum in Figure 10 shows the presence of plants as following: Mediterranean (14%), EuMediteranean (17%), Steno-Mediterrane (5%), Paleotemperuar (11%), Cosmpolite (6%), Eucaucasian (4%), Circumboreal (4%), Balkanic (5%) elements with the respective percentages.

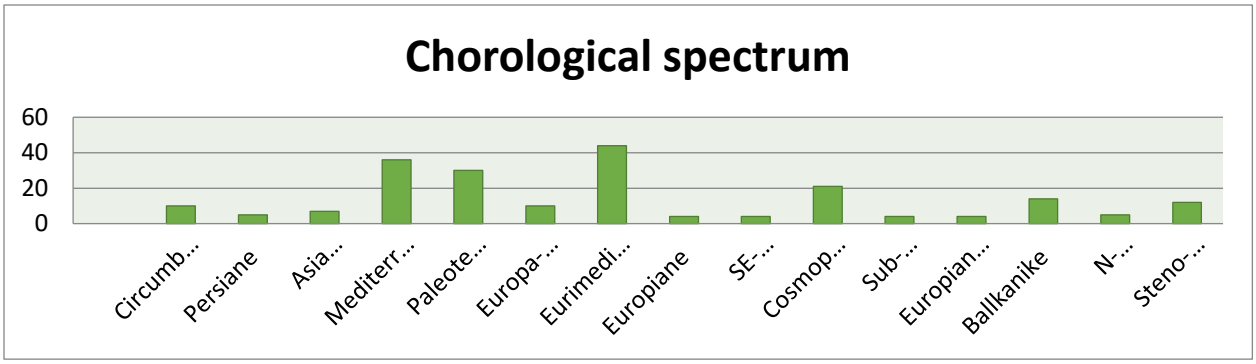

Figure 10. Chorological spectrum of the representative plant taxa

During our expedition, we observed that the areas along the river and between the cities are frequently utilized for anthropogenic purposes, particularly agriculture. According to the collected data, several taxa not typically associated with riparian zones were recorded, their occurrence explained by the influence of river meanders near urban settlements. Species such as: *Vitis vinifera L.*, *Brassica oleracea* L., *Lycopersicon esculentum* Mill. and *Solanum tuberosum* L are referred to as ruderal, and their cultivated areas extend along the river, overlapping with the native riparian vegetation. Within the surveyed sites, the species spectrum is dominated by native taxa (87%), followed by cultivated and naturalised species (5%) and introduced species (2%).

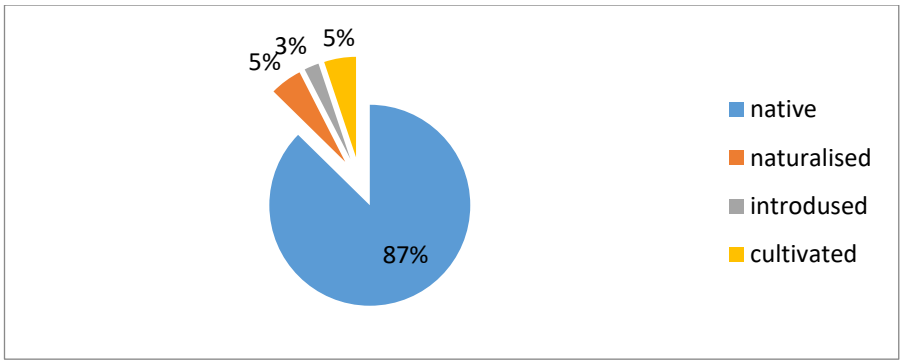

Figure 11. Plant taxa status: native, naturalised, introdused and cultivated

The following figure (Figure 12) illustrates the life-form spectrum of the collected taxa. The life-form spectrum demonstrates how the dominant elements mirror plant adaptation to environmental constraints, particularly those shaped by climate and topography. The most abundant life-forms in these riparian areas are hemicryptophytes, phanerophytes, and therophytes. All other life-form categories are represented to a much lesser extent, as illustrated in the graphic, supporting the conclusion that these areas are predominantly occupied by native riparian plants, with a side influence from anthropogenic activities.

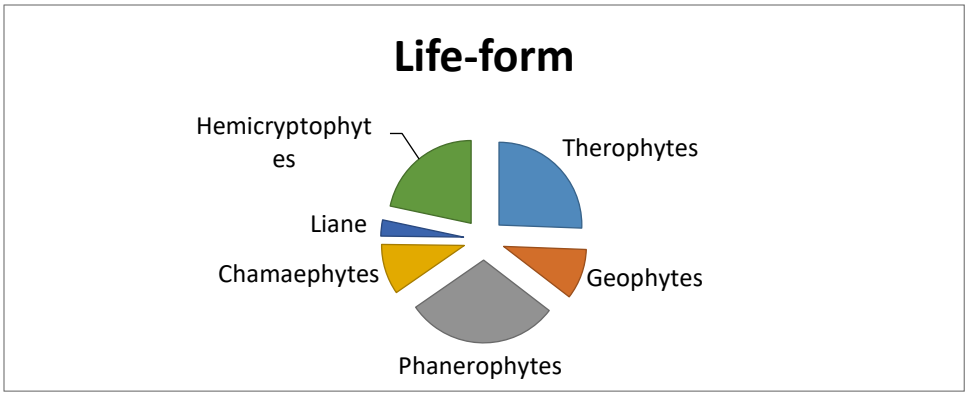

Figure 12. Life form of the collected plants

CONCLUSIONS

The riparian areas along the Shkumbin River represent highly diverse and ecologically dynamic habitats, supporting a wide range of plant taxa. The total plant taxa collected is 254 plants. The most representative families are Poaceae, Compositae, Lamiaceae and Fabaceae, which show a stronger influence on the floristic composition compared to the other families. The most abundant life-forms in these riparian areas are hemicryptophytes, phanerophytes, and therophytes. The presence of these life-formes in riparian areas shows the coexistence of species with different adaptive ability, reflecting the mediterranean climate and the ecological habitats in river banks. To complete the general overview of the collected data and the derived conclusions, we identified the chorological elements wich influence the most, including mediterranean, stenomediterranean, eurimediterranean, cosmopolitan, and paleotemperate elements. The floristic survey recorded predominantly native species (87%), with smaller proportions of cultivated, naturalised and introduced species, reflecting both the natural character of the area and the influence of human activities. Ruderal species and cultivated plants are distributed along the river, intermingling with the native riparian vegetation, which indicates continuous anthropogenic influence, especially from agriculture and urban development. These data will be added to further studies of these environments, extended to different time periods such as summer and autumn, to provide a more complete presentation of them.

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HORTICULTURAL PRACTICES IN POT CULTIVATION OF RHODODENDRON SPECIES: A CASE FROM TURKEY

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ABSTRACT

Rhododendron species, known for their striking flowers and foliage, are widely cultivated in temperate climates and are valued for their ornamental appeal. Pot cultivation of shrub or tree form Rhododendrons offers significant advantages for urban ornamental horticulture, particularly in limited spaces. Despite their aesthetic qualities, successful pot cultivation of Rhododendron species requires careful management due to their preference for acidic soil conditions and sensitivity to environmental stressors. In recent years, pot cultivation of Rhododendrons has gained popularity in Europe, as well as in parts of Asia and North America. However, there is currently no established commercial production in Turkey. This study examines selected horticultural practices related to the pot cultivation of Rhododendron species naturally occurring in Turkey, with a focus on propagation, microbial biostimulant application, and the use of pruning and growth regulators. Additionally, the study highlights the commercial potential of Rhododendrons and their suitability for balcony, terrace, and municipal landscape applications. Recommendations are also provided for future research and commercialization strategies.

Key Words: Woody ornamental plants, Adaptation, Propagation, PGRs, Microbial inoculants

INTRODUCTION

The genus Rhododendron (Ericaceae) represents one of the most species rich groups of woody ornamental plants, comprising 1,091 recognized species and natural hybrids according to the most recent taxonomic revisions (Royal Botanic Gardens, 2025). The native range of the genus extends from the temperate regions of the Northern Hemisphere to the tropical mountain zones of Asia, reaching as far as northeastern Australia (Lin et al., 2019). Within this global diversity, *Rhododendron* species are especially diverse in Asia, with nearly 600 species occurring in China and approximately 320 tropical *Vireya* species distributed across Southeast Asia and northeastern Australia (Chang et al., 2021). In Türkiye, the Eastern Black Sea Region constitutes the main center of diversity for the genus, where five native species *R. ponticum* L., *R. luteum* Sweet, *R. caucasicum* Pall., *R. smirnowii* Trautv., and *R. ungernii* Trautv. are naturally distributed. Alongside these species, four naturally occurring hybrids have also been identified: *R.* × *sochadzeae* (*R. ponticum* × *R. caucasicum*), *R.* × *filidactylis* (*R. ponticum* × *R. ungernii*), *R.* × *rosifaciens* (*R. smirnowii* × *R. ungernii*), and *R.* × *davisianum* (*R. smirnowii* × *R. caucasicum*).

The genus Rhododendron comprises both evergreen and deciduous taxa, with most species growing as shrubs and a few attaining the stature of small trees. They are renowned for their large, showy flowers, which confer high ornamental and economic importance (Altun, 2021). Pot cultivation has attracted increasing attention in urban horticulture, particularly in East Asia, where studies such as those conducted on *Rhododendron* shrub communities in Nanjing highlight the practical use of these species in containerized urban greening (Guo et al., 2022). However, these blooms typically last only a few days to several weeks each year, making foliage the most consistently visible and enduring ornamental trait. Consequently, leaf morphology, color, texture, and variegation play a decisive role in determining the aesthetic and commercial value of cultivars, particularly in pot cultivation where compactness and year-round decorative qualities are highly desirable (Figure 1). Table 1 summarizes Rhododendron cultivars that are particularly valued for their ornamental foliage and their suitability for pot cultivation. Traits such as variegation, indumentum, seasonal color changes, and compact growth habit provide year-round decorative appeal, making these cultivars highly attractive for terrace, balcony, and nursery markets. For example, 'Blattgold' and 'Goldflimmer' are prized for their strong leaf variegation, while 'Hydon Velvet' and 'Silbervelours' are notable for their dense pubescence on the leaf underside, enhancing texture and visual depth. Cultivars such as 'Wine and Roses®' and 'Rusty Dane' add ornamental value through striking young leaf coloration and dense pubescence.

Figure 1. Selected *Rhododendron* cultivars with attractive foliage for pot cultivation (Schröder Rhododendron GmbH & Co. KG, 2023)

Cultivars distinguished by their ornamental foliage hold a central place in global pot cultivation markets: in Western Europe (Belgium, the Netherlands, Germany, and the United Kingdom), they dominate balcony and terrace landscaping; in East Asia (China, Japan, and South Korea), foliage diversity is closely tied to local garden traditions; and in North America (Oregon, Washington, and North Carolina), compact forms with striking leaves are preferred for nursery production. The widespread adoption of such cultivars underscores the global horticultural importance of foliage traits in potted Rhododendron production (RHS, 2023; JARS, 2021; Fine Gardening, 2022) (Figure 2).

Table 1. Rhododendron Cultivars with Ornamental Foliage and Suitability for Pot Cultivation Information compiled from RHS (2023), JARS (2021), Fine Gardening (2022)

Cultivar	Leaf Characteristics	Pot Cultivation Traits
Blattgold	Dark green leaves with golden-yellow variegation.	Compact growth, highly decorative foliage for balcony and terrace pots.
Hydon Velvet	Underside densely covered with cinnamon-brown indumentum.	Compact form, velvety foliage enhances ornamental value in containers.
Goldflimmer	Green leaves with golden-yellow central variegation.	Strong foliage contrast, ideal for ornamental pot cultivation.
Graziella	Narrow, lanceolate glossy green leaves.	Elegant foliage, suitable for modern container displays.
Elizabeth	Young leaves showing reddish-brown tones.	Seasonal color change enhances decorative value in pots.
Silbervelours	Underside covered with silvery hairs, velvety appearance.	Unique foliage texture, attractive in pot displays.
Queen Bee	Rusty-brown tomentum on the underside.	Compact growth habit suitable for limited-space container cultivation.
Wine and Roses®	Young leaves reddish-purple, strong ornamental contrast.	High visual appeal in pot culture due to leaf color contrast.
Rh. roxieanum var. *oreonastes*	Needle-like narrow leaves with distinctive venation.	Exotic foliage form, suited for collectors' container displays.
Rh. taliense 'Honigduft'	Underside densely hairy with pale indumentum.	High ornamental foliage value, suitable for pot cultivation.
Rosa Perle	Small glossy green leaves, compact growth.	Compact form, ideal for small decorative pots.
Rusty Dane	Rusty-brown indumentum on the underside.	Distinctive leaf texture, strong ornamental potential in pots.

Figure 2. Global pot cultivation areas of *Rhododendron* spp. green shading indicate the main production centers in Europe (Belgium, Netherlands, Germany, United Kingdom, Poland, Czech Republic), orange shading indicates Asia (China, Japan, South Korea), and purple shading indicates North America (USA: Oregon, Washington, North Carolina). The map was prepared by the authors based on horticultural production reports.

Building upon the general information presented above regarding Rhododendron species woody plants exhibiting shrub or tree forms the subsequent sections explore the cultivation requirements for container-grown plants, key production challenges, and emerging approaches. The overall production potential is assessed through a combination of theoretical insights and practical experiences, with a particular emphasis on applications conducted in Türkiye.

1.1. Propagation and Rooting

1.1.1. The Role of Plant Growth Regulators (PGRs)

Propagation techniques are particularly critical in pot production, where uniformity, rooting efficiency, and plant quality determine commercial success. Likewise, cutting experiments showed that genotype, auxin type, and substrate composition strongly affect adventitious rooting, with IBA applied in peat–perlite substrates producing the best results across several cultivars (Ferus et al., 2017). Plant growth regulators (PGRs) further enhance production efficiency. Paclobutrazol reduces shoot elongation, improves compactness, and stimulates branching (Ranney et al., 1994), while thidiazuron (TDZ) and naphthaleneacetic acid (NAA) have been effective in in vitro regeneration systems (Wu et al., 2023). Recent studies reported that NAA was superior to IBA for rooting success in *R. micranthum* cuttings (Oh et al., 2023), whereas IBA remained more effective than IAA for adventitious rooting in *R. fortunei* (Wei et al., 2018). TDZ combined with optimized LED lighting improved microshoot proliferation and metabolite accumulation in *R. mucronulatum* (Zaytseva et al., 2023). Air-layering experiments on Turkish native species (*R. ponticum, R. luteum, R. ungernii, R. caucasicum*) demonstrated that both indole-3-butyric acid (IBA) concentration (0, 500, 1500 ppm) and season significantly affected rooting parameters, including root length, volume, diameter, and dry weight. The highest rooting efficiency (100%) was obtained for *R. ungernii* and *R. caucasicum*, particularly in autumn with 1,500 ppm IBA (Altun, 2023) (Table 2).

1.1.2. The Role of Microbial Inoculants

Alongside PGRs, beneficial microorganisms are increasingly recognized as sustainable tools in containerized rhododendron production. Ericoid mycorrhizal fungi (*Oidiodendron maius*) accelerate rooting and nutrient uptake in *R. fortunei* and *R. ovatum* (Wei et al., 2016, 2020). Long-term inoculation studies further improved N, P, and Ca accumulation in containerized cultivars (*R. hybridium* ‘Anneke’) (Jarosz, 2021). Endophytic bacteria such as *Pseudomonas lurida* and *Bacillus velezensis* produce IAA and GA, increase phosphorus solubilization, and suppress fungal pathogens, underscoring their dual role as PGPR and biocontrol agents (Biswas et al., 2024). Similarly, bio-based fertilizers enhanced soil microbial activity and growth in potted azaleas (*R. simsii*) (Teng et al., 2024) (Table 2).

Table 2. Effects of PGRs and Microbial Inoculants on Rooting and Propagation of Rhododendrons in Pots

Topic	Key Findings	Cultivar / Material	Reference
In vitro regeneration & PGR optimization (TDZ+NAA)	High shoot proliferation and rooting achieved with TDZ+NAA; efficient protocol.	*R. decorum* (flower bud explants)	Wu et al., 2023, Horticulturae
Auxin effects on cutting propagation	NAA (150–250 mg/L) superior to IBA in rooting %, root traits, survival.	*R. micranthum* (stem cuttings)	Oh et al., 2023, Horticulturae
Microshoot rooting response to auxins	IBA more effective than IAA for adventitious rooting in vitro.	*R. fortunei*	Wei et al., 2018, Industrial Crops & Products
TDZ + LED effect on microshoot culture	0.5 µM TDZ + LED increased shoot proliferation and biomass; 0.1 µM TDZ + LED enhanced accumulation of taxifolin, rutin, and other phenolics in *R. mucronulatum* microshoots	*R. mucronulatum*	Zaytseva et al., 2023, J. Plant Growth Regulation
Effect of ErM (Oidiodendron maius)	Accelerated rooting; IAA/Trp pathways activated.	*R. fortunei* (microcuttings)	Wei et al., 2020, Horticulture Research
Nutritional contribution of ErM (3 years)	Increased N, P, Ca uptake in container culture.	*R.hybridium* 'Anneke'	Jarosz, 2021, Agriculture
PGPR endophytes	Pseudomonas & Bacillus isolates produced IAA/GA, showed antifungal activity.	*R. griffithianum, R. arboreum*	Biswas et al., 2024, Current Microbiology
Microbial fertilizer	Improved soil biology and plant performance.	Azalea 'Carnation'	Teng et al., 2024, Frontiers in Microbiology
Plant Growth Regulators (Paclobutrazol)	Paclobutrazol reduces shoot elongation, ensures compact form; promotes flowering.	Potted *Rhododendron* spp.	Ranney et al., 1994
Air-layering + IBA	IBA (0–1500 ppm) and season significantly influenced rooting.	*R. ponticum, R. luteum, R. ungernii, R. caucasicum*	Altun, 2023, BioResources
Auxin/substrate & genotype effects	IBA + peat/perlite substrate → higher rooting success depending on genotype/time.	Various *Rhododendron* spp.	Ferus et al., 2017, J. Applied Horticulture

1.2. Cultivation Practices

Successful propagation and cultivation of *rhododendrons* in pots requires specialized practices, as these species are highly sensitive to substrate pH; neutral to alkaline conditions often impair root development and overall growth performance. These plants inherently thrive in acidic soils typically within a pH range of 5.0 to 6.0 but exhibit significant stress responses when grown in neutral to slightly alkaline conditions (Turner et al., 2020). High pH

environments adversely affect root morphology and limit nutrient uptake, leading to reduced growth, chlorosis, and lower survival rates. Greenhouse studies reinforce these findings, showing that neutral pH substrates (e.g., pH 6.3) result in stunted root and shoot growth, chlorophyll reduction, and impaired overall health in *R. fortunei* hybrids (Wang et al., 2023). Water management strongly affects pot production. Koniarski and Matysiak (2013) showed that regulated deficit irrigation (RDI) at 75% ETp produced the highest visual quality, while moderate deficits enhanced bud formation and compactness without negatively affecting photosynthesis. This suggests that controlled irrigation can serve as a practical alternative to pruning and PGRs while conserving water. French and Alsbury (1986) reported progress on forcing *rhododendron*s as pot plants under controlled greenhouse conditions. Unlike earlier work that relied on supplementary lighting and growth regulators, their approach utilized naturally budded plants grown over two seasons, followed by forcing at higher temperatures (21/18 °C, day/night). This method significantly shortened the forcing period, allowing flowering up to three weeks earlier than previous trials, with plants blooming in time for Valentine's Day. The study highlighted the potential of producing high-quality potted *rhododendron*s with flowers on nearly every shoot, while reducing reliance on growth regulators and heated greenhouse space, thereby offering a commercially viable production strategy (Table 3).

Table3. Academic Studies on Rhododendron Cultivation in Pots

Topic	Key Findings	Cultivar/Material	Reference
Irrigation (RDI)	Controlled water deficit (50–75% ETp) makes plants more compact and aesthetic; can enhance flowering; saves water.	'Catawbiense Boursault', 'Old Port'	Koniarski & Matysiak, 2013
Substrate and pH management	Acidic medium (pH 4.5–5.8) is suitable for growth and bud set; high pH may cause Fe chlorosis.	Various *Rhododendron* species	Turner et al., 2020; Wang et al., 2023
Photoperiod / Forcing	Naturally budded plants forced in greenhouse flowered 3 weeks earlier.	*R. simsii*, other hybrids	French & Alsbury, 1986

1.3. Recent Advances in Propagation

In recent years, grafting has emerged as an alternative propagation technique for potted Rhododendron production, particularly in commercial nurseries. As shown in Figure 3, selected cultivars (e.g., 'Alfred') are grafted onto vigorous rootstocks such as 'Cunningham's White'.

Figure 3. Sequential steps of grafting-based propagation of *Rhododendron*s, including scion and rootstock preparation, tying, rooting, and transplanting into nursery beds (Schröder Rhododendron GmbH & Co. KG, 2023)

After the graft union is secured, the young plants are transferred to rooting beds where initial root formation occurs, followed by transplantation into pots under controlled nursery

conditions. Compared with conventional cutting propagation, grafting offers higher uniformity, improved stress tolerance, and greater adaptation to intensive production systems, making it an increasingly preferred method in modern horticultural practice.

1.4. Pot Cultivation Research in Northeastern Türkiye

In Türkiye, native rhododendrons are among the most characteristic elements of the Eastern Black Sea flora; however, no studies have so far focused on their propagation and pot cultivation. To address this gap, a BAP-supported project was initiated with the aim of developing preliminary protocols for the containerized production of native species under controlled conditions. Within the scope of the project, plant materials of five native species (*R. ponticum* L., *R. luteum* Sweet, *R. caucasicum* Pall., *R. ungernii* Trautv., and *R. smirnowii* Trautv.) were collected from natural populations in Artvin, Rize, and Trabzon provinces during the spring and autumn seasons of 2023–2024.

To overcome the well-known challenges in rhododendron propagation, the project combines both ex vitro and in vitro approaches. In ex vitro trials, shoot cuttings were subjected to different rooting strategies, including auxin (IBA), mycorrhiza, rhizobacteria, and their combinations, and then planted in peat–perlite mixtures under greenhouse conditions. In vitro micropropagation experiments were conducted using shoot tip explants, with various sterilization protocols evaluated to minimize contamination. In addition, culture media supplemented with BAP, 2-iP, and IBA are being tested for their effects on shoot initiation, multiplication, and rooting. Furthermore, paclobutrazol applications are being carried out on rooted cuttings to obtain compact plants suitable for pot production. Different doses and application methods (dipping, soil drench, foliar spray; with and without pinching) are being evaluated in terms of their effectiveness in controlling plant height, increasing leaf number, thickening stem diameter, and promoting compact plant form.

This project represents the first research in Türkiye to investigate the pot cultivation potential of native rhododendrons. Although the trials are still ongoing, the study is expected to provide a foundation for future conservation-oriented research and to create new opportunities for the ornamental plants sector.

CONCLUSIONS

Rhododendrons are a group of woody ornamentals of high ecological significance and remarkable ornamental value. In Türkiye, particularly in the Eastern Black Sea Region, the natural diversity of these species represents both a genetic resource to be conserved and an underutilized potential for the ornamental plant sector. However, their commercial-scale pot production has remained limited due to rooting difficulties, susceptibility to abiotic stresses, and the absence of standardized cultivation practices.

The existing body of research provides valuable insights into propagation techniques, growing media, irrigation and fertilization management, and the role of growth regulators. Nevertheless, most of these studies have focused on a limited number of taxa or specific cultivation aspects, leaving major gaps in comprehensive protocols that could support commercial production. In recent years, the use of microbial biostimulants, growth regulators such as paclobutrazol, and advances in in vitro culture systems have emerged as promising strategies to overcome these challenges.

In this context, the ongoing BAP-supported project in Türkiye represents a systematic attempt to develop pot cultivation protocols for native rhododendrons by integrating ex vitro

cutting propagation, in vitro micropropagation, and paclobutrazol-based growth regulation. Although the trials are still in progress, this work provides an important foundation for future studies.

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EVALUATION OF THE ANTIOXIDANT ACTIVITY OF DAUCUS CAROTA L. ESSENTIAL OIL FROM EL-TAREF -ALGERIA

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ABSTRACT

This study is designed to promote the Algerian sector specializing in the production of essential oils by highlighting the pharmacological potential of certain essential oils, including *Daucus carota* L., produced by a unit located in the wilaya of El Taref (El Kala region) called Flor' Est. The antioxidant activity was assessed using the DPPH free radical method. Free radical scavenging activity was measured by mixing 100 µL of each of the methanolic solutions of the tested essential oils at different concentrations with 1300 µL of a methanolic solution of DPPH (0.004%). After a 30-minute incubation period at room temperature, the absorbance was read at 517 nm. The inhibition of the DPPH free radical by ascorbic acid was also analyzed for comparison.

Our results show that the antioxidant activity increased with increasing concentrations. It seems that vitamin C (ascorbic acid) is the most effective and powerful antioxidant than the essential oil studied with a value of 0.0056 mg/ml. The IC50 of *Daucus carota* L. equal to 14.11 mg/ml. The search for active molecules with low side effects is essential. Such molecules are present in plants that sometimes constitute the only source of treatment for poor populations. This work has revealed the importance of *Daucus carota* L. species, which deserve an honorable place in Algerian medicine.

Keywords: *Daucus carota* L., Essential oil, Antioxidant activity.

INTRODUCTION

Aromatic plants are promising and constitute a great source of natural antioxidants for the food industry. Indeed, the oxidation of lipids in food products not only induces a reduction in the nutritional value of the food, but also has effects recognized as harmful for the consumer and which can be associated with risks of century diseases in humans. The negative effects of synthetic antioxidants encourage their replacement by natural agents.

The antioxidant phytochemicals from plants, particularly essential oil components, flavonoids, and other polyphenols, have been reported to inhibit the propagation of free radical reactions, to protect the human body from disease (Kinsella et al., 1993 ; Terao et al., 1997).

Daucus is a worldwide genus of herbaceous plants of the family Apiaceae of which the best-known species is the cultivated carrot. The genus comprises about 20 species divided into five sections (Saenz et al., 2003).

The genus *Daucus* appears to have its center of dispersion in the Mediterranean Region, particularly in North Africa, where strong speciation has taken place. Except the common carrot *D. carota* L., which is cultivated throughout the world, there is one Australian species, naturalized also in Europe and three other species in the American continent; all others are

Mediterranean species (Saenz et al., 2003). *Daucus carota* has been subject to several investigations (Flamini et al., 2003).

Traditionally, Carrot Essential Oil "CEO" is use to treat urinary calculus, cystitis, gout, and lithuria (Thomas et al., 2001). CEO also has anti-tumor, anti-cancer properties, and antioxidant activity against a wide range of malignancies and diseases (Tawil et al., 2015). Many the medicinal properties are claimed for Iran's wild *D. carota,* characterized with nephroprotective, wound healing, and aphrodisiac activity (Bahrami et al., 2018).

Previous studies dealing with members of this genus revealed that the main constituents were: sabinene with monoterpene hydrocarbons for *D. carota* ssp. *gummifer* from Spain, whereas α-pinene with monoterpene hydrocarbons predominated in *D. carota* ssp. *carota* from Poland. However, monoterpene hydrocarbons (α -pinene, limonene, and sabinene) were dominating in *D. muricatus* and *D. sahariensis* from Algeria (Zaibet et al., 2015).

The chemical composition of the essential oils of *D. aureus* from Western Algeria has also been previously reported (Meliani et al., 2013). The oils were characterized by the presence as main components of sesquiterpene hydrocarbons (54.3%) in the aerial parts (Table 1).

Table 1. Percentage major components, in essential oil, of *D. aureus* growing in Western Algeria (Meliani et al., 2013)

Populations*	Group 1							Group 2						
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14
β-pinene	1.5	1.1	0.9	0.6	0.5	1.2	2.7	2.9	2.8	2.7	2.1	2.2	1.5	1.6
Myrcene	2.6	2.8	1.2	2	2.2	2.6	2.2	2.4	2.3	2.1	2.6	2.7	1.2	1.7
(E)-Decenal	0.9	0.2	0.6	0.5	1.2	1.1	1.5	1.6	1.2	1.1	2.2	1.7	1.5	1.4
Germacrene D	56.7	67.2	66.1	65.4	58.9	44.5	39.5	18.2	17.9	18.9	11.3	17.5	15.4	17.9
7-epi-α-selinene	0.9	0.6	1.2	0.9	1.7	1.4	1.9	2.6	2.7	2.6	2.9	2.5	1.9	2.5
Germacrene B	1.1	0.5	0.9	0.7	1.1	2.1	2.2	2.5	2.7	2.9	2.8	2.7	2.8	2.5
Spathulenol	1.3	0.6	0.8	1	1.2	2	2.1	5.2	5.7	5.6	7.7	6.2	8.4	8.4
Caryophylene-oxyde	1.2	1.9	0.9	1.1	1.8	3.2	4.4	7.3	8.3	7.3	8.3	8.9	8.6	9.5
Globulol	0.8	0.3	0.2	0.5	0.8	1.3	0.8	2.3	3.2	3.8	3.4	2.6	3.6	2.4
Viridiflorol	1.2	1.1	0.9	0.9	1.7	1.6	2.3	4.5	5.5	5.8	4.2	3.5	4.7	3.2
Guaiol	0.7	0.3	0.2	0.5	0.4	1.1	1.2	1.3	2.1	1.9	1.4	1.2	2.3	2.5
Copaborneol	1.2	0.3	0.6	0.3	0.6	1.6	0.8	1.1	2.6	2.7	2.7	1.1	2.3	1.2
Cadin-4-en-7-ol	1.2	1.7	1.6	1.5	1.2	1.6	2.9	4.1	4.5	4.7	4.3	5.5	5.6	4.4
β-Cadinol	2.5	1.2	1.5	2.2	2.2	1.5	1.9	3.9	2.3	4.2	4.6	5.2	4.3	4
Bulnesol	0.7	0.2	0.1	0.3	0.5	0.9	1.1	1.5	1.6	1.9	1.6	1.6	1.1	1.7
(Z)-α-Santanol	1.4	2.1	2.2	1.5	1.4	2.8	2.7	6.5	5.1	5.6	4.3	5.1	4.9	4.7

* S1= Saf Saf; S2= Chetouane; S3= Ain Fezza; S4= Laourit; S5= Mansourah; S6= Zarifet; S7= Khemis; S8= El Emir Abdelkader; S9= Ghazaouet; S10= Sidi youchaa; S11= Beni Saf; S12= Oulhaça; S13= Rachgoun; S14= Oulad Benayed (B'hira).

The aim of our study is to promote the Algerian sector specializing in the production of essential oils by highlighting the pharmacological potential of certain essential oils, including *Daucus carota* L., produced by a unit located in the wilaya of El Taref (El Kala region) called Flor'Est.

MATERIAL AND METHOD

Plant material

The species *Daucus carota* L. was harvested in the region of El Taref (northe astern Algeria) in April 2023. The identification of the plant was done with the key to determining the flora of Quezel and Santa (Quezel et al., 1963). Specimens were kept at the Laboratory Medical Botany, Department of Pharmacy, Faculty of Medicine Annaba-Algeria.

Essential oil

Essential oil of *Daucus carota* L. has been procured from production unit of essential and vegetable oils "Elexir Est" Algeria.

Determination of antioxidant activity with 2,2-di-phenyl-1-picrylhydrazyl (DPPH) method

Free radical scavenging ability of essantial oil was measured from the bleaching of a purple-coloured methanol solution of DPPH. Based on a pre-examination, ten concentrations are used. The DPPH solution was prepared by solubilizing 0.4 mg of DPPH in 100 ml of methanol. Briefly,1300 µL of a 0,4 mg/100ml solution of DPPH radical in methanol was added to 100 µL of essential oil at different concentrations. The mixture was shaken vigorously for 1min by vortexing and leftto stand at room temperature in the dark for 30min. Absorbances were measured at 517nm with a spectrophotometer against a blank. Ascorbic acid wasused for comparison as standard antioxidants. The percentage inhibition of activity was calculated as :

$$\%Inhibition=[(Ablank-Asample)/Ablank]\times 100.$$

Where Ablank is the absorbance of the controland Asample is the absorbance of the test extract.IC50 value (mg/mL) is the concentration of the sample scavenging 50% of the DPPH radical (Ounaissia et al., 2021).

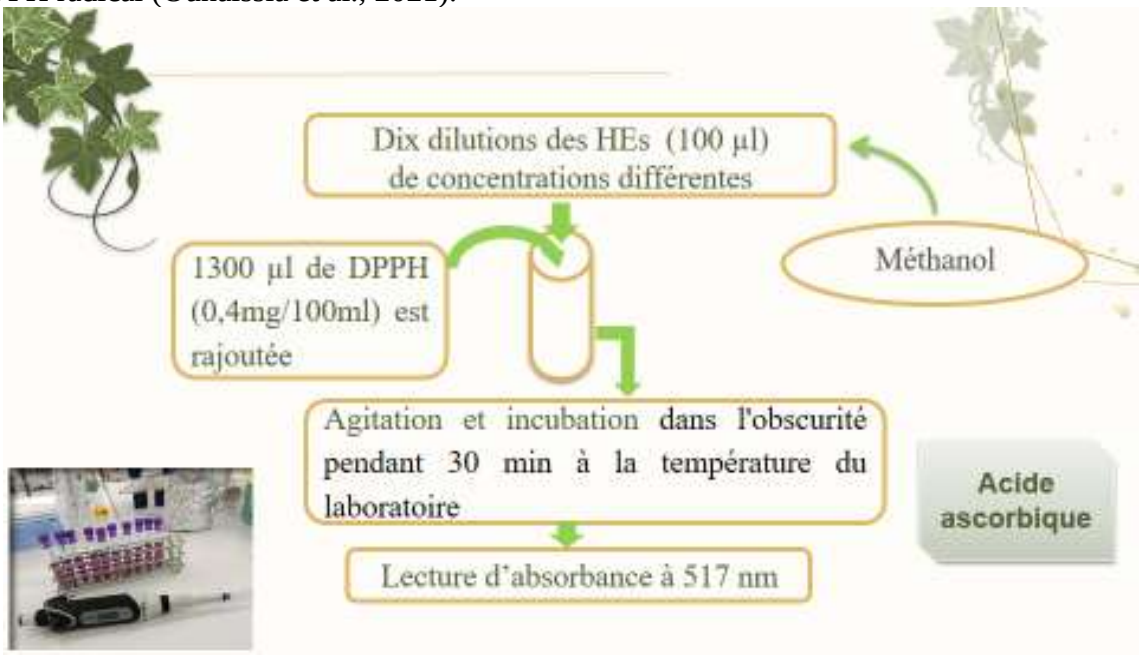

Figure 1. Operating protocol

RESULTS AND DISCUSSION

The results of the antioxydant activity are shown in Figure 2.

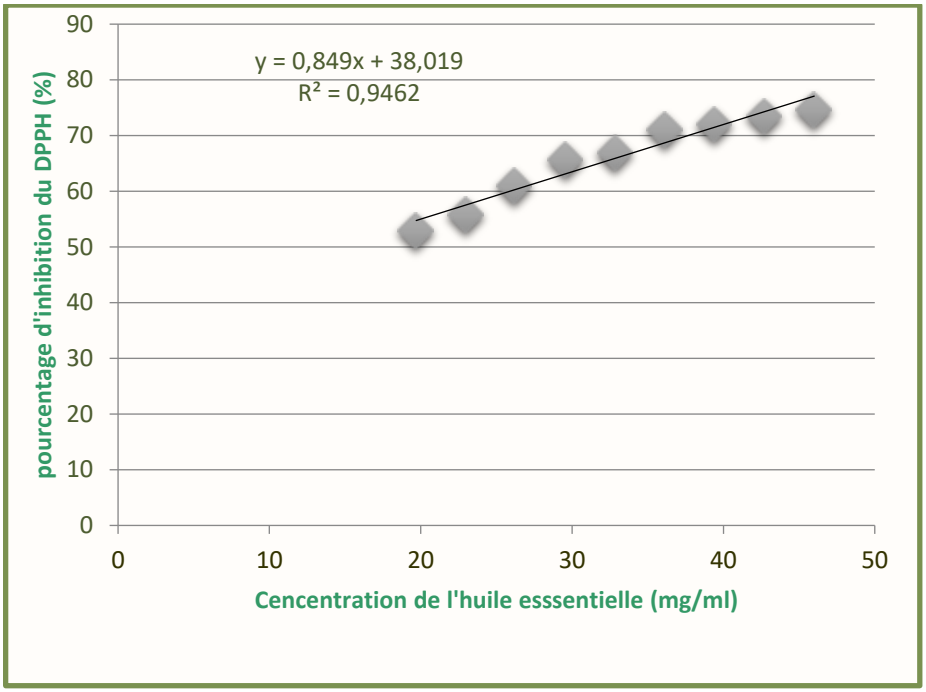

Figure 2. Percentage of DPPH free radical scavenging by *Daucus carota* L. essential oil

The results obtained by our study carried out on the species of *Daucus carota* L., are divergent from those of (Ihamdane et al., 2021) found a value of IC50 = 0.073 mg/ml. In our study, it was found that the radical scavenging activity expressed by the IC50 value was 14.11 mg/ml. This value is better than the results obtained in another similar study, which found an IC50 value of 30.5 mg/ml. (Sbai el otmani et al., 2018). Our results are in agreement with those of (Zafar et al., 2021) who affirm the antioxidant activity of *Daucus carota* L. essential oil, with an inhibition rate of 74% (74.61% in our study). In another study on the chemical composition and antioxidant activity of Essential Oil and methanolic extract of Algerian Wild Carrot *Daucus carota* L. subsp, the results showed that Wild Carrot Essential Oil has significant antioxidant activity (Ksouri et al., 2014).

Lebanon wild carrot essential oil extract from the dried mature umbels exhibited antioxidant activity in all the assays (DPPH, FIC, and FRAP assays). The IC_{50} value for the DPPH assay was determined to be 2.1 mg/mL. Nevertheless, it was a weaker antioxidant in comparison to Trolox (IC_{50} value of 0.18 mg/mL) (Shebaby et al., 2013). carrot essential oil from Lebanon wild plantsis characterized by significant antioxidant properties (DPPH activity, IC_{50} = 0.29 mg/mL) (Shebaby et al., 2015).

The carrot essential oil from Algerian carrot leaves and seeds were found to be effective antioxidants in several in vitro assays. The carrot leaf extract provided the highest radical-scavenging activity, with lower IC_{50} values (83 mg/mL) than seeds (136 mg/mL). Theantioxidant activity of the CEO was lower than that of the synthetic antioxidant ascorbic

acid (IC_{50} value of 3.73µg/mL) (Ihamdane 2021) .The tested EOs from Moroccan *D. carota* demonstrated a low antioxidant potential (IC_{50} = 73.31 mg/mL) (Ihamdane et al., 2021).

The essential oil from Oum Elbouaghi (Algeria) revealed the lowest antioxidant activity, IC_{50}= 970±0.53 µg/ml; the values for the Setif and Constantine sites were 713 ± 0.047 and 783 ± 0.16 µg/ml, respectively.

This variation in antioxydant activity can be attributed to the chemical composition of the essential oils. It has been reported that components with phenolic structures such as thymol or carvacrol, were responsible for the antioxidant activity of many essential oils and a scant antioxidant activity is given to monoterpene and sesquiterpene hydrocarbons (Teixeira et al., 2013 ; Rubberto et al., 2000).

Other factors, mainly related to the species, may interact: the season and location of the plant harvest, the part distilled, and the extraction protocol for these secondary metabolites.

CONCLUSIONS

Plant extracts have great potential as antioxydant compounds. Thus, they can be proposed as a natural additive in food industry to increase the shelf life of food stuffs and in pharmaceutical industries.The results of this study suggest that *Daucus carota* L. essential oil possesses antioxydant propertie.

This result suggests that the essential oil from *Daucus carota* have a potential use in the food industry as food preservative. Nevertheless, further studies are needed to explore other bioactivities of *D. carota* essential oil and to elucidate also the composition of non-volatile compounds.

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RECENT ADVANCES IN ALMOND MICROPROPAGATION AND FUTURE PERSPECTIVES

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ABSTRACT

Almond is a major nut crop cultivated extensively worldwide due to its high nutritional value and significant economic importance. However, conventional propagation methods have limitations, such as slow production rates, low rooting efficiency, and high risk of pathogen transmission. Micropropagation is an effective biotechnological tool that enables the rapid production of genetically uniform, disease-free, and high-quality plant material. This review examines the protocols reported in the literature on the micropropagation of almond and almond × peach hybrid genotypes. Explant types, medium composition, plant growth regulators, sterilization procedures, and culture conditions are comprehensively evaluated for their effects on propagation success. Evidence indicate that cytokinins (BA, TDZ) and auxins (IBA, NAA) play critical roles in shoot proliferation and rooting, although success rates vary considerably among genotypes. Additionally, light regimes, carbohydrate sources, and hormone optimization have been identified as crucial determinants of propagation efficiency. This review highlights the need for standardized, genotype-specific, and highly efficient protocols and is expected to guide future micropropagation studies on almond and its hybrids.

Keywords: Almond, auxins, cytokinins, **genotypic effect**, micropropagation, plant growth regulators

INTRODUCTION

Almond (*Prunus dulcis* Mill.), belonging to the subgenus Amygdalus of the genus Prunus (Rosaceae), is commercially cultivated worldwide (Madam et al., 2011). It is believed to have originated in the arid mountainous regions of Central Asia (Ladizinsky, 1999). Almond, one of the oldest domesticated fruit tree species, was first cultivated nearly 4,000 years ago in Iran, Türkiye, Syria, and Palestine, from where it spread to Greece, North Africa, and Italy, and later was introduced to North America by early settlers (Kester & Asay, 1975). Almonds are grown economically between latitudes 30–44° in the Northern Hemisphere and 20–40° in the Southern Hemisphere, at altitudes of 600–1000 m (Özbek, 1978).

Breeding programs in perennial fruit trees require decades to accomplish due to the long juvenile phase and high labor intensity. Such efforts are highly demanding, continuous, and costly. Considering the juvenile sterility of fruit trees, breeding studies in horticultural crops often take 20–25 years and require large experimental areas. Thus, the use of biotechnological approaches has become a necessity rather than a choice (Balık, 2024). Micropropagation, allowing rapid clonal multiplication under controlled conditions, is recognized as a

breakthrough technique in horticultural plant production. The method involves culturing plant cells or tissues under sterile and nutrient-rich media. The main advantages of micropropagation include year-round rapid propagation of cultivars, elimination of diseases, maintenance of genetic stability, suitability for large-scale commercial production in limited space, and conservation of rare genotypes. Moreover, the ability to manipulate growth conditions enhances propagation efficiency. Therefore, micropropagation emerges as a strategic biotechnological approach for meeting global demand for high-quality plant materials (Meena et al., 2024).

The aim of this review is to analyze research studies on the micropropagation of almond and almond × peach hybrids reported in the literature. Different protocols, medium compositions, plant growth regulators, and genotypic influences on micropropagation efficiency are discussed. Furthermore, recent advances in this field are compiled to provide a comprehensive perspective that can guide both researchers and practitioners.

STUDİES ON THE MİCROPROPAGATİON OF ALMOND AND ALMOND × PEACH HYBRİD GENOTYPES

Rugini and Verma (1983) developed an effective *in vitro* protocol to achieve rapid clonal multiplication from the difficult-to-root almond cultivar 'Ferragnes'. Shoot tips measuring 0.4–0.7 mm were used, and ~55% of the multiplied shoots were successfully rooted *in vitro* and transferred to soil. At initiation, shoots were maintained for 3–4 consecutive subcultures on a medium promoting slow growth, then transferred to Murashige and Skoog (MS) medium (0.9% agar, 0.7 mg L^{-1} 6-benzylaminopurine, BAP; 0.01 mg L^{-1} α-naphthaleneacetic acid, NAA). Under these conditions, up to sixfold proliferation per shoot was obtained at 20-day subculture intervals and sustained for at least 24 months. Shoot elongation was promoted by reducing BAP to 0.2 mg L^{-1} and omitting NAA. Shoots were divided into microcuttings with 2–3 leaves for rooting trials. Rooting was achieved on Bourgin and Nitsch (BN) medium with macronutrients halved, solidified with 0.9% agar and supplemented with either 1 mg L^{-1} NAA or 1 mg L^{-1} indole-3-butyric acid (IBA). Root induction proceeded rapidly in darkness with ~55% success. Subsequent root development was completed in liquid medium without auxin, with sterile vermiculite as a support. A proportion of the plantlets was successfully acclimatized to soil conditions. Kamali et al. (2001) sought to facilitate clonal propagation of the rootstock GF-677 via tissue culture. Shoot tips collected in April were surface-sterilized with mercuric chloride. The most suitable culture medium was a modified Knop formulation, and 1 mg L^{-1} BA yielded the highest multiplication. The greatest rooting percentage occurred on LS medium containing 0.3 mg L^{-1} NAA and 1.6 mg L^{-1} thiamine when shoots were exposed to 7 days of darkness. Multiplied plantlets were first transferred to Jiffy-7 and then to pots. Channuntapipat et al. (2003) investigated *in vitro* shoot proliferation in almond cultivars Nonpareil 15-1 and Ne Plus Ultra, and the almond × peach hybrid rootstock Titan × Nemaguard. Shoot tips (~0.7 cm, 3–5 leaves) were cultured on suitable proliferation media. For Nonpareil 15-1, the best response was on MS (pH 5.7) containing 0.049 mM IBA, 3 mM BAP, 0.058 M sucrose and 0.7% agar. For Ne Plus Ultra, optimal proliferation was obtained on MS (pH 5.7) with 0.049 mM IBA, 5 mM BAP, 0.088 M sucrose and 0.7% agar. For Titan × Nemaguard, the highest multiplication occurred on MS with 10 mM BAP, 0.088 M sucrose and 0.7% agar. Shoots (~2 cm) of the rootstock rooted at 88% on half-strength MS (pH 5.7) with 2.4 mM IBA, 0.088 M sucrose and 0.7% agar after 1 week in darkness plus 2 weeks in light. Micrografts (~1.5 cm) onto the rootstock survived at 50% (Nonpareil 15-1) and 65% (Ne Plus Ultra). Rooted stocks and micrografts were acclimatized successfully, with 92% survival after transfer to potting mix. Antonopoulou et al. (2005) assessed riboflavin (vitamin B_2) for *in vitro* rooting of the almond × peach hybrid rootstock GF-677, testing 0 (control), 0.5, 1.0, 1.5 and 2.0 mg L^{-1}. After four weeks, riboflavin did not stimulate rooting; rooting frequencies were

markedly lower than the control, and chlorosis with apical necrosis appeared at the two highest concentrations. Morini and Perrone (2006) evaluated a short light/dark regime (4 h light/2 h dark) on rooting in several fruit rootstocks: GF-677 (peach × almond), Mr.S. 2/5 (*Prunus cerasifera*), MM 106 (apple; Northern Spy × Paradise M1) and BA 29 (*Cydonia oblonga*). Controls received a 16 h light/8 h dark photoperiod. Under 4/2 cycling, 100% rooting was achieved in GF-677, Mr.S. 2/5 and MM 106. In BA 29, short cycles increased rooting by ~65%. All rootstocks under 4/2 exhibited higher root numbers and lengths and rooted ~4–5 days earlier than controls. In BA 29, 0.2 mg dm^{-3} IBA under 4/2 yielded rooting equivalent to 0.4 mg dm^{-3} IBA under 16/8. The authors concluded that 4/2 cycles enhance auxin effect and promote rhizogenesis. Kamali et al. (2006) determined optimal media for micropropagation of GF-677 and almond seedlings. For GF-677, the best multiplication occurred on modified MS with 0.7 mg L^{-1} BA + 0.01 mg L^{-1} NAA, whereas seed genotypes performed best on MS with 1 mg L^{-1} BA + 0.01 mg L^{-1} NAA. Shoot elongation of GF-677 was maximized on modified Knop with 0.1 mg L^{-1} BA, while seed genotypes elongated better on modified MS with the same BA level. Rooting of GF-677 reached up to 80% on LS with 0.3 mg L^{-1} NAA in darkness. By contrast, rooting in seed genotypes was problematic both conventionally and *in vitro*. Neslihan (2007) aimed to establish an efficient *in vitro* regeneration protocol for GF-677. Nodal explants were cultured on MS with BA (0.5–2.0 mg L^{-1}). High shoot induction and tillering occurred particularly on MS with 1 mg L^{-1} BA. Young leaves from *in vitro* shoots were cultured on MS with TDZ or BA alone or combined with NAA. Pronounced white callus formed at shoot bases, and adventitious shoots regenerated from these calli were transferred to MS containing 0.0, 0.1 or 0.5 mg L^{-1} BA for elongation. The most effective elongation medium was MS with 0.5 mg L^{-1} BA, 30 g L^{-1} sucrose and 5.5 g L^{-1} agar. Elongated shoots were rooted on ½ MS with 0.05–2.0 mg L^{-1} IBA; 1 mg L^{-1} IBA yielded 84.2% rooting and 66.4% acclimatization. Akbaş et al. (2009) developed an *in vitro* shoot-multiplication protocol for the cultivar 'Yaltsinki' using apical shoot tips from mature trees. Explants were cultured on MS with various BA and kinetin levels. The highest multiplication (16.10 shoots per explant) was obtained on MS with 30 g L^{-1} sucrose, 7 g L^{-1} agar and 1.0 mg L^{-1} BA—the most effective BA level for multiple shoot induction. Combining 1.0 mg L^{-1} BA + kinetin with three auxins (IAA, IBA, NAA at 0.25–0.5 mg L^{-1}) showed that NAA suppressed new shoot formation, whereas 1.0 mg L^{-1} BA + 0.5 mg L^{-1} IAA yielded 11.25 shoots per explant. Among sucrose concentrations (20–50 g L^{-1}), 30 g L^{-1} gave the best result (15.40 shoots per explant). Sevgin (2010) optimized rooting in the micropropagation of economically important but difficult-to-root almond cultivars (Nonpareil, Ferragnes, Tuano) alongside GF-677. Meta-topolin (mT) outperformed BA for shoot number and length. Rooting was not achieved in Ferragnes and Nonpareil. The highest rooting (64%) occurred in GF-677 on 1 mg L^{-1} IBA. Phloroglucinol did not improve rooting but promoted shoot growth. Rooting peaked (67%) at the eighth subculture. Dejampour et al. (2011) developed an efficient micropropagation protocol for the almond × peach rootstock HS314, proposed as an alternative to GF-677. Surface-sterilized nodal explants were cultured on modified DKW with 3% sucrose, 100 mg L^{-1} phloroglucinol, 0.7% plant agar and 0.5 mg L^{-1} BAP, then transferred to combinations of 0.5–2.0 mg L^{-1} BAP and 0.0–0.5 mg L^{-1} IBA. Maximum shoot number occurred at 2.0 mg L^{-1} BAP. For rooting, shoots were placed on DKW with 0.5–4.0 mg L^{-1} IBA or NAA; 2.0 mg L^{-1} IBA yielded the greatest root number and length. Halving DKW salts increased rooting from 66% to 85%. Rooted plantlets were acclimatized with high success. Sharifmoghaddam et al. (2011) optimized micropropagation of GF-677. After sterilizing actively growing shoots, explants were cultured on MS with various regulator combinations. After four weeks, data on callus formation, multiplication rate, shoots per explant and shoot length were recorded. MS + 0.01 mg L^{-1} IBA + 2.0 mg L^{-1} BA was optimal for initiation and proliferation. Autumn was identified as the best season for explant collection. Rooting was highest on MS with 0.5 mg L^{-1} IBA. Özbek (2011) achieved clonal multiplication

from shoot tips in the rootstocks 'Garnem', GF-677, 'Cadaman' and 'Myrobalan 29-C' under *in vitro* conditions and evaluated resistance of regenerated plants to root-knot nematodes (*Meloidogyne incognita, M. javanica*). S and modified MS (MST) media were used with constant 0.05 mg L^{-1} GA_3 and BAP at 0–2.0 mg L^{-1}. Substantial differences were reported among clones in multiplication capacity and nematode resistance. Ünek et al. (2011) studied *in vitro* multiplication potential of the clonal hybrid rootstock 'Garnem', using apical and axillary buds on MS with BAP, kinetin (Kin), 2iP and TDZ at various concentrations under 24 ± 2 °C, 16-h photoperiod and 3000–4000 lux. Plant growth regulators significantly affected proliferation; the highest shoot number occurred with 1.0 mg L^{-1} BAP, whereas higher BAP reduced the multiplication coefficient. Aghaye and Yadollahi (2012) optimized the micropropagation of GF-677 on MS with BAP at 0–2.0 mg L^{-1} (five replicates per treatment). BAP at 1.0 mg L^{-1} produced significantly greater shoot number (2.98) and shoot length than other concentrations and control (shoot number 0.4). Shoots (3–5 cm) were rooted under various treatments; the highest rooting occurred on 3 mg L^{-1} IBA (mean 2.167 roots; 11.53 cm root length). A one-week dark pre-treatment improved rooting relative to continuous light. Abbasi et al. (2019) examined micropropagation of *P. scoparia* using nodal segments (3–5 cm) sterilized with 10% sodium hypochlorite. For proliferation, explants were cultured on MS with 7.0 g L^{-1} agar, 30.0 g L^{-1} sucrose and BA at 0–4.0 mg L^{-1} or TDZ at 0–7.0 mg L^{-1}. Shoot proliferation occurred only at 2.0 and 4.0 mg L^{-1} BA and at 2.0 mg L^{-1} TDZ, with the latter giving the highest shoot number, leaf number and leaf color quality. For rooting, shoots (2–3 cm) were transferred to ½ MS with 30.0 g L^{-1} sucrose, 7.5 g L^{-1} agar and IBA or NAA at 0–1.0 mg L^{-1}. IBA significantly affected rooting percentage, root number and length; the best response was at 0.50 mg L^{-1} IBA, whereas other IBA levels and all NAA treatments failed to induce roots. Thus, MS + 2.0 mg L^{-1} TDZ for multiplication and ½ MS + 0.50 mg L^{-1} IBA for rooting are recommended for *P. scoparia*. Ritterbusch et al. (2020) addressed *in vitro* propagation of the almond × peach hybrid rootstock G × N-9, valued for resistance to several *Meloidogyne* spp. While autumn hardwood cuttings were recommended, demand increased for *in vitro* propagation. The study evaluated BAP and kinetin (KIN), carbon sources (sucrose, sorbitol) for proliferation/elongation, and IBA for rooting and acclimatization. BAP × KIN interactions significantly improved shoot multiplication and elongation. Media with 15 or 30 g L^{-1} sorbitol yielded the highest multiplication (5.75 ± 0.19 and 6.39 ± 0.21 shoots per explant). The highest root numbers occurred on QL medium with 0.6–1.2 mg L^{-1} IBA. After 30 days of acclimatization, 1.2 mg L^{-1} IBA plants had the shortest roots yet the greatest mean root number (8.35 ± 0.41). Continuous IBA use supported a robust root system, critical for successful acclimatization. Kodad et al. (2021) conducted an *in vitro* study to promote shoot formation from nodal explants of local almond ecotypes ("Beldi") from eastern Morocco. Explants were cultured on MS or Heller media supplemented with auxin (IBA) and cytokinins (BAP, TDZ, KIN) at different concentrations. TDZ was superior to other regulators; 1 mg L^{-1} TDZ produced the highest shoot proliferation (63.6 ± 0.63%). For genotype S1 on MS, the mean number of regenerated shoots reached 13 ± 0.54. In rooting, the highest rooting (60.41 ± 0.81%) and roots per shoot (7.3 ± 1.36) were obtained for genotype S2 on half-strength MS with 1 mg L^{-1} IBA. Approximately 80% of plantlets acclimatized successfully without morphological abnormalities. Borkheyli et al. (2021) optimized micropropagation of the widely used clonal rootstock GF-677 by testing medium type, plant growth regulators and carbohydrate sources. Proliferation was carried out on MS, WPM and DKW media with 0–2.0 mg L^{-1} BA. Rooting employed the same media with 0–2.0 mg L^{-1} IBA. A separate experiment compared carbohydrate sources. The highest shoot number and fresh shoot weight occurred on MS with 1.0–2.0 mg L^{-1} BA. Rooted shoot percentages were greatest on MS or WPM with 1.0–2.0 mg L^{-1} IBA; maximum roots per plantlet were recorded on WPM with 1.0 mg L^{-1} IBA. Sorbitol enhanced shoot proliferation relative to sucrose; average root number and length were highest

with sorbitol and sucrose media, respectively. Post-transfer survival during acclimatization reached 67%. Koaym et al. (2022) assessed growth regulator effects on shoot multiplication and rooting in four cultivars (Shami Furk, Dafadii, Ferragnes, Fournat). The aim was to identify combinations maximizing shoot number/length and rooting percentage/roots per plant/root length. Optimal sterilization (highest survival, no contamination) was achieved with 0.5% NaOCl for 10 min plus 0.1% $HgCl_2$ for 30 s in Dafadii (75%) and in Shami Furk and Fournat (62.5%). In Ferragnes, 0.1% $HgCl_2$ for 1 min gave the best response (50%). For multiplication, MS with 1.0 mg L^{-1} BA + 0.1 mg L^{-1} IBA + 0.2 mg L^{-1} GA_3 performed best for Shami Furk (5.31 shoots/explant; 6.23 cm) and Dafadii (3.67 shoots/explant; 4.98 cm). Ferragnes responded best to MS with 1.0 mg L^{-1} BA + 0.2 mg L^{-1} GA_3 (3.84 shoots/explant; 4.67 cm), whereas Fournat showed no meaningful improvement. For rooting, 1.0 mg L^{-1} IBA yielded the highest rooting in Shami Furk (75%), Dafadii (40%) and Ferragnes (35%). Acclimatization success reached 85% in Shami Furk and 50% in Ferragnes. Haghi et al. (2023) noted that seedling (seed-derived) rootstocks are common in fruit production but cause non-uniformity. They tested IBA at 0, 1500, 2500 and 3500 mg L^{-1} ex vivo for rooting of cuttings of the promising peach × almond hybrid rootstock TF92 (Karaj, 2019; heated beds). IBA significantly affected rooting percentage, root number, root dry weight and rooting rate; the highest rooting (78.45%) occurred at 2500 mg L^{-1}, recommended for TF92 cuttings. *In vitro*, the highest percentage of rooted plantlets was obtained on MS with 1 mg L^{-1} IBA, which the authors recommend for TF92 *in vitro* rooting. Dungarwal and Sangeeta (2023) reviewed factors influencing *in vitro* micropropagation of fruit trees: explant characteristics, sterilization, phenolic exudation control, medium composition, carbon sources/additives, environmental conditions (light, temperature), plant growth regulators, pH and acclimatization substrates. Using very small explants (~0.5 mm, e.g., shoot tips) is effective for virus-free cultures. The most successful sterilization employed 10–30% sodium hypochlorite with two drops of Tween-20 for 15–20 min. Explants include shoot tips, axillary buds, single-node segments, leaf discs, flower buds and seeds. Pre-soaking explants in an antioxidant solution (150 mg L^{-1} citric acid + 100 mg L^{-1} ascorbic acid) for 2–24 h minimized phenolic exudation. Setting medium pH to 5.7 improved development and rooting. Optimal growth occurred at 25–28 °C under a 16 h light/8 h dark photoperiod. Sucrose remains the most common carbon/energy source. Full-strength basal salts generally support better growth and multiplication; however, for species with high phenolic exudation (e.g., date palm, mango, apple), liquid media are advantageous at initiation and rooting. Low concentrations of auxins, cytokinins and gibberellins are typically sufficient; BAP and 2iP are common cytokinins, while NAA and IBA are relatively less stable in rooting media. Auxin/cytokinin combinations are generally more effective than gibberellins for multiplication. A 1:1:1 (v/v) mixture of vermiculite:sand:peat is recommended for acclimatization. Razavi and Aghbolaghi (2023) optimized micropropagation across 11 *Prunus dulcis* genotypes (Mamaei, Shekoufeh, Fragnies, and breeding lines A1, A2, A3, A4, A5, A7, A8, A9), focusing on optimal explant type and sterilization. Responses varied with genotype. For example, in A1, herbaceous shoots yielded a lower multiplication rate (30.12%) than woody shoots (40.20%), whereas in A7 the reverse was true (herbaceous 40.13% vs woody 36.20%). Overall, woody shoots performed better in A1, A3, A8, A9, SH, M and F. Sterilization efficacy was also genotype-dependent: 25% NaOCl for 15 min was superior for A7, A8, A9 and F, while 50% NaOCl for 10 min was preferable for other genotypes. Genotype × sterilization × explant-type interactions significantly influenced multiplication capacity, growth and rate. Hammud et al. (2024) aimed to establish protocols for 'Ferragnes' and 'Ferraduel'. MS media with BAP at 1.0 or 2.0 mg L^{-1} were tested. In 'Ferragnes', 1.0 mg L^{-1} BAP was more suitable, increasing leaf number, whereas 2.0 mg L^{-1} promoted shoot elongation. Explants initiated growth by day 5 under both BAP levels, and ~73% reached subculture size by week 3. IBA concentrations did not significantly affect rooting percentages; however, 0.1 mg L^{-1} NAA improved rooting. Rooting success

reached 23.07% (mean 2.6 roots) in 'Ferragnes' and 62.50% (mean 2 roots) in 'Ferraduel'. Ahmadi et al. (2024) developed micropropagation protocols for *Amygdalus scoparia* L. and *A. lycioides* Spach (Rosaceae). Nodal explants were cultured on MS with factorial combinations of BA (0, 0.25, 0.5, 1, 2, 3 mg L^{-1}) and IBA (0, 0.1, 0.25, 0.5 mg L^{-1}) in a completely randomized design (four replicates). In *A. lycioides*, the highest regeneration occurred with 0.5 mg L^{-1} BA + 0.25 mg L^{-1} IBA; in *A. scoparia*, with 2 mg L^{-1} BA + 0 mg L^{-1} IBA. Shoot number followed similar trends, underscoring species-specific hormonal requirements. Gök (2024) evaluated *in vitro* multiplication and rooting performance of 62 almond genotypes selected from Nevşehir, Niğde and Mersin-Mut, and developed an effective sterilization protocol, reducing contamination to 0.73%. Significant differences among genotypes were observed for micropropagation performance, and several previously unreported observations were documented. Eliwa et al. (2025) examined the effects of IBA (0.0, 0.5, 1.0, 2.0, 3.0, 4.0 mg L^{-1}) on *in vitro* rooting and acclimatization in the peach rootstocks 'Okinawa', 'Nemared' and 'Garnem'. 'Garnem' achieved the highest rooting rate, roots per plant and root length overall. At 2.0 mg L^{-1} IBA, rooting reached 100% (Garnem), 83.33% (Okinawa) and 75% (Nemared). 'Garnem' produced the most roots per plant (12.33) at 4.0 mg L^{-1} IBA, whereas maxima in 'Nemared' and 'Okinawa' were 8.00 and 5.00 at 3.0 mg L^{-1} IBA. Root lengths varied significantly with IBA; 'Garnem' had the longest average roots (5.33 cm), followed by 'Okinawa' (2.49 cm) and 'Nemared' (1.43 cm). However, 4.0 mg L^{-1} IBA induced abnormal roots and callus in all three genotypes. Acclimatization survival was 93% (Garnem), 90% (Okinawa) and 75% (Nemared). Gök et al. (2025) assessed eight selected almond genotypes on full-strength and half-strength NRM media with IBA at 0, 1, or 2 mg L^{-1}. The tallest plants were obtained in genotype 48-6 (2.04 cm), and the highest leaf number in 33-B-44 (12.26 leaves). Rooting was observed in genotype 33-B-54 from Mersin-Mut on ½ NRM with 2 mg L^{-1} IBA. As these materials are products of selection breeding, these are the first reports for these genotypes. Bai et al. (2025) optimized an *in vitro* propagation protocol for seedling production of *A. pedunculata*. The optimal sterilization sequence was 70% ethanol (30 s) followed by 2% NaClO (20 min). Shoot induction occurred on MS with 0.5 mg L^{-1} 6-BA and 0.1 mg L^{-1} 2,4-D; proliferation was supported on MS with 0.1 mg L^{-1} 6-BA and 0.3 mg L^{-1} IBA. Rooting was induced on ½ MS with 0.3 mg L^{-1} IBA + 0.1 mg L^{-1} NAA. Acclimatization was conducted on a peat:perlite:vermiculite mixture (6:3:1, v/v/v), yielding high ex vitro survival. This protocol enables large-scale production and supports both industrial utilization and conservation of this economically valuable species.

Micropropagation studies in almond and almond × peach hybrids therefore constitute a rich literature encompassing diverse genotypes, media and applications. An evaluation of the reviewed studies indicates that explant type, medium composition, combinations of plant growth regulators, and culture conditions vary considerably. These factors directly influence shoot proliferation, rooting success, and acclimatization efficiency. Protocols developed for widely used rootstocks such as GF-677, Garnem, HS314, and G×N-9 are particularly noteworthy, as they enhance multiplication capacity and facilitate adaptation to ex vitro conditions. Nevertheless, genotypic variation remains a decisive factor in propagation performance, while additional elements—including light regimes, carbohydrate sources, and antioxidant applications—play critical roles in determining the overall success of *in vitro* culture.

CONCLUSIONS

Micropropagation studies on almond and almond × peach hybrid genotypes demonstrate that propagation success is strongly influenced by genotype, culture medium composition, plant growth regulators, and culture conditions. Evidence from the literature highlights the critical roles of cytokinins (BA, TDZ) and auxins (IBA, NAA) during shoot proliferation and rooting, although success rates vary considerably among genotypes. Moreover, light regimes, carbohydrate sources, antioxidant applications, and sterilization protocols are also decisive factors shaping overall culture performance.

The limited rooting observed in certain genotypes underscores the necessity of genotype-specific optimization. By comprehensively evaluating micropropagation research on almond and its hybrids, this review provides a valuable reference for both researchers and practitioners. Future studies should prioritize the development of standardized, genotype-responsive, and highly efficient protocols. Such advances will be essential for improving commercial propagation systems, producing disease-free plant material, and conserving rare genotypes. Ultimately, this review contributes to the more effective planning of future micropropagation research in almond.

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ASSESSING THE IMPACT OF GLOBAL WARMING ON SOIL HEALTH AND AGRICULTURAL PRODUCTIVITY

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ABSTRACT

Global climate change imposes increasing and multifaceted pressures on soil health and fertility. Rising temperatures, irregular precipitation patterns, and the accumulation of greenhouse gases in the atmosphere significantly affect the physical, chemical, and biological properties of soils. Elevated temperatures accelerate the decomposition of soil organic matter, reduce soil organic carbon, and increase the risk of salinization and erosion. Intense and erratic rainfall events disrupt soil aggregate stability, lower cation exchange capacity, and enhance nutrient leaching. Conversely, reduced rainfall and drought limit soil moisture availability and nutrient uptake, while suppressing biological nitrogen fixation. Although increased atmospheric CO_2 levels may stimulate microbial activity and nutrient cycling, they can also lead to net carbon losses from the soil. This review explores the complex effects of climate change on soil health, focusing on structural degradation, shifts in pH, salinization, nutrient cycling disruptions, and microbial imbalance. Furthermore, the study discusses mitigation and adaptation strategies (such as conservation agriculture, organic matter management, and integrated nutrient management) that can help reduce climate-related soil degradation. Implementing climate-resilient soil management practices is essential to sustain long-term soil health and agricultural productivity.

Keywords: Climate change, Greenhouse gases, Carbon cycle, Sustainable agriculture

INTRODUCTION

Soils underpin terrestrial life and global food systems by regulating water and nutrient cycles, storing vast amounts of carbon, and hosting extraordinarily diverse biota that drive decomposition and fertility. Soils contain more carbon than the atmosphere and terrestrial vegetation combined, positioning them as a critical yet vulnerable component of both the climate system and agricultural productivity. Recent studies and guidance from agricultural and climate agencies emphasize that the size and persistence of this soil carbon pool confer both opportunity and risk: management can enhance sequestration, but warming and land-use pressures can accelerate losses (Keller and Handler 2024). Anthropogenic climate change, which has resulted in approximately 1.1 °C of global warming relative to the 1850–1900 baseline, has already led to widespread and multifaceted impacts on both natural and managed ecosystems. There is high confidence that continued warming will exacerbate risks to soil health and food security. The IPCC's Sixth Assessment Synthesis Report (2023) highlights that

increasing temperatures, shifting precipitation regimes, and more frequent extremes are altering hydrologic and biogeochemical processes that determine soil health, with cascading effects on crop yields and rural livelihoods.

Warming directly influences the soil carbon balance by accelerating microbial metabolism and decomposition, potentially shifting soils from net sinks to net sources of CO_2 in sensitive regions. Recent whole soil warming experiments and model data analyses published since 2023 highlight the significant vulnerability of soil organic carbon, including deeper soil layers that were previously considered relatively stable. These studies consistently report increased carbon emissions under sustained warming and underscore considerable uncertainty in both the magnitude and timing of these losses (Ren et al. 2024). Hydroclimatic change compounds these thermal effects. Intensifying heavy-precipitation events increase erosion, destabilize aggregates, and enhance nutrient leaching, while prolonged drought reduces soil moisture, suppresses biological nitrogen fixation, and impairs microbial functioning. Emerging assessments and regional projections show rising erosion risk under warmer, more variable climates, with implications for water quality and long-term soil fertility (Yin et al. 2025; Lua et al. 2024; IPCC 2023). At the farm and landscape scales, these physical and biogeochemical stresses translate into measurable risks for crop production. A 2023 article in *Nature Reviews Earth & Environment* notes that warmer temperatures, elevated CO_2, and altered water availability jointly modulate cereal yields, often exposing heat and water-stress thresholds that management must address. Complementary experimental evidence suggests that climate change and intensified cropland use can reduce soil multifunctionality, defined as the concurrent provision of nutrient cycling, carbon storage, and water regulation. This reduction in multifunctionality may, in turn, constrain agronomic resilience (Sünnemann et al. 2023)

Given soils' centrality to both mitigation (through carbon storage) and adaptation (through water retention, nutrient buffering, and biological resilience), safeguarding soil health is a prerequisite for maintaining agricultural productivity under climate change. This review synthesizes current understanding of how warming, altered precipitation, and rising atmospheric CO_2 interact to affect soil physical structure, chemistry (pH, salinity, cation exchange capacity), and biology (microbial communities and nutrient cycling). It also evaluates evidence based strategies such as conservation agriculture, organic matter management, and integrated nutrient management, which have the potential to slow or reverse climate related soil degradation while maintaining agricultural productivity.

CLIMATE CHANGE DRIVERS AFFECTING SOIL HEALTH

Rising temperatures

Global warming has led to significant increases in both air and soil temperatures, with profound implications for ecosystem processes and climate feedbacks. In China, analyses of five decades of temperature records (1962–2011) revealed that surface soil temperatures rose 31% more than air temperatures and that warming penetrated as deep as 3.2 meters, amplifying soil respiration rates by up to 28% and extending the growing season by approximately 20 days (Zhang et al. 2016). Similar long-term trends have been documented in other regions, such as North Rhine-Westphalia, Germany, where soil warming ranged from 0.36 °C to 0.47 °C per decade depending on depth (Dorau et al. 2022). Moreover, projections for the coming decades

imply continued soil warming, especially in deeper layers, potentially altering global temperature regimes and cascading into ecological disruptions (Bradford 2019; Lembrechts 2022). Taken together, these findings highlight the increasingly urgent need to incorporate soil temperature dynamics, alongside atmospheric warming, into climate models and ecosystem assessments, as soil warming acts both as a driver and an amplifier of climate change.

Altered precipitation regimes

Global warming is profoundly altering precipitation regimes worldwide, intensifying both the wet and dry extremes and restructuring seasonal patterns. Rising global temperatures increase the atmosphere's capacity to hold moisture, by approximately 2 to 3 percent per degree Celsius on a global scale, with extreme precipitation events intensifying at an even faster rate. This results in more severe rainfall events and prolonged dry periods, even as average precipitation shows an overall increasing trend (Douville, 2021). Climate models reveal that precipitation becomes less frequent yet more intense in the tropics, with heavy precipitation events increasing by up to 35 % per 1°C warming, despite an overall decline in light rainfall (Guo *et al* 2022). Furthermore, the annual range of precipitation widens significantly; maximum precipitation increases by roughly 3.6 % $°C^{-1}$, while minimum precipitation rises by only 0.2 % $°C^{-1}$ (Guo et al. 2012). Changes in wet and dry season patterns are also evident: across the tropics, many regions are transitioning from annual to biannual precipitation regimes, with wet seasons shortening by an average of about 7 days and beginning 6 days later, while dry seasons grow both longer and drier (Guo et al. 2012). Moreover, projections suggest that large precipitation accumulations, which have been rare in historical records, will become exponentially more likely under continued warming, with some events projected to increase by several hundred to over one thousand percent (Neelin et al. 2017). Collectively, these alterations not only pose escalating risks for flooding, drought, and agricultural disruption but also challenge water-resource management and infrastructure resilience in both regional and global contexts.

Drought

Anthropogenic global warming is exacerbating drought globally through a combination of elevated atmospheric evaporative demand (AED), altered precipitation dynamics, and soil moisture depletion. Recent high-resolution global drought data (1901–2022) demonstrate that increased AED (driven by rising temperatures) has amplified drought severity by an average of 40 %, and has helped expand drought-affected land by approximately 74 % in the period 2018–2022 compared to 1981–2017, with nearly 30 % of the terrestrial surface experiencing moderate to extreme drought in 2022 alone, 42 % of which is attributable to AED (Gebrechorkos et al. 2025). This pattern is echoed regionally; in the Iberian Peninsula, intensified evaporative demand has led to more frequent and severe hydrological droughts, significantly reducing streamflow in both natural and regulated river basins (Lorenzo, 2014). Projections indicate that under moderate emissions scenarios (SSP2-4.5), by mid-century nearly 70 % of global land could face declining soil moisture, increasing agricultural drought risk particularly in regions like India, China, Europe, and the United States (OECD, 2025). Moreover, observational and modeling evidence indicates that drought durations, particularly the longest annual dry spells, may lengthen by the end of the century, substantially exceeding midrange estimates when

models are calibrated with observational data. This convergence of rising temperatures, increased evaporation, and declining moisture storage highlights the urgent need for resilient water resource management to address more frequent, prolonged, and severe drought conditions.

Elevated atmospheric CO_2 (eCO_2)

Elevated atmospheric carbon dioxide, a central driver of anthropogenic climate change, exerts a fundamental influence on plant physiology, hydrology, and ecosystem carbon cycling. However, the magnitude and consistency of these effects remain highly dependent on environmental context. The dramatic rise in CO_2 from preindustrial ~280 ppm to over 427 ppm by 2024 underscores its critical role in enhancing the greenhouse effect and global warming (NOAA, 2024). On the positive side, eCO_2 enhances photosynthetic carbon uptake, thereby increasing terrestrial gross primary productivity (GPP) and improving leaf-level water-use efficiency by reducing stomatal conductance and transpiration (Lucas et al. 2019). Free Air Carbon Dioxide Enrichment (FACE) studies report biomass increases of approximately 20 to 30 percent, although these gains are often limited by soil nutrient availability, particularly nitrogen and phosphorus. These nutrient constraints account for a substantial portion of the variability observed in carbon fertilization responses (Fu et al. 2024). Additionally, elevated CO_2 can partially buffer plants against drought and heat stress by improving water status and mitigating oxidative damage, as seen in meta-analyses across diverse species and controlled experiments such as those involving Arabidopsis (Wang et al. 2022). That said, eCO_2 can also yield counterintuitive outcomes: enhanced leaf area may increase canopy transpiration, and changes in plant hydraulics (e.g., altered stomatal behavior or hydraulic conductance) may reduce drought resilience in certain contexts (Becklin et al. 2017). Moreover, the CO_2 fertilization effect may be waning globally due to nutrient constraints and ecosystem saturation, potentially weakening the land carbon sink and increasing reliance on mitigation strategies. Thus, although elevated carbon dioxide plays a critical role in modulating plant responses under climate change, its overall influence is determined by species-specific traits, environmental interactions, and resource availability. This underscores the need for nuanced, multifactor assessments in ecosystem modeling.

SOIL PROPERTY ALTERATIONS UNDER CLIMATE STRESS

The climate stress factors mentioned above reconfigure soils' physical, chemical, and biological properties in ways that directly govern fertility and yield stability.

Physical properties: structure, aggregation, and erosion susceptibility

Under climate stressors such as intensified precipitation, prolonged droughts, and fluctuating temperatures, soil physical properties undergo marked degradation. Elevated temperatures and extreme heat events can reduce cohesion among clay particles, shifting textures toward more silt-sized fractions and compromising structural integrity through induced cracking and dehydration of 2:1 clay minerals (Mangi et al. 2017). Drought exacerbates these effects by suppressing the production of root mucilage and microbial exudates that normally stabilize aggregates, leading to a decline in mean weight diameter (MWD) and aggregate

stability; the removal of vegetation during drought further accelerates macroaggregate breakdown and diminishes aggregate-associated organic carbon (Reinsch et al. 2024). When heavy rainfall follows such weakened soil states, erosion susceptibility increases markedly: less aggregated soils, particularly coarser-textured ones, show higher erodibility and decreased stability across successive rainfall events, with significant reductions in soil K-factor observed (Yao 2019). Moreover, intensifying land use changes under warming climates influence aggregation through altered thermal amplitudes and moisture regimes, which in turn affect porosity, bulk density, and infiltration potential (Li et al. 2023). Collectively, these climate-induced disruptions in soil structure, aggregation, and susceptibility to erosion threaten soil health, water retention, and ecosystem resilience.

Chemical properties: pH, salinity, cation exchange capacity (CEC), and nutrient leaching

Under climate stressors such as shifting precipitation patterns, rising temperatures, and sea level rise, soil chemical properties are significantly disrupted. Soil pH is highly sensitive to climate-driven changes in temperature, precipitation, and biogeochemical cycling. Increased precipitation and extreme rainfall events accelerate the leaching of base cations (Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}), which normally buffer acidity, leading to progressive soil acidification (Yaseen 2024). At the same time, enhanced mineral weathering and the oxidation of soil organic matter under warming release hydrogen ions (H^{+}) and mobilize aluminum (Al^{3+}), further lowering pH and impairing nutrient availability (Brinkman, 1990). In drought-prone regions, reduced leaching and the accumulation of soluble salts can, conversely, raise soil pH (alkalinization), particularly in calcareous soils where carbonates become concentrated in the profile (Bui, 2017). Moreover, rising atmospheric CO_2 and associated shifts in soil respiration influence carbonic acid equilibria, which can modify pH dynamics seasonally. Overall, climate stress exerts a bidirectional influence: acidification dominates in humid and high-rainfall systems, while alkalinization often occurs in arid and semi-arid landscapes both with profound consequences for nutrient cycling, microbial activity, and long-term soil fertility.

In coastal and semi-arid zones, sea-level rise and saltwater intrusion have increased soil salinity by between 1 % and 33 % over recent decades, while elevated evapotranspiration in arid regions inhibits salt leaching, promoting accumulation in the root zone (Rahman et al. 2018). Climate-induced warming accelerates the decomposition of soil organic matter (SOM), which critically underpins soil Cation Exchange Capacity (CEC) a determinant of the soil's ability to retain key nutrient cations. As SOM declines, especially in coarse-textured or low-clay soils, CEC diminishes, reducing nutrient retention and amplifying leaching losses when intense precipitation occurs (Mandal and Sathyaseelan, 2012). Together, these shifts diminish nutrient retention, elevate the risk of nutrient leaching, and impair soil fertility and ecosystem function under ongoing and future climate stress.

Biological properties: microbial diversity, processes, and symbioses

Climate-induced stressors such as warming, drought, and altered precipitation patterns have pronounced effects on soil microbial diversity, functional processes, and symbiotic relationships. Meta-analyses reveal that on average, global warming reduces soil bacterial and fungal diversity by approximately 16% and 20%, respectively, with projected declines reaching

56% (bacteria) and 81% (fungi) under high-emission scenarios by mid-century trends that threaten soil organic carbon (SOC) sequestration and ecosystem resilience (Sun et al. 2025). Long-term experimental observations in grasslands indicate that warming restructures microbial successional trajectories, making fungal communities increasingly divergent over time and emphasizing deterministic environmental filtering over stochastic assembly (Guo et al. 2018). Drought compounds these effects: prolonged moisture deficits and elevated temperatures significantly alter the composition of bacterial and fungal communities, reduce their richness, and disrupt established symbioses, including mycorrhizal colonization patterns (Seaton et al. 2012). At the functional level, changes in temperature and precipitation influence the complexity of microbial networks. In temperate steppes, warming has been shown to increase fungal network complexity and stability, particularly during cooler seasons, indicating a shift toward more interconnected, fungi-driven processing networks (Yu et al. 2024). Moreover, symbiotic relationships are altered: drought promotes arbuscular mycorrhizal fungi (AMF) colonization and structural adaptations like increased hyphal networks and vesicle formation, enhancing plant drought tolerance and nutrient uptake via osmolyte production and water relations adjustments (Silva et al. 2025). Collectively, climate stress reduces microbial diversity, reshapes community assembly, alters biogeochemical functioning, and reshapes symbiotic partnerships, with profound implications for nutrient cycling, plant resilience, and ecosystem stability.

CONSEQUENCES FOR AGRICULTURAL PRODUCTIVITY

Climate-induced soil degradation exerts cascading effects on agricultural systems, ultimately eroding crop yields, resource efficiency, and resilience.

Declines in Crop Yields and Soil Multifunctionality

Climate driven soil degradation, reflected in declines in soil organic carbon, increased erosion, loss of structural integrity, and reduced microbial function, significantly undermines both crop yields and soil multifunctionality. Intense warming and drought drive accelerated SOC decomposition and erosion, reducing moisture retention, nutrient availability, and key ecosystem services that support plant growth (e.g., carbon and nutrient cycling) (Cheng et al. 2017). Empirical modeling shows that warming reduces global yields of maize by 3.4%, wheat by 2.4%, rice by 0.3%, and soybean by 5%, while improving SOC can dramatically mitigate these losses bringing yield reductions down to near-neutral levels in many cases (Deng et al. 2023). Furthermore, precipitation reduction in semi-arid grasslands disrupts microbial biodiversity and network complexity, resulting in diminished soil multifunctionality such as nutrient provisioning, microbial growth efficiency, and organic matter decomposition (Wang et al. 2023). Conservation agriculture, which enhances soil health through practices like reduced tillage and increased SOC and microbial biomass, has been shown to sustain or even boost crop yields (e.g., 9.3% wheat yield increase) under long-term warming, highlighting how degraded soils may lose resilience in the absence of such practices (Teng et al. 2024). Together, these findings illustrate a clear mechanistic pathway: climate-induced soil degradation erodes the suite of soil functions that underpin crop productivity and ecosystem resilience, unless countered by proactive soil management strategies.

Nutrient Inefficiencies and Increased Fertilizer Demand

Climate driven soil degradation, including the loss of organic matter, erosion, altered moisture regimes, and disruption of nutrient cycling, exacerbates nutrient inefficiencies in agroecosystems and consequently increases fertilizer demand. Declining soil organic matter (SOM) and fertility force farmers to rely more heavily on synthetic fertilizers, yet as SOM diminishes, soils' physical and biological ability to retain nutrients also deteriorates, causing a substantial portion of applied nutrients to be lost to leaching or volatilization (Mosier et al. 2021). For example, degraded dryland agroecosystems often exhibit both nutrient depletion and reduced fertilizer-use efficiency, prompting escalating fertilizer applications that further increase greenhouse gas emissions (Iqbal et al. 2023). Furthermore, rising temperatures above critical thresholds (e.g., 28 °C) have been empirically linked to sharp increases in fertilizer use particularly nitrogen in irrigated regions, as farmers attempt to offset heat- and moisture-induced yield declines, even as nutrient uptake efficiency falls (Quan et al. 2024). Together, these dynamics reveal a vicious cycle: climate stress degrades soil, impairs nutrient retention, and forces greater fertilizer inputs, often with diminishing returns and increased environmental and economic costs.

Water Stress and Drought Vulnerabilities

Climate-driven soil degradation such as erosion, loss of organic matter, structural decline, and vegetation removal, significantly heightens water stress and drought vulnerability in agroecosystems. The degradation of soil structure and aggregation due to intensified erosion and compaction diminishes infiltration rates and reduces soil water–holding capacity, leading to faster runoff and reduced water availability during dry periods (Radulov and Berbecea, 2023). Degraded soils lacking organic matter retain less moisture and are more prone to moisture deficits under warming and erratic precipitation, which amplifies drought impacts on plant growth and ecosystem resilience (IUCN, 2024). Land-use changes such as deforestation and the conversion of forests and rangelands to bare or cultivated land further impair the land's capability to buffer drought by disrupting vegetation cover and hydrological buffering (Liu et al. 2025). Empirical evidence from drought studies also shows that soil drying reduces microbial function and alters soil carbon and nitrogen processes, degrading the soil habitat and making ecosystems less resistant to prolonged water deficits (De Silva, 2025). Together, these processes create a feedback loop whereby degraded soils lose both physical and biological resilience, leaving landscapes more susceptible to water stress and prolonged drought under changing climate conditions.

Enhanced Greenhouse Gas Emissions and Feedbacks

Climate-induced soil degradation through warming, drying, erosion, and land-use change leads to an increase in greenhouse gas emissions from soils, thus reinforcing a positive feedback loop that accelerates climate warming. Experimental warming studies demonstrate that a ~1.5 °C rise in temperature significantly elevates soil CO_2 emissions (~13%), nitrous oxide (N_2O) emissions (~35%), and methane (CH_4) emissions (~23–38%) in various ecosystems, collectively adding 1.8 gigatonnes CO_2-equivalent per year and undermining the mitigation potential of terrestrial carbon sinks (Liu et al. 2020). Decomposition of previously stable soil organic carbon including deep SOM and permafrost carbon is accelerated by microbial activity

under warming conditions, potentially releasing hundreds of petagrams of carbon over time and substantially reducing overall SOC storage (Cheng et al. 2017). Soil erosion and topsoil loss also enhance CO_2 emissions: an experimental study found that removal of the top 20 cm of soil increased seasonal CO_2 efflux from cropland, whereas adding soil reduced emissions, indicating that degradation itself can amplify GHG flux (Liang, 2018). In peat-rich ecosystems, draining and degradation of peatlands commonly undertaken for agriculture, transforms these systems from net carbon sinks into potent sources of CO_2 (and additionally CH_4 upon rewetting), making them a major driver of GHG emissions (Leifeld and Menichetti, 2018). Together, these processes illustrate that soil degradation triggers heightened emissions of CO_2, N_2O, and CH_4, creating self-reinforcing feedbacks jeopardizing both soil carbon stocks and broader climate stability.

Table 1. Climate Change Impacts on Agricultural Productivity

Process	Impact Mechanism	Productivity Consequence
Yield reduction	Stress from heat, drought, and fragmentation of multifunctionality	Lower output and food security risks
Nutrient losses	Leaching, runoff, low CEC	Reduced fertilizer efficiency, increased inputs
Water stress	Poor infiltration, structure loss, reduced water retention	Greater drought sensitivity, yield instability
Greenhouse feedbacks	SOC loss and N_2O emissions	Climate feedback loop and inefficiency

MITIGATION AND ADAPTATION STRATEGIES

Soil resilience to climate change depends on integrated, site-specific combinations of practices that protect soil structure, rebuild organic matter, improve nutrient and water use efficiency, and enhance biological function. Below we synthesize the evidence for key strategies and note important caveats.

Conservation agriculture (reduced/no-till, residue retention, cover crops)

Conservation agriculture (CA), anchored by the principles of reduced or no-tillage, residue retention, and diversified cropping (including cover crops), serves as a pivotal approach for both climate change mitigation and adaptation in agroecosystems. Reduced soil disturbance preserves structure and surface organic matter, enhancing water infiltration, reducing erosion, and promoting soil carbon accumulation (Zomer, 2017). Residue retention and cover cropping further bolster these benefits by maintaining soil moisture, regulating temperature extremes, and incrementally increasing soil organic carbon (SOC), soil aggregation, and nutrient cycling (Page, 2020). Meta-analyses reveal that CA often enhances yields modestly with cumulative

gains in maize of ~4 % while improving drought resilience and yield stability under variable rainfall (Corbeels et. Al. 2020). Significantly, CA practices substantially reduce nitrous oxide (N_2O) emissions by about 7 % with no-till alone, and up to 30 % when combined with residue retention and rotation and increase crop yield by over 9 %, offering synergistic mitigation and productivity gains (Zhu et al. 2025). In drought settings, implementing cover crops and residue retention has shown striking results for example, soybean yields up to 29 % higher, along with improved water-use and photosynthetic performance (Whippo, 2024). Altogether, conservation agriculture offers a multifaceted strategy: it mitigates greenhouse gas emissions, enhances soil health and resilience, and stabilizes crop productivity under climate stress though site-specific outcomes may vary.

Organic matter management: composts, manures, green manures, and biochar

Organic matter management, including the application of composts, manures, green manures, and biochar, is increasingly recognized as an effective strategy for mitigating and adapting to climate change impacts on agricultural productivity. The incorporation of composts and animal manures improves soil fertility by supplying essential macro- and micronutrients, enhancing cation exchange capacity (CEC), and stimulating beneficial microbial activity, which together bolster plant growth and resilience under climate stress (Goldan et al. 2023). Green manures, particularly legumes, contribute biological nitrogen fixation and enhance soil organic carbon (SOC) stocks, thereby improving soil aggregation, water-holding capacity, and nutrient cycling, which are critical under conditions of drought and variable rainfall (Mandal et al., 2004). Biochar, due to its stability and high carbon content, provides long-term carbon sequestration potential while also enhancing soil structure, nutrient retention, and water-use efficiency, and has been shown to reduce nitrous oxide (N_2O) and methane (CH_4) emissions from soils (Lehmann et al., 2011). Empirical studies demonstrate that co-application of biochar with organic manures or compost further improves soil physicochemical properties, crop productivity, and microbial community dynamics, highlighting the synergistic potential of integrated organic matter management strategies (Wang et al. 2019). Collectively, these practices not only enhance soil health and crop yields but also provide critical climate mitigation benefits by sequestering carbon and reducing greenhouse gas emissions, positioning them as vital tools for sustainable agriculture in a warming world.

Integrated nutrient management (INM) and precision fertilization

Integrated Nutrient Management (INM), which combines organic sources (e.g., manures, composts, crop residues) with judicious use of inorganic fertilizers, enhances soil fertility, synchronizes nutrient release with crop demand, and improves nitrogen use efficiency thereby reducing greenhouse gas emissions and enhancing resilience under climate variability Long-term field studies in rice–wheat systems demonstrate that INM significantly boosts soil organic carbon (SOC), aggregate stability, and soil health compared to sole inorganic treatments, while supporting sustained crop productivity (Paramesh et al. 2023). Empirical trials in dryland maize production reveal that INM using crop residue plus fertilizer substantially increases SOC (by up to ~52%) and enhances water retention, thereby bolstering drought resilience and productivity (Wang et al. 2023). Precision fertilization through spatial diagnostics, remote sensing, and targeted nutrient placement further refines INM by aligning

application timing and dosage with site-specific crop needs, minimizing nutrient losses (e.g., nitrate leaching, N_2O emissions), and enhancing fertilizer use efficiency (Bayu, 2020). Overall, the integrated application of INM and precision fertilisation offers a robust pathway for climate-smart agriculture, achieving dual goals of improved productivity and reduced environmental impact under changing climatic conditions.

Water management: mulching, micro-irrigation, and landscape retention

Effective water management strategies encompassing mulching, micro-irrigation, and landscape-level retention, play a critical role in enhancing agricultural resilience amid climate change. Mulching, whether organic (e.g., straw, gravel) or synthetic (e.g., plastic film), significantly reduces soil evaporation, moderates soil temperature, enhances moisture retention, suppresses weeds, and improves soil structure and fertility, thereby reducing irrigation demand and promoting water-use efficiency under arid and semi-arid conditions (El-Beltagi et al. 2022). Micro-irrigation systems, such as drip or trickle irrigation, deliver water precisely to the root zone, minimizing losses via evaporation or runoff; they enhance water-use efficiency, support deeper root development, and can be integrated with fertigation for optimized nutrient delivery (Bwire et al. 2024). The synergy between mulching and micro-irigation yields even greater benefits: combining mulching with drip systems can reduce water consumption by as much as 40–50%, decrease irrigation needs by 30%, and substantially boost crop yield and water productivity in vegetable production. At the landscape or catchment scale, soil mulching and micro-irrigation can lower the blue water footprint, delivering water savings of millions of cubic meters per year in some basins (Nouri et al. 2019). Furthermore, strategic landscape water retention such as enhancing soil organic carbon can markedly buffer yield losses under drought, as demonstrated in modeling studies showing that a 1% increase in soil organic carbon reduced potato yield loss from 16.4% to 7% during a severe drought (Heinz et al. 2025). Collectively, these practices form an integrated adaptation and mitigation framework, reducing vulnerability to shifting precipitation and water stress, stabilizing crop outputs, and enhancing efficiency and sustainability of agricultural systems in a warming climate.

Agroforestry, diversification, and perennial integration

Agroforestry, diversification, and perennial integration constitute complementary mitigation–adaptation strategies that increase the resilience and sustainability of agricultural landscapes by strengthening ecological function, buffering climatic shocks, and enhancing long-term productivity. Agroforestry by integrating trees and shrubs with crops and/or livestock modulates microclimate, reduces erosion and surface runoff, improves water and nutrient retention, and stores carbon in woody biomass and soil, thereby delivering both adaptation and mitigation co-benefits at field and landscape scales (Ghanbari et al. 2025). Crop and system diversification (spatial, temporal and genetic diversification, intercropping and mixed farming) reduces yield and income volatility, spreads climate and pest/disease risk across species and time, and improves soil health and biological pest regulation, making farm livelihoods less sensitive to extreme weather and market shocks (Mihrete and Mihretu, 2025). Integrating perennial species whether as tree crops, forages, silvopasture, or newly domesticated perennial grains reduces the need for annual tillage, increases below-ground carbon allocation through deeper, longer-lived root systems, curbs nutrient leaching and erosion, and can improve drought

resilience through more extensive water capture and use (Chapman et al. 2022). When combined in diversified, perennial-rich agroecosystems (for example agroforestry mosaics, perennial–annual polycultures, and mixed silvopasture), these approaches produce synergistic outcomes higher and more stable ecosystem services (carbon sequestration, water regulation, soil conservation), improved on-farm biodiversity, and reduced vulnerability of production systems to climate variability while also opening policy and market pathways for scaling adoption.

Biological and engineered soil amendments (microbial inoculants, engineered bio-products)

Biological and engineered soil amendments including microbial inoculants and cutting-edge bioengineered products offer promising pathways to bolster agricultural resilience amid climate change. Microbial inoculants, or plant growth–promoting microorganisms (PGPMs), improve soil health by enhancing nutrient cycling, increasing plant resilience to drought and salinity, and reducing reliance on synthetic fertilizers and pesticides a source of considerable greenhouse gas emissions (Díaz-Rodríguez et al. 2025). These inoculants help restore soil microbial communities degraded by intensive agriculture, enriching ecosystem functions crucial for climate mitigation and sustainable productivity (Campos-Avelar et al 2023). Furthermore, targeted applications of PGPMs in wheat under drought stress have demonstrated significant improvements in plant drought tolerance, biomass, and soil enzymatic activity highlighting their practical effectiveness under abiotic stress (Li et al. 2023). In parallel, engineered bio-products such as biochar produced via pyrolysis of organic biomass provide multifunctional benefits: sequestering carbon over centuries, enhancing soil water-holding capacity and nutrient retention, increasing microbial activity, and reducing emissions of nitrous oxide and methane (Shyam et al. 2025). Emerging biochar–microbe synergistic systems have shown spectacular results; for example, in calcareous soils, combining biochar with auxin-producing microbial inoculants (and L-tryptophan) improved canola yield by over 200%, while amplifying nutrient uptake and soil microbial activity (Mustafa et al. 2025). These methodologies underscore a powerful suite of mitigation and adaptation strategies: they enhance soil function, improve plant stress tolerance, and help sequester carbon all critical in transitioning toward climate-smart agriculture.

Policy, incentives, and carbon programs

Policy frameworks, targeted incentives, and carbon programs form a crucial triad for catalyzing climate-resilient agricultural transformations. Effective policy instruments ranging from subsidies, taxes, rural finance, land tenure reforms, extension services, to certification schemes, can significantly enhance the adoption of climate-smart agricultural (CSA) practices among smallholder and large-scale farmers, by reducing financial risk and aligning actions with public goods objectives (Van Asseldonk et al. 2023). Carbon programs, including credits for soil carbon sequestration or regenerative practices, offer a direct financial reward mechanism for climate-positive farming. Yet program design matters greatly: recent reporting highlights that in the U.S., while programs like the USDA's EQIP and related climate-smart initiatives channel billions in incentives, over half the funding may not effectively reduce greenhouse gas emissions; implementation gaps likewise limit program efficacy (The Guardian, 2024).

Similarly, corporate-led carbon credit initiatives, such as those by Indigo Ag, have supported farmers transitioning to regenerative agriculture by paying premiums for verified carbon credits, yet critics warn that delays in payments and inequitable cost-sharing can undermine farmer engagement (Reuters, 2024). Internationally, the European Union's Common Agricultural Policy (CAP) and eco-schemes offer adaptation and mitigation support via agri-environmental commitments and climate-related rural development funds, although recent critiques argue that policy alignment with climate goals remains inconsistent due to imprecise metrics and diluted incentives (European Commission, 2025). Collectively, these insights underscore that a blend of well-calibrated policies, robust incentive structures, and transparently governed carbon programs is essential for driving agricultural systems toward sustainability, resilience, and equitable climate mitigation.

FUTURE DIRECTIONS AND RESEARCH GAPS

Safeguarding soil health under climate change requires closing several scientific and implementation gaps. Below I synthesize the most important open questions highlighted in recent literature, then propose concrete research priorities to guide the next decade of work. Key thematic gaps are

1. Measurement, monitoring, reporting and verification (MRV) for soil carbon and other soil health indicators;
2. Mechanistic understanding of carbon stabilization and losses across depths and land-uses;
3. The soil microbiome as an actionable lever for resilience;
4. Scalable, location-specific practice packages and socio-economic pathways to adoption; and
5. Modelling and remote sensing tools for up-to-date, trustworthy decision support.

RECOMMENDATIONS

Safeguarding soil health in the era of climate change requires integrated approaches that bridge scientific research, land management, and socio-economic policy. Healthy soils underpin climate mitigation and adaptation by sequestering carbon, regulating water, and supporting biodiversity, yet they remain vulnerable to degradation from unsustainable practices, land-use change, and governance gaps. Embedding soil health explicitly into climate policies at national and international levels is therefore essential to meet long-term environmental and food security goals, as exemplified by initiatives such as the European Union's Green Deal.

At the same time, economic incentives play a pivotal role in aligning farmer and landowner decision-making with environmental objectives. Payment for ecosystem services, subsidies for agroecological practices, and programs that reward carbon sequestration can make sustainable land stewardship financially viable. Secure land tenure and robust governance frameworks further underpin these efforts, empowering communities to prioritize long-term soil health investments over short-term exploitation.

Moving forward, several priorities emerge for policymakers and practitioners. Developing standardized soil health indicators encompassing physical, chemical, and biological dimensions, will enable consistent monitoring and adaptive management across regions.

Greater investment in research and farmer education is equally vital, fostering innovation and knowledge transfer that accelerate the adoption of resilient practices. Moreover, the promotion of sustainable agricultural systems, including crop rotation, cover cropping, reduced tillage, and organic farming, offers proven pathways to strengthen soil functions and buffer climate extremes. Finally, broad-based public awareness and inclusive stakeholder engagement are critical to ensure that soil health remains a shared societal priority.

In sum, advancing soil health requires more than agronomic interventions; it demands coherent policies, supportive institutions, and active engagement across scales. By aligning science, practice, and governance, soil can be secured as both a natural capital asset and a cornerstone of climate resilience in the decades ahead.

CONCLUSION

Soil health is a cornerstone of sustainable development and climate resilience. Effective policies that integrate soil conservation into broader environmental and economic frameworks are essential for safeguarding this vital resource. By implementing the recommendations outlined above, governments can foster an environment where soil health is prioritized, leading to enhanced agricultural productivity, environmental sustainability, and climate change mitigation.

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PRE-TREATMENT WITH GILABURU EXTRACT ALLEVIATES H2O2-INDUCED CYTOTOXICITY IN ENDOTHELIAL CELLS

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ABSTRACT

Viburnum opulus L. (Gilaburu) is a traditional medicinal plant known for its antioxidant, anti-inflammatory and diuretic properties. It is rich in polyphenols, vitamin C, L-malic acid and other bioactive compounds that contribute to its potential as a natural protectant. In this study, the effects of Gilaburu (Glb) extract on oxidative stress-induced cytotoxicity in human endothelial cells were investigated. Treatment with Glb extract alone resulted in a significant, dose-dependent reduction in cell viability. Exposure to hydrogen peroxide (H_2O_2) caused marked cytotoxicity, simulating oxidative damage. However, pretreatment with a Glb extract of 100 µg/mL significantly improved cell viability compared to H_2O_2 alone, indicating a protective effect. These results suggest that Glb can attenuate oxidative stress and maintain endothelial cell integrity. Its traditional use in kidney and liver diseases, hypertension and menstrual cramps may be related to its antioxidant effects. Overall, *V. opulus* extract proves to be a promising natural cytoprotectant against oxidative damage in vascular cells.

Keywords: Cytotoxicity, Gilaburu, Hydrogen peroxide, Protective effect, *Viburnum opulus*

INTRODUCTION

Viburnum opulus L. is known in Turkey as “Gilaburu” and in Europe as “Guelder rose” (Cam M, Hisil Y, Kuscu A, 2007). *V. opulus* L. (VO) is a plant of the Viburnaceae family, formerly also used for Caprifoliaceae. Gilaburu (Glb) is a shrubby, perennial, fast-growing, white-flowered plant that can grow up to 2-4 meters long, sheds its leaves in winter, can live up to 300 years thanks to its ground shoots and starts to harvest 2-3 years after planting (Özer 2000, Çam 2005, Hızlısoy 2009).

Glb is used to treat kidney stones, liver, stomach, prostate and urinary bladder diseases, diabetes, mumps, rheumatism, hemorrhoids, nervous disorders, high blood pressure and menstrual disorders (Aksoy et al., 2004, Çam 2005). The Glb fruit contains vitamin K, viburnin, isovaleric acid, salicin, salinic acid, (+)-catechin, (+)-epicatechin, quercetin, resin and valeric acid, which gives the plant a unique odor. In addition to its use in the food industry, the Glb fruit, which contains a lot of vitamin C and antioxidant substances, is also used in the paint and printing ink industry (Kalyoncu et al., 2007).

Glb shows a strong protective (cytoprotective) effect against oxidative stress at the cellular level thanks to its rich phenolic compounds, flavonoids, anthocyanins and proanthocyanidins. Numerous studies have shown that Glb extract supports the antioxidant defense system by reducing cell damage caused by free radicals. In particular, a study on pancreatic MIN6 beta cells found that Glb fruit phenols increase cell viability against hydrogen

peroxide (H_2O_2)-induced oxidative stress, reduce the production of ROS (reactive oxygen species) and maintain mitochondrial membrane potential (Turkcan et al., 2020) In addition, in the acid-induced colitis model, Glb fruit suppresses oxidative tissue damage as well as inflammation in intestinal tissue; it was found to protect tissue integrity by increasing antioxidant enzyme levels (Koyuncu et al., 2020). These results show that the cell-protective effect of Glb extract is largely dependent on strengthening the antioxidant system and maintaining oxidative balance.

The aim of this study was to investigate the potential cytoprotective effects of Glb against oxidative stress-induced cytotoxicity in HUVEC cells. Specifically, the dose- and time-dependent effects of Glb extract on cell viability were to be investigated both alone and in the presence of H_2O_2, a known trigger of oxidative damage. The results contribute to the understanding of *V. opulus* as a potential natural antioxidant and cytoprotective agent for use in oxidative stress associated with vascular disease.

MATERIAL AND METHOD

This study used human umbilical vein endothelial cells (HUVECs) obtained commercially from the American Type Culture Colleciton (ATCC). HUVEC cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin (100 IU/mL and 100 µg/mL, respectively), and 2 mM L-glutamine. The HUVEC cells were maintained at 37 °C in a humidified atmosphere with 5 % CO_2. For cell viability experiments, cells were seeded in 96-well plates at a density of 2×10^4 cells/well and allowed to adhere overnight before treatment.

Cell viability was determined using the WST-1 assay (Biovision, Germany). For this purpose, HUVEC cells were treated with Glb at concentrations of 1, 10, 50, 100 and 200 µg/mL for 24 and 48 hours to determine dose- and time-dependent effects. To induce oxidative stress, cells were treated with H_2O_2 at concentrations of 50, 100 and 200 µM. To evaluate the protective effect, cells were pretreated with Glb (50, 100 and 200 µg/mL) for 24 and 48 hours and then exposed to 100 µM H_2O_2 for 24 or 48 hours. After the treatments, 10 µL of WST-1 reagents were added to each well and incubated for 1 hour. The absorbance was measured at 450 nm using a microplate reader. Cell viability was calculated as a percentage compared to untreated control cells.

All experiments were performed in triplicate and data were expressed as mean ± standard deviation (SD). Statistical analyses were performed using GraphPad Prism software (version 10). A one-way analysis of variance (ANOVA) was performed to determine significant differences between groups, followed by a Tukey post hoc test for multiple comparisons. A p-value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The effects of Glb and H_2O_2 on endothelial cell viability were evaluated by WST-1 analysis (Figure 1). Glb treatment resulted in a significant, concentration- and time-dependent decrease in human endothelial cell viability. At both 24 and 48 hours, cell viability was reduced in all Glb-treated groups compared to control, with statistical significance at each concentration (Figure 1A, $p<0.0001$). Exposure to 50 µg/mL and higher concentrations resulted in a significant decrease in viability, with 200 µg/mL reducing cell viability by more than 50% after 48 hours (Figure 1A). These results indicate that the Glb extract exerts cytotoxic effects on endothelial cells at higher concentrations and longer exposure times. This dose-dependent cytotoxicity may be attributed to the presence of bioactive compounds in the extract that exert prooxidant or membrane disrupting effects at higher concentrations, which is consistent with previous findings on plant-derived polyphenolic compounds (Wang et al., 2018; Galati and O'Brien, 2004). Furthermore, H_2O_2 exposure resulted in a progressive decrease in cell viability, with the most dramatic effect occurring at 200 µM, especially after 48 hours (Figure 1B). The cytotoxic profile of H_2O_2 served as a model for oxidative stress against which potential protective effects of Glb could be compared in subsequent experiments. These results provide a basis for evaluating the antioxidant or protective role of Glb against H_2O_2-induced oxidative damage in endothelial cells.

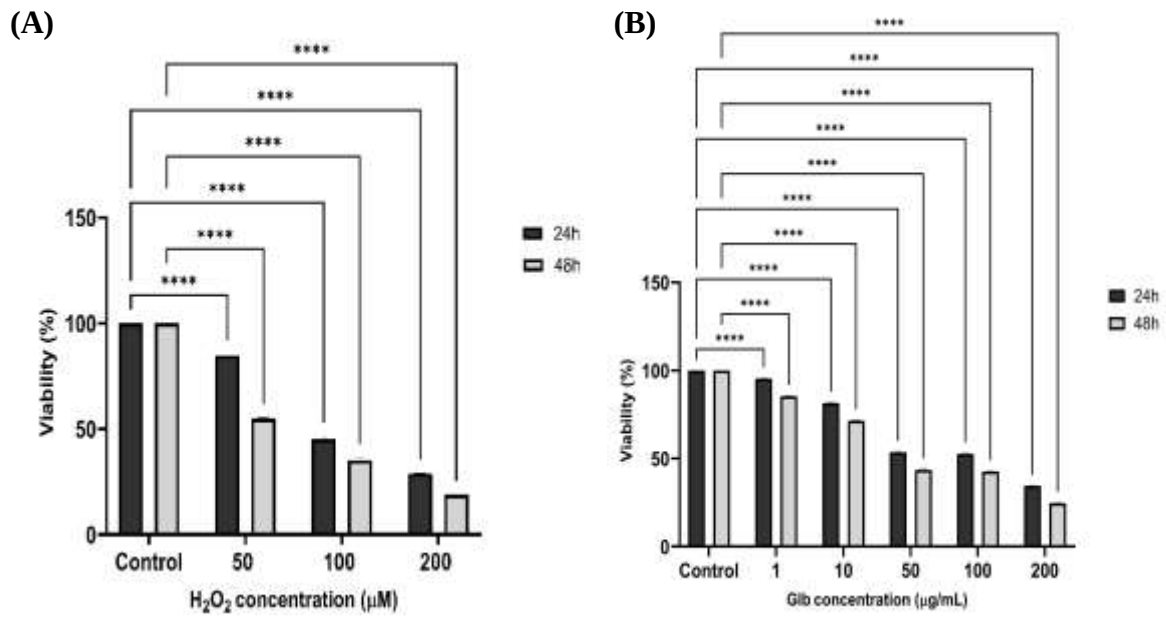

Figure 1. Effects of Glb and H_2O_2 on the viability of HUVEC cells. Cells were treated with increasing concentrations of (A) Glb (1–200 µg/mL) and (B) H_2O_2 (50–200 µM) for 24 and 48 hours. Statistical significance compared to control: ****$p < 0.0001$.

To evaluate the protective effect of Glb against oxidative stress, endothelial cells were pretreated with different concentrations of Glb before being exposed to 100 µM H_2O_2 (Figure 2). As expected, H_2O_2 alone caused a significant reduction in cell viability after both 24 and 48 hours. However, pretreatment with Glb significantly attenuated this cytotoxic effect. The 100 µg/mL Glb group showed the highest recovery of viability after 24 hours, indicating an optimal concentration for the protective effect (Figure 2). This protective effect is likely due to the

antioxidant components in Glb extract, such as flavonoids, phenolic acids or other redox-active molecules known to scavenge reactive oxygen species (ROS) or enhance endogenous antioxidant defense mechanisms (Rahman et al., 2021; Heim et al., 2002). Although the 200 µg/ml dose also provided partial protection, its effect was less pronounced, possibly due to intrinsic cytotoxicity at higher concentrations. These results demonstrate the potential antioxidant and cytoprotective properties of Glb against oxidative damage in endothelial cells. The observed decrease in protection at 200 µg/mL may reflect a biphasic (hormetic) dose-response curve in which antioxidant effects dominate at lower doses, while cytotoxic effects occur at higher concentrations, a phenomenon observed with other phytochemicals such as quercetin and resveratrol (Calabrese et al., 2010). The current results highlight the therapeutic potential of Glb as a cytoprotective agent against oxidative stress, provided that its concentration is carefully optimized. Future mechanistic studies are warranted to elucidate the specific molecular pathways through which Glb exerts these effects, including its influence on intracellular ROS levels, expression of antioxidant enzymes (e.g. SOD, catalase, GPx) and apoptotic signaling cascades.

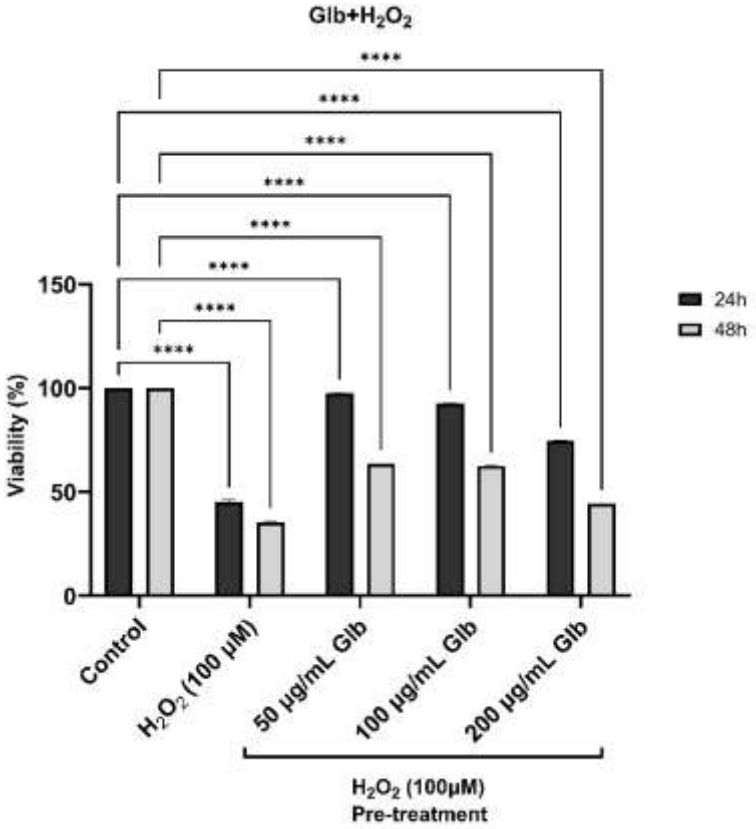

Figure 2. Protective Effect of Glb against H_2O_2-induced cytotoxicity in HUVEC cells. Cells were pre-treated with Glb at concentrations of 50, 100, and 200 µg/mL for 24 and 48 hours, followed by exposure to 100 µM H_2O_2 for 24 or 48 hours. Statistical significance: ****$p < 0.0001$ vs. control.

CONCLUSIONS

In summary, Glb shows promising cytoprotective properties, possibly due to its content of bioactive phytochemicals. However, the cytotoxic effects observed at high concentrations underline the importance of dose optimization for future therapeutic application. Further studies are required to elucidate the underlying molecular mechanisms and to assess the in vivo relevance of these results

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A PRELIMINARY ANALYSIS OF FUNCTIONAL TRAIT COMPOSITION IN ADRIATIC SEA FISHERIES

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ABSTRACT

Natural and anthropogenic drivers have both impacted marine ecosystems over the years, altering ecosystem structure and functionality. These external drivers can affect multiple levels of biological organizations, disrupting species interactions, recruitment dynamics and trophic structure, potentially leading to cascading effects throughout the ecosystem. In this context, trait-based approaches are becoming more widely acknowledged as useful instruments enhancing current ecosystem-based fisheries management. In this study, we investigated the temporal dynamics in the functional traits in the Adriatic fisheries landings over 5 decades (1970-2020). A landings dataset comprising 27 taxonomic groups was transformed into 18 functional trait categories related behaviour, ecology, distribution and biology. Dirichlet regression was applied to all trait categories in order to investigate the dynamics of individual traits. Over the last five decades, the community's trait composition has undergone notable transformations. Traits such as zoobenthivore, stenobathic, and winter spawning have become more prominent, whereas features like short lifespan, piscivore, and summer spawning have steadily diminished. Meanwhile, other characteristics including thermal tolerance and distribution range have remained largely stable, reflecting resilience to broader ecological shifts. Expanding the application of trait-based analyses may offer valuable insights for refining fisheries management strategies and safeguarding ecosystem functionality in the face of ongoing change.

Keywords: functional traits, fisheries landings, Adriatic Sea

INTRODUCTION

Marine ecosystems have been impacted by both natural variability and human activities, leading to alterations in ecosystem structure and functioning (Pecl et al., 2017; O'Hara et al., 2021). Through the continuous interplay of these external drivers, ecosystems have faced multiple consequences, often described in terms of changes in fish population abundance and species turnover (Barausse et al., 2011; Fortibuoni et al., 2017). Instead, the ecological significance of these processes lies in the resulting functional changes they induce, which ultimately determine ecosystem functioning (Beisner et al., 2003; Mouillot et al., 2013). Ecosystem integrity, therefore, depends not only on species abundances, but also on the traits and ecological roles of these species within the community. (Hooper et al., 2005).

In the Adriatic Sea, fishing pressure and warming have caused significant alterations in community composition. These include declines in large, long-lived piscivores and an increasing dominance of smaller, short-lived species that occupy lower trophic levels (Barausse et al., 2011; Sguotti et al., 2022). These processes have further facilitated the northward expansion of thermophilic species (Azzurro et al., 2019), consistent with broader trends observed across the Mediterranean basin (Fortibuoni et al., 2017). Such trait alterations, as observed in the Adriatic Sea, have important implications for fundamental ecosystem processes,

including functional redundancy and energy flow through food webs, which in turn influence ecosystem productivity and vulnerability to disturbances (Hooper et al., 2005; Palkovacs et al., 2012; Biggs et al., 2020). Given the strong influence of external drivers on ecosystem dynamics and functioning, trait-based approaches are increasingly recognized as valuable tools for strengthening ecosystem-based fisheries management (Barnett et al., 2019). By addressing the current limitations, they offer a pathway toward more effective and adaptive strategies to safeguard marine resources under changing environmental conditions (Costa et al., 2024)

In this study, we investigated five decades of Adriatic fisheries landings to examine temporal trends in trait modalities across biological, distributional, ecological, and behavioural categories, providing new insights into ecosystem responses to external drivers.

MATERIALS AND METHODS

Data

Fisheries landings in the Adriatic Sea were analyzed over 1970–2020 using Food and Agriculture Organization (FAO) data. Based on the requirements of the Data Collection Framework (DCF) and national reporting practices, these landings data are reported either at species level or at higher taxonomic levels (e.g., genus or family). To ensure data consistency during the whole study period, analyses were restricted to taxa with continuous records across the study period. Specifically, species with gaps exceeding 15 consecutive years were excluded from the analyses. In total, 27 groups were retained, including 11 at species level, 7 at the genus level, 5 at the family level and 4 taxa including species from more than one family. For groups aggregated above the species level, the dominant contributor to total landings was taken as representative.

Using available information from existing literature, an additional dataset was constructed including information regarding various categories of functional traits such as; biology, distribution, ecology and behaviour. Each trait category included up to five distinct modalities, resulting in a total of 61 trait modalities. Each species was assigned to only one modality per trait category, based on attributes characteristic of the adult stage. To quantify temporal changes in the functional structure of the fish community, the Community Weighted Mean (CWM) was calculated for each trait modality using the following equation:

$$CWM = \sum_{i=1}^{S} p_i x_i$$

where p_i represents the relative abundance of species i in a given year and x_i is a binary value indicating the presence (1) or absence (0) of a given trait modality for that species.

We calculated relative abundance by comparing each species' yearly catch to the total catch of all species in that year. This way, the influence of each species on trait expression reflects how important it was in the community. The method was applied to all 61 trait categories, giving yearly values of community traits from 1970 to 2020.

Statistical analyses

To investigate the dynamics of individual functional traits, we applied Dirichlet regression, which is particularly suitable for modelling continuous proportional data and reduces common violations of statistical assumptions often encountered in ecological analyses (Douma and Weedon, 2019). Models were fitted in R with the DirichletReg package, incorporating a smoothed effect of year as the predictor of trait modality proportions.

RESULTS AND DISCUSSION

To investigate temporal variation in trait modalities across functional categories, we applied Dirichlet regression, which revealed distinct patterns over the past five decades. Traits such as zooplankton feeding, stenobathic species, and winter spawners increased in prevalence, whereas short-lived species, piscivores, and summer spawners declined. Meanwhile, some modalities, including stenothermal, eurythermal, and globally distributed species, remained largely unchanged. Among the four categories, biological and ecological traits displayed the most contrasting dynamics, while distribution and behavioural traits showed stability in their dominant modalities.

Across the study period, community biological traits showed contrasting dynamics in relation to longevity, body size, and body form. Species with intermediate lifespans (10–19 years) increased significantly from the mid-2000s, becoming dominant, whereas those with 5–9 years longevity declined after peaking during the same period. Short-lived (<5 years) and long-lived (≥20 years) species remained consistently low and stable (Figure 1, left). Body size composition was characterized by the persistent dominance of the 20–50 cm group, while very large (>100 cm), large (50–100 cm), and small (<20 cm) species consistently represented a minor fraction, showing little temporal variation and suggesting structural stability in size distribution (Figure 1, middle). Similarly, body form composition remained largely unchanged, with atractoid species predominating throughout the whole time series and flat, rounded, and deep-bodied forms contributing only marginally (Figure 1, right).

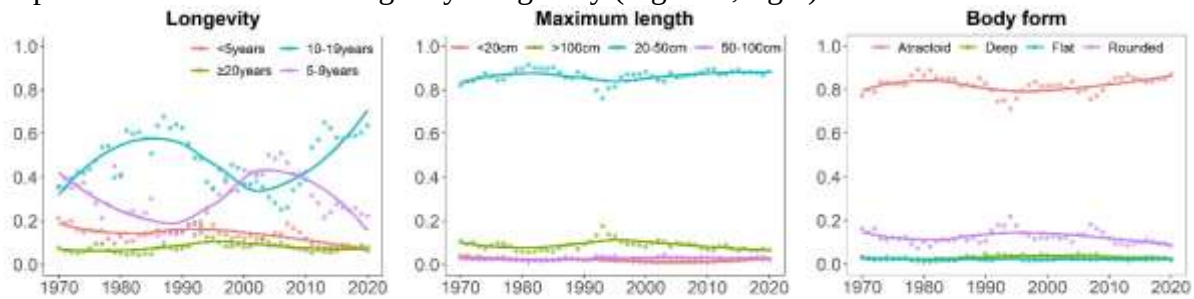

Figure 1. Temporal dynamics of biological trait categories, with fitted trajectories estimated using Dirichlet regression.

Community distribution traits displayed clear and persistent trends, without major shifts or reversals (Figure 2). Optimal temperature preferences were dominated by species associated with 15–20 °C, which remained stable, while both cooler (<15 °C) and warmer-affinity (20–25 °C, 25–30 °C) species contributed minimally. Depth composition was dominated by 50–200 m species, which increased slightly after the 1990s following an abrupt decline; >200 m species rose modestly until the early 1990s and then returned to initial levels, whereas 0–50 m taxa remained rare and stable. Habitat type was consistently dominated by pelagic species, which increased steadily since the 1990s, while benthic and benthopelagic species stayed in low levels. Distribution ranges were largely dominated by temperate species, with global and subtropical species contributing only marginally and showing little variation. Seabed morphology reflected an increasing dominance of water-column species alongside a decline in soft-bottom taxa and minor contributions from variable-substrate species. Spawning habitat was characterized by the persistent dominance of pelagic spawners, which increased steadily, whereas benthic spawners declined slightly and remained low in prevalence.

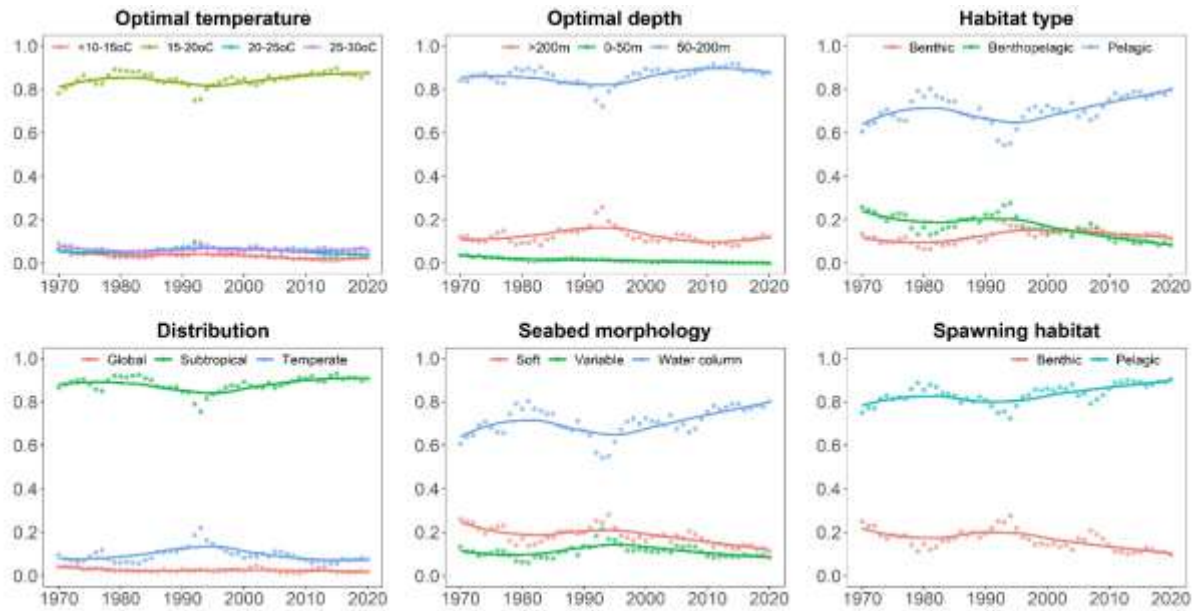

Figure 2. Temporal dynamics of distribution trait categories, with fitted trajectories estimated using Dirichlet regression.

Ecological traits exhibited more diverse patterns, with both stability and shifts across modalities (Figure 3). Diet remained dominated by zooplanktivores throughout the time series, with their contribution increasing further from the early 1990s, while piscivores declined. Trophic level was consistently dominated by level 3 species, which became even more prevalent over time, whereas level 4 species declined. Salinity range was likewise dominated by euryhaline species, which increased further from the early 1990s, while stenohaline taxa decreased. Temperature range showed a stable dominance of eurythermal species, with stenothermal species remaining at low levels. Depth range and spawning period were characterized by shifts in dominance between modalities: stenobathic species increased markedly from the early 2000s, overtaking eurybathic species, and winter spawners increased from the late 2000s while summer spawners declined; autumn, spring, and year-round spawners remained at consistently low levels.

By comparison, behavioural traits showed remarkable stability, with no major changes in the dominant modalities (Figure 4). The assemblage was consistently characterized by grazing species with high mobility that formed schools, while active predators, ambushers, solitary species, and taxa with lower mobility remained comparatively minor components.

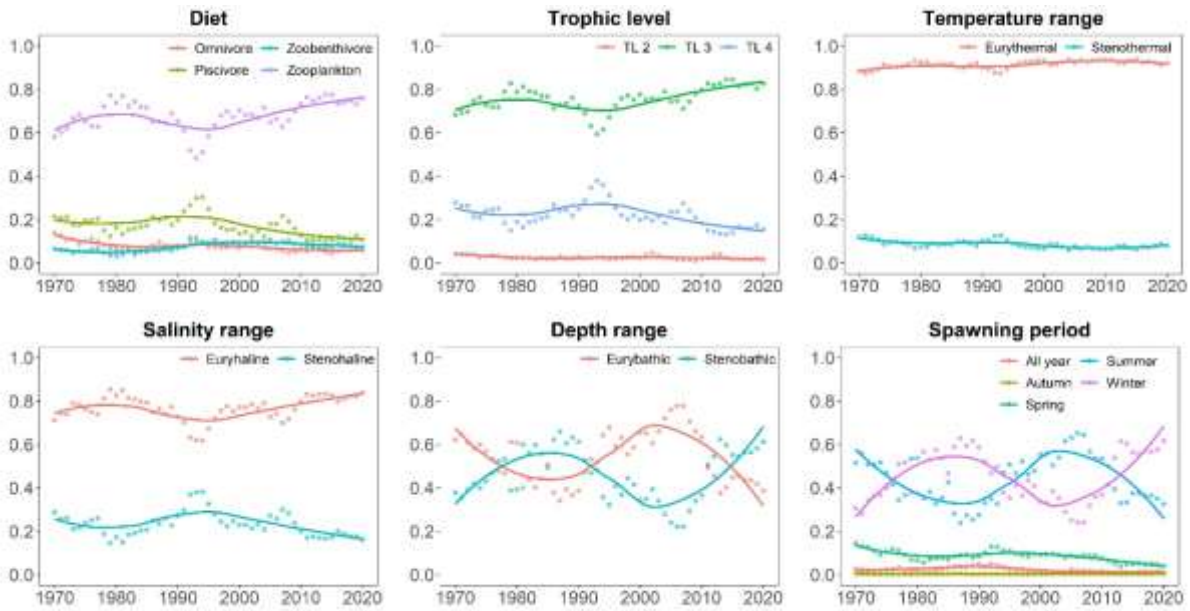

Figure 3. Temporal dynamics of ecological trait categories, with fitted trajectories estimated using Dirichlet regression.

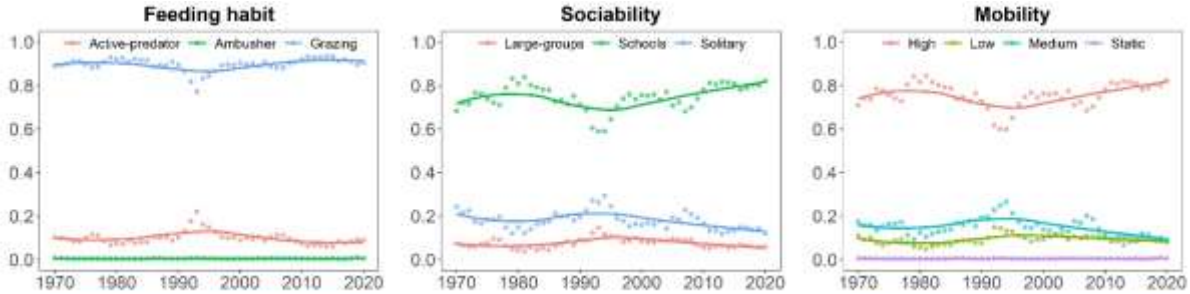

Figure 4. Temporal dynamics of behaviour trait categories, with fitted trajectories estimated using Dirichlet regression.

CONCLUSIONS

Overall, the analysis indicates that while some traits have shifted markedly in response to external pressures, others have remained stable, revealing both vulnerability and persistence within the Adriatic fish community. The growing dominance of smaller, short-lived, thermophilic species aligns with broader regional trends, whereas the stability of distributional and behavioural traits points to a degree of functional continuity despite shifts in species composition (Fortibuoni et al., 2017; Sguotti et al., 2022). Collectively, these findings highlight the value of trait-based approaches for uncovering ecosystem responses and supporting ecosystem-based fisheries management strategies.

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SHORT-TERM EFFECTS OF DIFFERENT ORGANIC FERTILIZERS ON SOIL CHEMICAL PROPERTIES AND PLANT GROWTH IN MELON AND WATERMELON CULTIVATION

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ABSTRACT

The integration of organic fertilizers into horticultural systems has gained increasing importance as a sustainable strategy to enhance soil fertility and crop productivity under challenging soil conditions. This study aimed to evaluate the effects of various organic fertilizers including manures from small ruminants, cattle, poultry, and vermicompost on the chemical properties of a sandy-calcareous soil and the growth performance of melon (*Cucumis melo L.*) and watermelon (*Citrullus lanatus Thunb.*). The study focused on how recommended fertilizer doses influence soil chemical quality and plant growth. The results indicated that organic fertilization altered soil electrical conductivity (EC) and available phosphorus (P) levels. Poultry and cattle manures led to notable improvements in soil nutrient content. Additionally, plant growth parameters such as stem diameter and fruit weight were positively affected by organic fertilizers, particularly poultry manure. Crop-specific responses were observed: melon plots exhibited higher soil EC and available P, while watermelon plants showed superior performance in stem diameter, fruit flesh thickness, and soluble solid content. In contrast, melon plants recorded higher chlorophyll levels. Overall, poultry manure emerged as the most effective input for enhancing plant growth, while vermicompost and cattle manure also contributed depending on the crop type. These results suggest that organic fertilizers, especially poultry manure, are valuable tools for improving soil fertility and crop productivity in calcareous soils.

Keywords: Calcareous soils, Fruit quality, Mediterranean climate, Organic amendments

INTRODUCTION

Sustainable agricultural systems are an emerging model that promotes the integrated use of organic materials as alternatives to chemical inputs are being developed to ensure more environmentally friendly and efficient food production. In this context, organic fertilizers that enhance soil fertility have come to the forefront. The core component of organic fertilizers, organic matter, serves as a primary source of energy and nutrients for soil-dwelling microorganisms. However, in Turkey and many other agricultural countries, inadequately matured animal manures characterized by high C/N ratios and the presence of root rot pathogens or weed seeds have long been used in crop production. These manures often fail to provide the expected agronomic benefits for both soil and plants (Karaman 2012).

In recent years, there has been a growing demand particularly in intensively cultivated horticultural and greenhouse production areas for thermally treated or fermented organic fertilizers. This trend reflects efforts to promote sustainable agriculture and to optimize the combined use of organic and chemical fertilizers. Nevertheless, these processed fertilizers are significantly more expensive than traditionally matured manures, due to additional processing, packaging, registration, and storage costs. Furthermore, their claimed benefits for practical use often rely more on manufacturer assertions than on robust scientific evidence. Therefore, it is essential to evaluate their effects on soil fertility and plant growth in order to better understand their potential contribution to sustainable agricultural systems and to promote their effective and economical use.

Melon (*Cucumis melo* L.) and watermelon (*Citrullus lanatus* Thunb.), among the most widely produced and consumed vegetables globally and in Turkey, are annual crops adapted to warm and temperate climates. While both crops are cultivated in open fields and greenhouses, only about 3% of melon and 2% of watermelon production is conducted under protected cultivation (Anonymous 2001; Taşkaya and Keskin 2004). These crops are valued not only for their edible parts but also for their seeds and rinds, which are utilized across the food, pharmaceutical, and cosmetic industries, making them strategic export commodities. Deep, well-drained, sandy-loam or loamy-sand soils with high water-holding capacity are ideal for watermelon cultivation, while poorly drained soils with shallow groundwater levels (below 1 meter) are unsuitable (Tuna and Özer 2005). Like many other cultivated plants, melons and watermelons respond positively to organic fertilization. Accordingly, the application of solid and/or organic fertilizers in the cultivation of these crops is considered important for improving both yield and quality, as well as enhancing soil health (Bayram et al. 2020).

In this study, the effects of processed organic fertilizers including sheep, cattle, and poultry manures, as well as vermicompost on the chemical properties of calcareous sandy soils and on plant growth in melon and watermelon cultivation were investigated. Additionally, the study aimed to assess how the optimum application rates recommended by organic fertilizer manufacturers for plant growth influence soil fertility. By comparing the effects of these commonly used organic fertilizers on both soil chemical parameters and plant growth indicators, this study identified the strengths and weaknesses of each fertilizer type. The findings offer valuable insights into the practical use of organic fertilizers in sustainable farming systems.

MATERIAL AND METHODS

Site Description

This study was conducted as a field trial at the Finike campus of Akdeniz University (36°18′31″N, 30°08′48″E; altitude: 40 m). According to meteorological data collected during the trial period, the site had an average daily temperature of 19.3 °C, an average daily sunshine duration of 9.10 hours, an average daily open surface evaporation of 125.58 mm, an average daily rainfall of 1.4 mm, an average daily wind speed of 1.6 m/sec, and an average relative humidity of 66.03%.

Soil samples collected to represent the trial area were analyzed prior to the experiment. The soil was classified as sandy loam in texture. It had a neutral pH and was classified as non-

saline. The soil also contained a high level of lime and was rated as sufficient in terms of humus content. Total nitrogen (N) and available phosphorus (P) levels were determined to be adequate, while the cation exchange capacity (CEC) was classified as low.

Organic Fertilizers and Fertilization Practices

In this trial, various heat-treated organic fertilizers (processed at 65–70 °C) were applied, including small ruminant manure (goat and sheep manure; T1), cattle manure (T5), poultry manure (T4), and vermicompost (T2) (Table 1). Additionally, control (T3) plots were established, which received only chemical fertilizers (detailed below) without any organic fertilizer input. The application rates of the organic fertilizers were determined based on the manufacturers' recommendations and supported by findings from previous studies (Maltaş 2023; Maltaş et al. 2024). This approach aims to develop practical organic fertilization strategies suitable for field conditions, specifically for melon and watermelon cultivation, while improving soil chemical properties and supporting plant growth.

Table 1. Some properties of organic fertilizers used in the experiment

Parameter	Organic fertilizer			
	Vermicompost	Chicken manure	Cow manure	Goat-sheep manure
Organic matter, %	45	62	35	70
EC (dS/m)	6.0	2.6	11.2	7.5
Organic C, %	26.1	35.96	20.3	40.6
Humic/fulvic acid, %	41.5	36	15	28
Total N, %	3.0	2.2	1	1.5
Total P, %	1.5	1.76	1.5	1.5
Total K, %	1.0	1.62	-	2
Humidity max., %	20	20	20	20
pH	6-8	6.2	7-9	8-10
C/N	9/1	16/1	20/1	27/1
C/P	17/1	20/1	13/1	27/1

The application rates were as follows: 4 Mg ha^{-1} for vermicompost, 8 Mg ha^{-1} for cattle manure, 6 Mg ha^{-1} for goat manure, and 4 Mg ha^{-1} for poultry manure. In addition to these inputs, chemical fertilizers certified for use in organic farming were applied through a drip irrigation system to support plant nutrition throughout the experimental period. These chemical inputs were selected based on the initial soil analysis and estimates of nutrient removal by the crops (Zengin 2023).

Previous studies have demonstrated that the use of organic mulching materials (such as straw, sawdust, and compost) can improve microclimatic conditions and enhance the effectiveness of organic fertilizers (Gao et al. 2019; Teasdale and Mohler 2000). Mulching has been shown to promote soil biological activity, thereby improving nutrient uptake efficiency by plants and contributing to sustainable farming practices (Li et al. 2019). Therefore, in this study, a mulch was applied to the experimental plots to maximize the effectiveness of organic fertilizers.

Implementation of the Experiment

In this study, melon and watermelon cultivation was carried out using four types of heat-treated organic fertilizers. The experimental setup was established according to a randomized block design with three replications. The organic fertilizers were applied uniformly to the plots based on their respective application rates (in dry weight).

Melon (*Cucumis melo L.*) and watermelon (*Citrullus lanatus Thunb.*) seedlings were procured from a commercial nursery. Melon seedlings (Anka F1) were transplanted at a spacing

of 2.0 m between rows and 0.3 m within rows, while watermelon seedlings (Peykan F1) were planted at 2.0 m between rows and 0.6 m within rows. Both crops were cultivated over a vegetation period of approximately 90–100 days.

Throughout the growing period, all essential cultural practices such as weed control, hoeing, and plant protection treatments were carried out. In addition, irrigation and fertigation were performed using a drip irrigation system. The daily irrigation requirements of the plants were calculated based on climatic parameters (including rainfall, temperature, and relative humidity), soil texture and moisture, and plant needs (e.g., signs of wilting). These water requirements were integrated with the optimized fertilization strategy to ensure proper crop management.

Sampling and Analysis

Soil samples were air-dried and sieved through a 2 mm mesh prior to analysis. To characterize the initial soil conditions, samples were collected from the 0–30 cm soil depth using an auger before the experiment began. These samples were analyzed for texture, pH, electrical conductivity (EC), lime content, organic matter, total nitrogen (N), available phosphorus (P), and cation exchange capacity (CEC). Additionally, to evaluate the effects of organic fertilizer applications on soil chemical properties, samples collected at harvest were analyzed again for organic matter, total nitrogen, available phosphorus, and CEC.

The methods used for analyzing the above-mentioned soil parameters were as follows: soil texture was determined using the hydrometer method (Bouyoucos 1955); pH and EC were measured in a 1:2.5 soil-to-water suspension (Jackson 1967; Anonymous 1982); lime content was determined using the calcimeter method (Çağlar 1949); organic matter was measured using the modified Walkley-Black method (Black 1965); total nitrogen was determined using the Kjeldahl method (Kacar 1995); available phosphorus was analyzed according to the Olsen method (Olsen and Sommers 1982); and CEC was determined using the ammonium acetate method (Kacar 1995).

At the end of the experiment, pomological and physiological traits were assessed in plant and fruit samples (Figure 1 and Figure 2). For each treatment replication, measurements and evaluations were performed on three randomly selected plants per plot. The results were expressed as the average of these three plants.

The evaluated pomological characteristics included: stem base diameter (cm; measured with a caliper), fruit fresh weight (g; measured with a precision balance), and yield (kg da^{-1}; calculated). The physiological parameters included: soluble solids content (SSC), fruit juice pH, total chlorophyll content (measured with a chlorophyll meter), and rind thickness (mm; measured with a caliper).

Figure 1. Views of selected physiological and pomological traits in watermelon plants (A: Soluble solids content, B: Chlorophyll content, C: Fruit flesh thickness, D: Stem base diameter, E: Fruit juice pH, F: Fruit weight)

Figure 2. Views of selected physiological and pomological traits in watermelon plants (A, C: Harvested fruits; B: Fruit juice pH)

Statistical Analyses

The numerical data obtained in this study were statistically analyzed using the SPSS 23.0 software package. The significance levels of soil chemical properties and plant pomological and physiological parameters were determined through analysis of variance (ANOVA) at the 5% significance level. Parameters showing statistically significant differences were further evaluated using Duncan's multiple range test for mean separation. In addition, Pearson correlation analysis was performed to determine the relationships among the measured parameters (SPSS 2008).

RESULTS AND DISCUSSION

Soil Chemical Properties

At the end of the growing season, the effects of different organic fertilizer treatments on soil cation exchange capacity (CEC), organic matter (OM) content, total nitrogen (N%), and available phosphorus (P, mg/kg) were evaluated for watermelon cultivation (Table 2). According to the statistical analysis, changes in CEC, total N, OM (%), and available P values in watermelon plots were found to be statistically non-significant.

Table 2. Effects of applications on CEC, OM, total N and available P contents in watermelon soil

Treatments	CEC (meq/100)	% OM	% N	P (mg/kg)
T1	26.54	2.26	0.16	22.17
T2	29.33	2	0.15	19.47
T3	31.04	2.1	0.16	13.36
T4	28.11	2.17	0.18	18.52
T5	30.58	1.92	0.18	38.54
ANOVA (%5)	NS[1]	NS	NS	NS

[1] NS; not significant.

In contrast, in melon cultivation plots, although changes in CEC, total N, and OM (%) were also statistically non-significant, the variation in available phosphorus content was statistically significant at the $P < 0.05$ level (Table 3). Among the treatments, T4 (48.60 mg/kg) resulted in the highest increase in available phosphorus content compared to the other applications.

Table 3. Effects of applications on CEC, OM, total N and available P contents in melon soil

Treatments	CEC (meq/100)	% OM	% N	P (mg/kg)
T1	32.82	2.29	0.15	13.31 b[1]
T2	30.99	2.26	0.16	23.72 ab
T3	30.10	1.85	0.14	9.36 b
T4	29.32	1.97	0.13	48.60 a
T5	29.68	2.00	0.17	40.04 ab
ANOVA (%5)	NS	NS	NS[3]	2.966 **[2]

[1] Differences between values not indicated by the same letter are significant at the 5% level. [2] **; significant at the 1% level. [3] NS; not significant.

Previous studies have indicated that increasing doses of poultry manure significantly contribute to the enhancement of soil nitrogen content (Okur et al. 2008), while farmyard manure has been found to have a more pronounced effect on available phosphorus levels in soils (Agbenin and Goladi 1997). In the present study, the available phosphorus level in soils under melon cultivation (T4: 48.60 mg/kg) was approximately 20% higher than that in watermelon soils.

Göksu and Kuzucu (2017), in their study investigating the effects of different vermicompost doses on yield and quality parameters in watermelon, reported that vermicompost and chemical fertilizer applications increased the soil ion exchange capacity.

However, in the current study, organic fertilizer treatments did not result in statistically significant changes in CEC for either melon or watermelon soils.

Similarly, Kılıç and Sönmez (2019), in their study on the effects of increasing doses of various organic materials (vermicompost, poultry manure, farmyard manure, leonardite) on soil fertility parameters in lettuce cultivation, found that the effects on soil organic matter content were statistically significant. In contrast, the findings of the current study showed that different organic matter applications had no statistically significant effect on soil organic matter levels in either melon or watermelon cultivation.

Plant Growth Properties

At the end of the cultivation period, among the yield and quality parameters obtained from the watermelon plant, leaf chlorophyll content (SPAD), fruit flesh thickness, fruit juice pH, and soluble solid content were found to be statistically non-significant (Table 4). However, collar diameter was found to be statistically significant at the $P < 0.01$ level, with higher values observed in T1 and T4 (23.41 mm and 23.23 mm, respectively) compared to other treatments. Fruit weight was also found to be statistically significant at the $P < 0.05$ level, with higher fruit weights recorded in T2, T4, and T5 (7.38 kg, 8.13 kg, and 7.52 kg, respectively).

Table 4. Effects of treatments on SPAD, root collar diameter (RCD), fruit weight (FW), fruit flesh thickness (FFT), fruit juice pH (FJpH) and soluble solid content (SSC) in watermelon

Treatments	SPAD	RCA (mm)	FW (kg)	FFT (cm)	FJpH	SSC
T1	37.13	23.41 a[1]	6.99 ab	16.13	5.34	8.38
T2	38.99	17.74 c	7.38 a	15.53	5.60	9.26
T3	34.48	15.95 c	5.50 b	17.55	5.70	8.43
T4	34.71	23.23 a	8.13 a	14.29	5.49	8.70
T5	31.01	20.08 b	7.52 a	10.31	5.46	8.71
ANOVA (%5)	NS[4]	21.282 ***[3]	3.575**[2]	NS	NS	NS

[1] Differences between values not indicated by the same letter are significant at the 5% level. [2] **; significant at the 1% level. [3] ***; significant at the 0.1% level. [4] NS; not significant.

Regarding the melon plant, leaf chlorophyll content (SPAD), fruit flesh thickness, fruit juice pH, and soluble solid content were also found to be statistically non-significant (Table 5). However, root collar diameter was found to be statistically significant at the $P < 0.05$ level, with higher values observed in T1, T3, and T4 (14.67 mm, 14.83 mm, and 14.85 mm, respectively). Fruit weight was found to be statistically significant at the $P < 0.01$ level, with the highest fruit weight observed in T1 (2.02 kg).

Üçok et al. (2019), in their study investigating the effects of different organic fertilizer applications on yield, quality, and plant nutrient contents in lettuce (*Lactuca sativa* L. var. crispa), reported no significant differences in chlorophyll content among the treatments. Similarly, in our study, although the differences between applications were not statistically significant for both watermelon and melon plants, the chlorophyll value in melon was found to be approximately 20% higher than in watermelon.

Table 5. Effects of treatments on SPAD, root collar diameter (RCD), fruit weight (FW), fruit flesh thickness (FFT), fruit juice pH (FJpH) and soluble solid content (SSC) in melon

Treatments	SPAD	RCA (mm)	FW (kg)	FFT (cm)	FJpH	SSC
T1	42.53	14.67 ab[1]	2.02 a	9.78	5.93	4.80
T2	36.74	13.22 b	1.20 b	9.12	5.79	4.13
T3	48.32	14.83 ab	1.42 b	11.65	5.60	4.13
T4	44.78	16.85 ab	0.95 b	9.98	5.85	4.37
T5	40.28	16.15 a	1.45 b	10.97	5.86	3.97
ANOVA (%5)	NS	1.701 **[2]	5.448 ***[3]	NS[4]	NS	NS

[1] Differences between values not indicated by the same letter are significant at the 5% level. [2] **; significant at the 1% level. [3] ***; significant at the 0.1% level. [4] NS; not significant.

Özen (2018) investigated the effects of different doses of mushroom compost, leonardite, and vermicompost on lettuce yield and quality, and reported that increasing doses of organic material applications resulted in increased collar diameter. In a study conducted on watermelon (*Citrullus lanatus* (Thunb.) Matsum & Nakai), it was reported that different doses of vermicompost (0, 300, and 600 kg/da) did not have statistically significant effects on fruit flesh thickness, fruit juice pH, and soluble solid content (Göksu and Kuzucu 2017). Similarly, in our study, although the differences between treatments were not statistically significant in both watermelon and melon, the collar diameter, fruit flesh thickness, and soluble solid content in watermelon were found to be 40%, 40%, and 100% higher, respectively, compared to melon. In addition, the fruit weight of watermelon was found to be six times higher than that of melon. One of the main reasons for this large difference is the physiologically larger fruit size of watermelon compared to melon.

Correlations Between Soil Properties and Plant Growth

Based on the numerical data obtained from the trial, relationships between soil chemical properties and plant growth parameters were examined. Accordingly, in melon soils, a negative correlation was observed between fruit flesh thickness and organic matter content (r = -0.539*), while a positive correlation was found between root collar diameter and organic matter content (r = 0.728**). These correlations suggest that soil organic matter may play a dual role in influencing plant morphological traits. While the positive correlation between root collar diameter and organic matter content implies a supportive role of soil organic matter in promoting vegetative growth and stem development, the negative correlation between fruit flesh thickness and organic matter content might indicate a potential trade-off between vegetative and reproductive growth processes under certain soil conditions. This could be due to nutrient partitioning or changes in water-holding capacity and aeration associated with increased organic matter. However, these relationships are likely to be influenced by plant species, growth stage, and environmental factors.

CONCLUSION

This study aimed to evaluate the effects of various organic fertilizers including manures from small ruminants, cattle, poultry, and vermicompost on the chemical properties of a sandy-calcareous soil and the growth performance of melon (*Cucumis melo* L.) and watermelon (*Citrullus lanatus* Thunb.). The study focused on how recommended fertilizer doses influence soil chemical quality and plant growth. Poultry and cattle manures stood out in improving soil

chemical properties in watermelon cultivation areas, whereas vermicompost and poultry manure significantly enhanced plant growth parameters. For melon cultivation, cattle manure was most effective for soil chemical improvements, while small ruminant, poultry, and cattle manures showed prominent positive effects on plant development. Overall, poultry manure emerged as the most effective input for enhancing plant growth, while vermicompost and cattle manure also contributed depending on the crop type. These results suggest that organic fertilizers, especially poultry manure, are valuable tools for improving soil fertility and crop productivity in calcareous soils. Nonetheless, expanding these findings through comprehensive studies involving different crops, soil types, cultivation locations, and production systems (e.g., greenhouse or organic farming) will be essential to deepen understanding and broaden the applicability of these organic fertilization strategies. These results underscore the critical role of optimized organic fertilization in promoting sustainable agricultural productivity, supporting both soil health and crop performance in Mediterranean agroecosystems.

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SMART BIOPOLYMER-BASED PACKAGING SYSTEMS AND CURRENT APPLICATIONS IN FOOD QUALITY TRACEABILITY

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ABSTRACT

Recently, sustainable packaging technologies have become an important research area in response to the growing demand for food safety and quality monitoring systems. Within this context, smart biopolymer-based packaging systems offer innovative solutions for monitoring quality and safety in food industry due to their eco-friendly structures and superior functional properties. Biopolymers derived from renewable resources (starch, cellulose, etc.) are being integrated with advanced sensor and indicator technologies, to create smart packaging systems. These systems respond to environmental changes and provide real-time information to consumers, thus significantly enhancing food safety and reducing food waste. Smart biopolymer-based packaging systems essentially consist of two basic components: the packaging material and the smart mechanism. Edible sensors and indicators, which provide consumers with information about the food's storage conditions, quality, and packaging environment, are integrated into the packaging as the smart mechanism. Edible sensors detect parameters such as temperature, pH, and freshness in foods and transmit data to control systems, while indicators provide direct visual information about the current state of the food. Such integrations enhance the functionality of packaging and support informed consumer decisions by providing more information. Current studies have focused on developing smart packaging tools such as time-temperature indicators for monitoring freshness and shelf life of frozen foods, gas-sensitive indicators for perishable products like meat and fish, and color-changing films that respond to pH changes. Additionally, advanced technologies such as biosensors, barcodes, and electronic noses monitor chemical and microbiological properties of foods, enabling early detection of potential risks and timely intervention. Smart biopolymer-based packaging offers sustainable and innovative solutions for meeting the quality and safety monitoring requirements of the food industry, thanks to its advantageous properties and superior performance. This study highlights the significance of smart biopolymer-based packaging systems in food quality traceability, current technological applications, consumer expectations, challenges, and future opportunities.

Keywords: Smart biopolymer-based packaging, Food quality, Traceability, Biosensors, Shelf life

INTRODUCTION

The rapid expansion and increasing complexity of the international food supply chain have made it more challenging to maintaining the quality and integrity of food products (Hong et al., 2025). With the increasing public awareness of food safety, consumers have been paying more attention to assessing the quality and safety of food before consumption (Zhao et al., 2023). However, traditional food packaging only provides consumers with information about the composition and expiration date of food, and lacks any monitoring mechanism capable of

detecting microbial spoilage during the shelf life. This highlights the need for more innovative and functional packaging solutions to ensure food safety (Ahmad et al., 2025).

Petroleum-based plastics derived from fossil fuels are widely used in food packaging. Packaging materials such as polyethylene, polyvinyl chloride, and polyethylene terephthalate, are commonly used for packaging products across various sectors. However, compounds like polytetrafluoroethylene, polyvinylidene, epoxy, and acrylic-based lacquers, which impart hydrophobic properties to polymers, are considered unsuitable for food contact, and their use in food packaging is restricted (Niemczyk-Soczynska & Sajkiewicz, 2025; Sabu Mathew et al., 2025). Although traditional packaging materials offer several advantages, their usage is gradually declining, as they pose serious environmental threats and contribute significantly to climate change (Hong et al., 2025; Shafi et al., 2025). As a result, the development of bio-based packaging materials has become increasingly important and a top priority in the packaging industry (Sabu Mathew et al., 2025; Zhao et al., 2023).

Recent studies have focused on developing next-generation smart packaging systems that can detect food quality and freshness throughout the shelf life, contain natural, functional, and antimicrobial bioactive compounds, and are produced from edible sources to contribute to sustainability (Ahmad et al., 2025). Researchers have focused on the use of biopolymer nanocomposite materials in smart food packaging due to their potential to address environmental concerns and ensure food safety. Natural sources, such as animals, plants, and microorganisms, are utilized in the production of these polymer materials (Mishra et al., 2024).

Food packaging materials made from biopolymers extends shelf life by reducing microbiological growth. These materials not only provide protection against water vapor, gas permeation, and moisture but also offer a sustainable solution to address growing environmental concerns due to their biodegradable properties. Furthermore, several researchers have reported that packaging can be further improved by adding various additives to biopolymer films (Chia et al., 2022; Eker et al., 2025; Kanphai et al., 2025; Murugan et al., 2025; Pilehforooshha et al., 2025; Rami et al., 2024).

Biopolymer-based smart packaging systems provide consumers with information about the quality and safety of the product through sensors that monitor internal conditions of the packaging or indicators that detect spoilage, rather than directly monitoring the external sensory attributes of the products (da Silva Ponciano et al., 2025; Sundaresan et al., 2025). Additionally, they contributes to tracking the history of the food from production to consumption (Yılmaz & Yılmaz, 2017). In these systems, the integration of radio frequency identification (RFID), electronic tags, and sensors into food packaging enables more effective monitoring of quality control points, thereby increasing information flow and transparency (Dimou et al., 2024). These packaging systems, which can provide real-time information about the product by detecting microbiological and chemical changes, not only offer physical protection but are also considered innovative technologies in terms of sustainability and food safety, with added functions such as portion control and marketing convenience (Dağbağlı & Kayaardı, 2021; Hepsağ & Varol, 2018). This study addresses food quality traceability, smart biopolymer-based packaging systems, their application in monitoring food quality, current practices, challenges encountered, and future perspectives.

TRACEABILITY IN THE FOOD CHAIN

Traditional packaging systems are designed to protect food against biochemical contamination and to provide consumers with ease of use during storage, handling, and transportation (Siciliano et al., 2024). However, in recent years, this approach has shifted, and packaging has become important not only for protection but also being functional and interactive with consumers. Growing consumer concerns about food safety and freshness, along

with regulatory requirements, have made it necessary for packaging to incorporate advanced systems capable of monitoring the condition of food. Today, the primary goal in food packaging production is to develop innovative solutions that extend the shelf life of food by controlling various biological and physical factors (Li et al., 2025).

Recently, the use of technologies such as coatings, antimicrobial packaging, and modified atmosphere packaging has become widespread in the field of food packaging. However, these packaging methods alone do not provide real-time monitoring of the current condition of food (Mkhari et al., 2025). The traceability of highly perishable foods until they reach consumers is particularly important. Ensuring food safety throughout the supply chain in the food industry is made possible through smart food traceability. The integration of this technology into packaging not only ensures food safety but also helps prevent food waste (Yu et al., 2022). Billions of tons of food are wasted annually due to losses occurring during storage, logistics, transportation, and sales processes within the food supply chain. This not only increases the risk of global food scarcity but also causes serious negative impacts on the environment and human health due to wasted greenhouse gas emissions during the production process (Moustafa et al., 2025). In recent years, new packaging systems have been developed by combining active and intelligent packaging as a novel approach. These innovative technologies enable real-time monitoring of the product and contributes to ensuring food safety and reducing food waste (Huang & Wang, 2025).

Ideal traceability with smart packaging is made possible through the use of indicators, sensors, tags, Internet of Things (IoT), radio frequency identification (RFID), near-field communication (NFC), and other similar technologies that provide information about the condition and quality of the food (Du et al., 2025; Yu et al., 2022). Real-time monitoring can provide information about the product's condition throughout the supply chain, including factors such as temperature, humidity, and microbial spoilage (Du et al., 2025).

In conclusion, smart packaging systems monitor factors such as temperature, humidity, and microbial activity to ensure food traceability throughout the supply chain and optimize quality control processes (Du et al., 2025; Yousefi et al., 2019). Packaging technologies supported by RFID, NFC, and IoT-based systems reduce spoilage risk, prevent product losses, and provide consumers with more reliable information (Kadirvel et al., 2025; Sobhan et al., 2025; Zuo et al., 2022). Moreover, effective shelf life management contributes to reducing food waste and promoting sustainability (Huang & Wang, 2025). In this context, smart packaging emerges as a crucial tool that is not only protective but also enhances traceability and increases transparency within the food chain (Butuc et al., 2025).

SMART BIOPOLYMER-BASED PACKAGING SYSTEMS

Smart biopolymer-based packaging systems are environmentally friendly packages produced from edible and biological sources, such as starch, chitosan, and cellulose. Through integrated indicators or sensors, these systems can monitor critical food parameters in real time, including freshness, temperature, pH, humidity, gas composition, and microbial activity (Huang & Wang, 2025; Mafe et al., 2025). They not only ensure food safety but also combine active and smart components to contribute to sustainability (Amin et al., 2022; Chaudhary et al., 2022). These smart components, integrated into the bio-based polymer matrix, provide consumers with visual feedback—especially through natural color-changing indicators—enabling reliable monitoring of food quality and freshness (Rodrigues et al., 2021).

Biopolymer matrices combined with sensor indicators are commonly used in smart packaging systems. For example, sodium carboxymethyl cellulose stands out as a preferred material for smart packaging due to its transparency, excellent film-forming ability, biodegradability, and high moisture barrier properties (Zhao et al., 2023). These types of bio-

based smart packaging materials can provide consumers with information about the product's condition by reacting to components such as pH, gases, temperature, relative humidity, and enzymes (Huang & Wang, 2025).

In the literature, various smart indicators and biosensors have been developed for detecting pH, temperature, gas concentrations (e.g., O_2, CO_2), humidity, and microbial metabolites (Gam et al., 2025; Huang & Wang, 2025; Panwar et al., 2024; Shao et al., 2021; Yousefi et al., 2019). For example, pH indicators visually reflect chemical changes, such as acidification or alkalization, that occur during food spoilage through color changes, while gas sensors indirectly monitor microbial activity by tracking oxygen or carbon dioxide levels in the packaging atmosphere (Amorim et al., 2022; Gupta et al., 2024; Heo & Lim, 2024; Saliu & Pergola, 2018). Biosensors, on the other hand, can directly provide consumers with information about the product's condition by detecting the presence of specific pathogens or toxins through enzymatic reactions or antigen-antibody interactions (Inês & Cosme, 2025; Khan et al., 2025; Moran et al., 2016). The chemical and physical stability of these indicators and sensors within the biopolymer matrix, their durability under environmental conditions, and their ability to maintain long-term performance are critical for the reliability of the system (Teixeira et al., 2025; Ye et al., 2025). In conclusion, biopolymer-based smart packaging systems play a crucial role in preventing food waste and enhancing consumer safety by enabling the early detection of spoilage, monitoring freshness, and determining the safe consumption period (Akhila et al., 2024; Huang & Wang, 2025).

USE OF SMART BIOPOLYMER-BASED PACKAGING SYSTEMS IN FOOD QUALITY TRACEABILITY

Smart packaging systems are expected to significantly transform the food sector through enhanced traceability, improved sustainability, and industrial integration. However, in some countries, limited knowledge and lack of education in some countries hinder the widespread industrial adoption of these technologies. When combined with environmentally friendly and biodegradable materials, smart packaging not only helps reduce environmental concerns associated with conventional packaging but also contributes to decrease the carbon footprint (Alizadeh Sani et al., 2024).

Smart biopolymer-based packaging systems are classified into two categories as indicators and biosensors that provide smart functionality to the packaging. Indicators provide consumers with information about the product's condition by reflecting physiological or microbial changes occurring in the food (Shao et al., 2021), whereas biosensors deliver information about the product by converting signals generated from the interaction between embedded bioreceptors and specific analytes into stimulus understandable to consumers through a transducer (Nastasijevic et al., 2025). Figure 1 shows the indicators and sensors used to monitor food quality traceability.

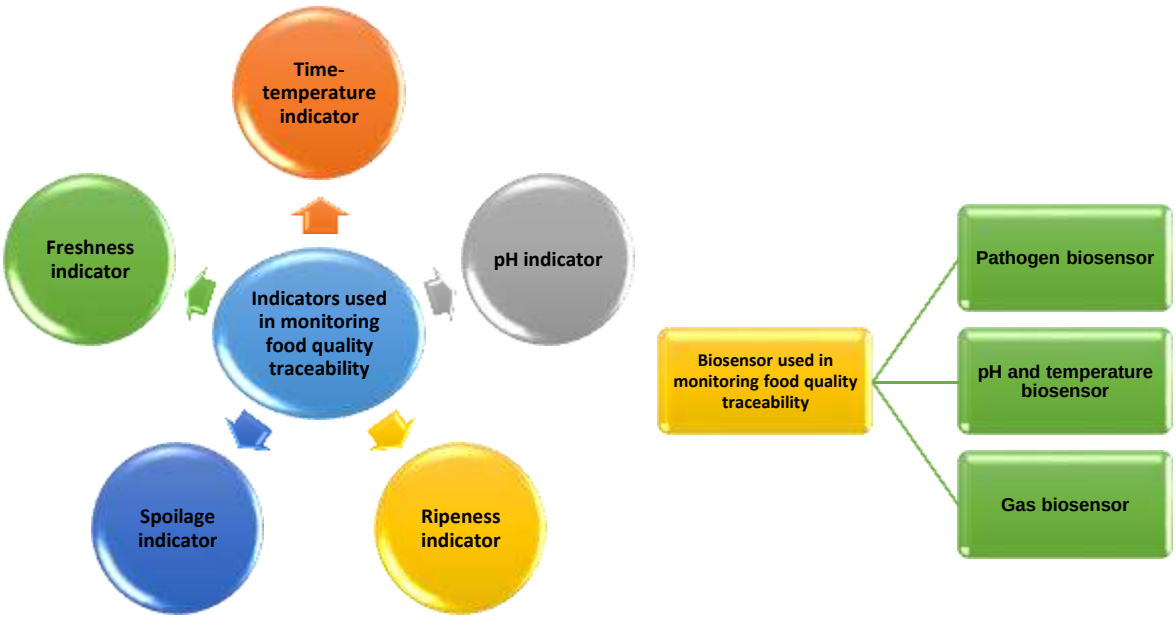


Figure 1. Indicators and sensors used in monitoring food quality traceability

(Bodkhe et al., 2025; Palanisamy et al., 2025)

Indicators

Indicators provide consumers with real-time monitoring of food quality and play a crucial role in detecting the freshness of target foods (such as meat, fish, and dairy), as well as changes occurring in the internal or external environment of the packaging. These systems, which deliver real-time information about product quality throughout the supply chain, stand out as innovative packaging solutions with a communication function. The working principle of indicators is based on the color change of dye molecules that are sensitive to environmental factors such as pH, gases (particularly volatile amines and CO_2), and humidity released during food spoilage (Panwar et al., 2024; Shao et al., 2021). These dyes are typically integrated into biodegradable polymer carriers to provide consumers with visual and real-time notifications about the freshness of the product (Yu et al., 2023). Indicators used in smart packaging systems are classified as freshness, time–temperature, pH, ripeness, and spoilage indicators (Kadirvel et al., 2025; Kang et al., 2024). Packaging systems that are sensitive to pH and gases can inform consumers about the level of food spoilage by responding to gases, such as ammonia and ethanol, released during deterioration, through visible color changes. Temperature-sensitive packaging materials reflect temperature fluctuations, facilitating the monitoring of products requiring cold chain transportation. Meanwhile, relative humidity-sensitive systems help maintain product quality by preserving the internal moisture balance within the package. Enzyme-responsive materials convey information about the product's condition by releasing functional compounds in the presence of specific enzymes (Huang & Wang, 2025).

Freshness indicator

Freshness indicators are systems that detect qualitative or quantitative changes in the concentration of one or more substances (e.g., glucose, volatile nitrogen compounds, biogenic amines, carbon dioxide, or sulfur compounds) within the packaging that correspond to food deterioration (Becerril et al., 2021; Palanisamy et al., 2025). These halochromic indicators can provide consumers with real-time information about the chemical and microbial quality of food (Huang et al., 2025)These systems, which provide information about the freshness of the

product through color changes depending on the packaging environment, are used to monitor factors such as the catalytic activity of endogenous enzymes causing spoilage, microbial growth, temperature, humidity, and oxidation in foods (Zhu et al., 2024).

Smart packaging systems generally use a polymeric or biopolymeric carrier matrix and pH-sensitive dyes that change color by reacting with chemical substances, such as basic nitrogen compounds and organic acids released during food spoilage (Almasi et al., 2022). Common synthetic dyes used in these systems include cresol red, bromocresol purple, and xylene (Li et al., 2025). However, the toxicity and potential carcinogenic risk of these synthetic dyes raise serious health concerns regarding their use in food packaging materials. Therefore, in recent years, studies on the use of natural pigments derived from fruits and vegetables have increased (Almasi et al., 2022). Natural dyes include anthocyanins, alizarin (Ezati et al., 2019; Gam et al., 2025), carotenoids, curcumins (Li et al., 2025), shikonins (W. Sun et al., 2018) and chlorophylls (Hailu et al., 2025a). These natural dyes are considered a more advantageous option than synthetic alternatives for freshness indicators due to their edibility, biodegradability, and low cost (Huang et al., 2025).

Anthocyanins, used as natural indicator dyes in smart packaging, are important pigments that have attracted considerable research interest. Especially during the storage of protein-based foods such as fish and meat, the accumulation of volatile basic nitrogen compounds causes the packaging environment to become alkaline. Under alkaline conditions, anthocyanins change to blue or green, allowing visual monitoring of pH changes; when the pH shifts from neutral to acidic values, they change from purple to red or pink, providing information about the degree of food spoilage (Yi et al., 2025).

Functional packaging films containing carrageenan can be used to monitor spoilage and extend the shelf life of foods. There are studies evaluating carrageenan within smart packaging systems that provide visual alerts through color changes for packaging foods such as meat, fish, and bananas (Han et al., 2025; Jia et al., 2025; J. Liu et al., 2018; Ramadas et al., 2024; J. Wang et al., 2024). Table 1 summarizes various studies on the utilization of freshness indicators in smart packaging systems.

Table 1. Various studies on the utilization of freshness indicators in smart packaging systems

Pigment	Food	Film Material	Color Change	Reference
Carrageenan	Beef	Sodium carboxymethyl cellulose-based film	From pink to yellow	Cao et al., 2024
Curcumin	Shrimp	Hydrogel film	From yellow to red	Zhang et al., 2021
Red cabbage-Beetroot extract	Shrimp	Sodium alginate	From red to yellow	Öztürk & Gündüz, 2025
Cresol red and Bromocresol purple	Fish	Polytetrafluoroethylene and polyvinylidene fluoride	From yellow to purple	Kim et al., 2023

In a study, smart indicator films based on carrageenan and sodium carboxymethylcellulose, containing composite anthocyanins (purple cabbage and dragon fruit extract) encapsulated with zein and gellan gum were developed to monitor beef freshness during storage at 4°C. It was reported that the film color changed from pink to yellow in the presence of ammonia, which is released during meat spoilage, and that these color changes were clearly observed during the spoilage (Cao et al., 2024).

Zhang et al. (2021) developed an agar and polyvinyl alcohol-based indicator containing curcumin to monitor shrimp freshness. During the storage period (4°C for 3 days), the indicator

color changed significantly from yellow to red due to shrimp spoilage, and a distinct color change was observed in the presence of ammonia.

An ammonia-sensitive, colorimetric freshness indicator was developed to assess the freshness of fish during storage. The indicator, formulated using cresol red and bromocresol purple, monitored the spoilage process in various fish species stored under different temperatures through ammonia detection. It was reported that the indicator successfully tracked the freshness of the fish by exhibiting a color change from yellow to purple as the storage period increased (Kim et al., 2023).

Time-temperature indicator

Time-temperature indicators are systems that visually reflect changes in food quality and shelf life by monitoring the temperature history of food exposure. These indicators change color in response to a specific time and temperature combination. This color change parallels the spoilage process of the product. Time-temperature indicators can simplify cold chain monitoring and reduce the risk of spoilage (Gao et al., 2020).

Time-temperature indicators are generally reliable tools for predicting food quality attributes; however, their predictive performance can sometimes be limited. This is mainly due to the instability of the chemicals used in their formulation and the differences in temperature sensitivity between the indicator and the food product. To address this, storage studies are conducted to establish a calibration curve that correlates the indicator's color changes with the actual quality of the food. However, when there is a mismatch in temperature sensitivity between the food and the indicator, the calibration curve generated under constant temperature conditions may no longer be valid. This creates challenges in developing a consistent and representative curve. For the indicator to be considered accurate and reliable, it is crucial that it must be capable of reflecting food quality consistently with a single calibration curve, even under variable conditions (Upadhyay et al., 2024).

In a study, an application was developed for the digitally monitor time-dependent temperature changes of ready-to-eat salads under both laboratory and real-time supply chain conditions. Sliced carrots were identified as the main component limiting the product's shelf life. The predictions from the time-temperature indicator generally aligned with the actual shelf life of the product. However, deviations were observed in cases where temperature fluctuations occurred. These deviations were particularly associated with rapid color changes in the indicator at 2°C and 7°C, resulting in shorter predicted shelf life compared to the actual product (Waldhans et al., 2025a).

(Yar et al., 2025) developed a packaging film incorporating a time-temperature indicator composed of amaranth leaf extract, polyvinyl alcohol, and citric acid to monitor beef freshness. It was reported that the packaging film is not only capable of detecting temperature fluctuations in the environment but can also be effectively used to assess the beef freshness.

In another study, an application was developed to predict the shelf life of pork sausages throughout the supply chain using time-temperature indicators. In the study, a spoilage kinetics model based on microbial parameters was created for pork sausage, and this model was integrated with data obtained from the time-temperature indicator to form a dynamic system. The findings showed that the time-temperature indicators and the product spoilage kinetics were comparable, demonstrating that the developed application is conceptually valid for predicting the shelf life of pork sausage. Additionally, it was reported that technical improvements to the system could further enhance the accuracy of shelf life predictions (Waldhans et al., 2025b).

pH indicator

Various metabolites released during food spoilage provide important information about the freshness and safety of the product. In particular, metabolites such as CO_2, histamine, and dimethylamine are directly related to spoilage, and the pH changes caused by these metabolites can negatively affect the quality of the food. The detection of such metabolites has become possible through the pH and gases sensitive indicators incorporated in smart packaging systems. These indicators enable real-time monitoring of product quality, offering reliable information to both producers and consumers (Zhu et al., 2024).

In recent years, biodegradable and non-toxic plant-based natural pigments have gained attention as alternatives to synthetic pigments (such as bromocresol purple, methyl red, cresol red), which pose potential risks to both the environment and human health (Jebel et al., 2025). Moreover, natural pigments obtained from the valorization of agricultural and food industry waste offer a sustainable alternative. These pigments are typically incorporated into biopolymers such as polysaccharides, lipids, proteins, or polyvinyl alcohol to form films, which are effectively used in smart packaging systems (Panwar et al., 2024).

The integration of pH-sensitive pigments into smart packaging systems has emerged an effective method for monitoring food spoilage. During storage, changes in pH interact with the conjugated structures or phenolic components of these pigments, resulting in visible color changes. Since these color changes can be easily perceived visually by the consumer, they offer the advantage of providing quick and practical information about the freshness of the product (Parlak et al., 2024).

The main reasons for using pH-sensitive natural pigments as indicators in smart packaging systems include their non-toxic nature, water-soluble structures, pH responsive color-changing properties, and even anticancer effects (Zhao et al., 2023). In this context, natural pigments such as anthocyanin, curcumin, alizarin, shikonin, and betalain are commonly used in pH-sensitive smart packaging applications (Liu et al., 2025; Parlak et al., 2024).

Alizarin, a natural indicator dye, is a non-toxic compound derived from plant extracts and can be used as a pH indicator in smart packaging systems. Alizarin dissolves easily in polysaccharide-based films without requiring chemical modification, making it a safe and suitable pigment for use in polymer-based smart packaging systems (Li et al., 2024). Shikonin is another notable compound in smart packaging applications due to its pH-sensitive nature and antimicrobial properties. However, its use in smart packaging systems remains limited because of challenges such as poor water solubility, low stability, and difficulties in maintaining its functionality (Sun et al., 2025b) Table 2 presents various studies on the utilization of pH indicators in smart packaging systems.

Table 2. Various studies on the utilization of pH indicators in smart packaging systems

Pigment	Food	Film Material	Color Change	Reference
Anthocyanin	-	Cellulose Chitosan	From blue-green to yellow	Hailu et al., 2025a
Curcumin	Chicken fillet	Artocarpus lakoocha starch-based film	From yellow-red to orange	Mali & Pandey, 2024
Blueberry and blackberry pomace	-	Chitosan	From red to purple	Kurek et al., 2018
Plum peel	Chicken	Sodium alginate, Gelatin	From red to yellow-green	Chen et al., 2023

In a study, an intelligent packaging film was developed using curcumin as a pH indicator and starch derived from *Artocarpus lakoocha* seed waste. Although the film exhibited reduced

mechanical properties, increases in water vapor transmission rate and crystallinity was observed. Moreover, the film demonstrated antioxidant and antibacterial activities in meat products and proved to be an effective pH indicator (Mali & Pandey, 2024).

Hailu et al. (2025) developed pH-sensitive smart packaging films by combining anthocyanins obtained from pea flowers and red cabbage extracts with a cellulose-chitosan matrix. Their study reported that these films possess superior properties and can be used as freshness indicators in food packaging.

In another study, chitosan-based smart packaging films were developed using blueberry and blackberry pulp extracts. The films exhibited distinct color changes depending on pH variation, with blueberry films changing from rose to blue-green, while blackberry films shifted from red to dark violet. The most pronounced color changes were observed in the pH range of 4–7, which reflects food spoilage. Therefore, it was concluded that these films have potential for use in detecting food spoilage (Kurek et al., 2018).

Chen et al. (2023) developed pH-sensitive films containing sodium alginate, gelatin, and plum peel extract to monitor the freshness of chicken meat. It was found that the developed films exhibited a distinct color change from red to yellow-green over a pH range of 1 to 14, demonstrating high pH sensitivity. Furthermore, during the storage of chicken at 4°C for 6 days, the film's color changed from orange-pink to yellow-green as spoilage progressed, indicating its effectiveness used as a smart packaging material for monitoring the freshness of chicken meat.

A pH-sensitive biopolymer film containing betacyanin and carbon dots was developed using soy protein and methylcellulose to monitor the spoilage of shrimp. In the study, the pH-sensitive color change properties of the developed biofilms were examined. As the spoilage of shrimp progressed, the indicator color shifted from pinkish brown to yellow, reflecting an increase in environmental pH (Ebrahimi et al., 2025).

Ripeness indicator

Ripeness indicators are systems that visually show consumers whether a food is ready for consumption, its ripeness, and freshness. These indicators respond to gases released by fruits and vegetables, especially ethylene, by changing their color. This helps consumers easily select products at their ideal ripeness, thereby contributing to the reduction of food waste (Palanisamy et al., 2025). In the reliable assessment of fruit ripeness, it is important for the indicator to detect ripening markers, such as ethylene gas, on-site and to be cost-effective. Accordingly, researchers have recently focused on developing odor visualization technologies. However, dye-based sensors used to detect the low concentrations of ethylene gas released from fruits and vegetables may be insufficient in detection processes due to limitations such as low signal output and high-dimensional data outputs (You et al., 2024).

In a study on the detecting banana ripeness and delaying spoilage through the integration of machine learning techniques and IoT technologies, it was reported that the machine learning algorithms provided high accuracy in predicting the ripening stages of bananas (Rajini & Voola, 2025). In another study, an ethylene gas-sensitive indicator containing aluminum and nitrogen-doped carbon quantum dots embedded in an agar film was developed to determine banana ripeness. It was reported that this indicator demonstrated successful results in the qualitative detection of ethylene gas produced during ripening (Lohrasbi Nejad & Shekarchizadeh, 2025).

Spoilage indicator

Spoilage indicators are used to detect microbial activity by interacting with enzymes or secondary metabolites produced by microorganisms responsible for food spoilage. Compounds such as diacetyl, amines, carbon dioxide, ammonia, and hydrogen sulfide, produced by microorganisms, react with indicators integrated into packaging systems, causing visible color

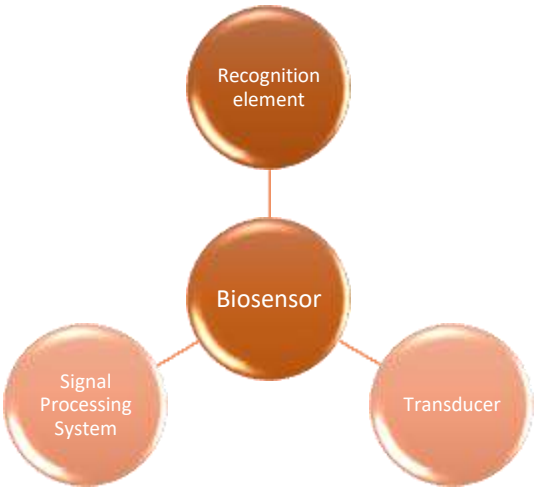
changes (Pereira et al., 2024). In addition to these visual indicators, electrochemical sensors, RFID tags, and biological and enzymatic analysis methods are also used for detecting food spoilage. However, the high cost of these systems along with challenges in production and packaging integration, limits their widespread use in packaging applications. Therefore, researchers continue to work on developing practical, low-cost, and user-friendly spoilage indicators that enable on-site and real-time detection of spoilage in foods (Liu et al., 2025)

Liu et al. (2025) conducted a study in which a bacterial nanocellulose film produced from fermented sour whey, was functionalized with anthocyanins derived from *Clitoria ternatea* to design a pH-sensitive smart label system for detecting spoilage in meat. The study demonstrated that the color changes exhibited by the film in response to spoilage could enable real-time monitoring of quality changes in meat through smart packaging systems.

In a study, a smart packaging system was designed by integrating a pH indicator containing encapsulated bromothymol blue as a label into a polyethylene packaging film to monitor spoilage during storage (4°C, 15 days) of chicken fillets. It was reported that the developed films successfully detected pH changes associated with microbial spoilage in chicken fillet samples by exhibiting color changes (Ibrahim et al., 2025).

Sensors

Smart packaging systems utilize various sensors to monitor quickly, accurately, and reliably environmental factors that impact product quality . These systems, particularly used in the packaging of dairy products, seafood, and fresh food, significantly contribute to reducing food waste and protecting consumer health by monitoring food content (Wang et al., 2025). One of the most notable technologies in these systems is biosensors. Biosensors are manufactured using materials that can react electrochemically and change color, and are integrated into packaging materials to detect chemical and microbial changes in foods and monitor their current condition (Sobhan et al., 2025).



Şekil 3. Basic components used in biosensor production (Feng et al., 2025)

Figure 3 shows the basic components used in the production of biosensors. Different types of biosensors are used for the detection and analysis of physiological changes occurring in food products, such as food toxins, chemical contaminants, spoilage indicators, food enzymes, food pathogens. In this context, optical, electrochemical, acoustic, and gas biosensors are particularly notable (Bodkhe et al., 2025; Rodrigues et al., 2021a; Sobhan et al., 2025; Upadhyay et al.,

2024). These biosensors operate in different ways according to their signal transduction mechanisms during analyte detection, and this classification is detailed in Figure 4.

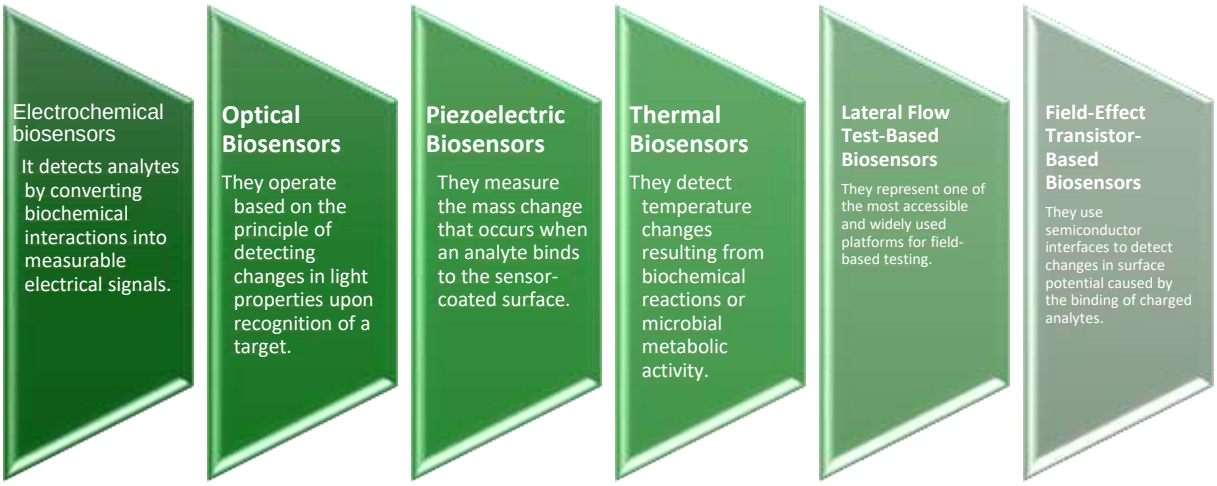


Figure 4. Biosensors classified according to their signal transduction mechanisms (Bodkhe et al., 2025; Damborský et al., 2016; Hammond et al., 2016; Pohanka, 2017)

Recently, colorimetric sensors, which allow for simple and equipment-free detection of analytes, have become the focus of research. These sensors enable easy detection of spoilage in food through color changes resulting from interactions with analytes, and they can also be integrated into or attached to packaging (Najafi et al., 2025). However, a large portion of current sensors are made from nonbiodegradable and nonrenewable synthetic materials. Considering environmental impacts, this situation is increasingly driving the demand for the development of sustainable, eco-friendly smart biosensor materials (Rodrigues et al., 2021b). In smart food packaging sensor applications, pathogen biosensors that detect the presence of pathogens, pH and temperature biosensors that monitor changes in acidity, and gas biosensors that detect gases produced as a result of spoilage are generally used (Mani et al., 2025).

Pathogen biosensor

Pathogen biosensors are electrochemical biosensors developed based on target-specific biological recognition elements such as enzymes, antibodies, and nucleic acids to detect the presence of pathogens in food. These biosensors operate by producing electrochemical signals that determine microbial load and concentration levels through the binding of biological recognition elements and electrochemical transducer materials to pathogen cells (Sobhan et al., 2025).

Jeyaraman and Eltzov (2025) developed an alginate-gelatin-based biosensor system for the rapid and specific detection of *Bacillus subtilis* in food. It has been reported that this system, tested in complex food matrices such as rice and milk, can respond rapidly even at low bacterial concentrations and may be useful for early contamination detection.

A triple-signal biosensor has been developed for the simultaneous and sensitive detection of both live and dead forms of *Listeria monocytogenes*. The developed biosensor could detect live bacteria with a detection limit of 1 CFU/mL and total bacteria with a detection limit of 5 CFU/mL. Therefore, it is reported that the biosensor can be used for the simultaneous and highly sensitive detection of both live and dead bacteria in terms of food safety (Zhang et al., 2025).

pH and temperature biosensors

Thermochromic materials used in smart food packaging systems serve as temperature sensors due to their ability to change color in response to temperature variations. These sensors exhibit irreversible color changes during food storage, providing continuous monitoring throughout the product's shelf life. When the food reaches a critical temperature, the color change becomes permanent, offering visual information to the consumer about storage conditions and supporting safe consumption (Pereira et al., 2024).

Yildiz et al. (2021)developed curcumin-loaded chitosan and polyethylene oxide-based electrospun nanofibers to monitor the freshness of chicken meat and investigated the potential use of these materials as halochromic pH sensors. The curcumin-loaded nanofibers exhibited a color change from bright yellow to red on chicken breast, effectively providing a visual indication of spoilage. The study reported that the developed nanofibers could be used as an efficient sensor system for real-time monitoring of chicken meat freshness.

Wang et al. (2024) developed a label-free and novel DNA sequence-based poly-dA20 pH-sensitive colorimetric biosensor system working with gold nanoparticles for the evaluation of food freshness. Visible color changes in the gold nanoparticle solution were observed during the storage (4 °C and 25 °C) of meat and sea bass samples, corresponding to pH variations. The study demonstrated that the developed pH-sensitive biosensor could be effectively used for detecting food spoilage.

Gas biosensors

Gas sensors consist of three main components: the sensing, counter, and reference electrodes, which work together to detect gases. When the sensing electrode reacts with the gas present in the product, the counter electrode balances this reaction, while the reference electrode measures the potential change. This setup ensures the reliability and sensitivity of the sensor (Upadhyay et al., 2024). Gas sensors used in food packaging provide information to consumers about the product's condition by producing distinct color changes when reacting with metabolites, thereby contributing to the proper assessment of the product (Zhu et al., 2024).

Among the semiconductor metal oxide compounds used in gas detection sensors, compounds such as tin dioxide (SnO_2), zinc oxide (ZnO), tungsten trioxide (WO_3), copper oxide (CuO), and nickel oxide (NiO) are commonly used for the detection of hydrogen sulfide (H_2S) gas released during meat spoilage (Fang et al., 2025). Additionally, studies using pH indicators such as methyl red and brilliant cresol blue for the detection of ammonia (NH_3) gas released during the spoilage of fish and meat products are also found in the literature (Liu et al., 2025).

Fang et al. (2025) developed composite biosensors containing titanium oxide doped with copper oxide and nickel oxide, as well as quantum dots, for the evaluation of meat freshness. These biosensors enabled effective and rapid monitoring of meat freshness by detecting hydrogen sulfide gas with a sensitivity of 100 ppb. The study reported that the sensor demonstrated superior gas detection performance.

FUTURE PERSPECTIVES AND CHALLENGES

In the field of biopolymer-based food packaging, numerous research and industrial studies are ongoing on the integration of bioactive compounds such as metal nanoparticles, plant-derived polyphenols, and volatiles into polymer matrices (Periyasamy et al., 2025). However, although the indicators and sensors developed within the scope of smart packaging systems have shown promising results under laboratory conditions, they have not yet been commercialized and mostly remain at the research stage. The high cost of indicators and sensors, the challenges in integrating them into production and packaging processes, and the adverse effects of thermal treatments applied in large-scale film production on their properties

all slow down the commercialization process. Therefore, it is necessary to develop durable, low-cost indicators and sensors suitable for commercial use (Huang et al., 2025).

Researches on sustainable packaging systems offer significant advantages both in meeting consumer demands and in integrating environmentally friendly materials. Advances in sensor technologies and data analysis not only facilitate food preservation and transportation but also have the potential to bring about a fundamental transformation in the field of packaging through the use of alternative technologies (Baneshi et al., 2024). However, although biopolymer-based packaging materials are a promising alternative, they must overcome significant challenges, such as good mechanical performance, sustainable raw material supply, and market acceptance, to replace traditional plastic packaging (Venkatesan et al., 2025).

CONCLUSION

Smart biopolymer-based packaging systems have significant potential in food packaging due to their advanced features, including user-friendliness, environmental compatibility, and the ability to provide information about the product. These systems are considered advanced technological applications, as they monitor quality parameters such as freshness, pH, and the presence of pathogens in food. Although laboratory studies have demonstrated the practical benefits of these systems, their production on an industrial scale remains limited.

There are certain technical challenges that hinder the widespread adoption of biopolymer-based smart packaging in the food industry. Disadvantages such as poor mechanical strength, inadequate moisture resistance, and low thermal stability limit their large-scale application. In addition, the scalability and efficiency of production methods are also important factors that need to be considered.

The integration of advanced production techniques with biopolymer-based smart packaging films significantly contributes to sustainability goals and supports the circular economy approach. The use of components derived from renewable biomass not only increases resource efficiency but also positively impacts the development of bioeconomy applications.

Consequently, the widespread use of biopolymer-based smart packaging systems requires attention not only to technological advancements but also to issues such as material safety, biocompatibility, data security, and regulatory compliance. Further research and development efforts are necessary to overcome current limitations and support the advancement of this field.

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ENHANCING ENERGY EFFICIENCY IN AGRICULTURAL PRACTICES

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ABSTRACT

Energy efficiency is one of the areas that must be carefully addressed in the agricultural sector due to its direct relationship with the long-term viability of economic growth and social development goals, as well as its critical role in lowering greenhouse gas emissions. Energy efficiency refers to determining how to reduce energy consumption while maintaining the same production and service levels. This study examines the importance of energy efficiency in the agricultural sector and the energy efficiency practices that can be implemented at various stages of agricultural production. Understanding energy consumption is essential before assessing energy efficiency initiatives. Agriculture uses energy either directly or indirectly. Determining and documenting the energy inputs, as well as creating the required energy plans, are essential. The use of tractors and agricultural equipment, irrigation systems, greenhouse heating and cooling systems, and fertilizer and pesticide application are examples of energy-intensive activities in the agricultural sector. Furthermore, educating producers about energy efficiency and the importance of tracking and revealing energy consumption raises awareness and helps efficiency initiatives succeed. Increasing energy efficiency in farms typically costs less than installing new energy production systems. The development and implementation of integrated energy efficiency measures are also critical for production sustainability. Energy efficiency in farms and throughout the supply chain will help offset rising energy costs for heat, electricity, gas, and liquid fuels. This is also critical for increasing profits while protecting natural resources, minimising environmental impact, and ensuring the long-term viability of agricultural production.

Keywords: Energy, Smart farming, Energy-saving techniques, Sustainable food systems, Energy efficiency

INTRODUCTION

Agriculture is a critical sector that enables the production of food required for human survival. This sector is extremely important in economic, social, and environmental terms. Modern agricultural practices, which use various methods to meet the increasing food demand, have largely replaced primitive agriculture (Finan, 2019). Modern agricultural production processes have resulted in a shift from the widespread use of human or animal power to mechanical energy using fuels (Sial et al., 2021). This agricultural sector has become dependent on energy sources such as oil and electricity, just as other sectors have (Bekhet & Abdullah, 2010). In this context, energy efficiency is crucial because it influences various operations, including resource consumption and production.

ENERGY EFFICIENCY

Energy is the capacity to perform tasks. Renewable and non-renewable (fossil) energy sources are typically classified into two major categories. Although coal, oil, and natural gas are non-renewable energy sources, solar, wind, biomass, and hydroelectric power are renewable energy sources. Fossil fuels continue to provide a significant amount of today's energy needs, which poses major challenges to the sustainability of the environment (BP, 2020; IEA, 2021). Therefore, energy efficiency is becoming increasingly important.

Energy efficiency refers to achieving the same or higher production level by consuming fewer existing energy resources (EnVer, 2023). Usually expressed as a percentage between 0% and 100%, energy efficiency is the quantity of energy produced for a specific energy input. Energy efficiency can be increased at many stages of the energy production–consumption chain. Energy efficiency is a key policy component in many developed nations (ETKB, 2023; Yildiz et al., 2017).

Energy efficiency refers to efforts to reduce or, if possible, eliminate unnecessary energy consumption. Energy efficiency does not imply reducing or restricting energy demand; rather, it involves lowering the amount of energy consumed per product rather than the rate of energy consumption (ETKB, 2023).

ENERGY USE AND AGRICULTURE

Energy is a critical input in all stages of agricultural production. In the agricultural sector, energy use in plant and animal production processes is classified into two types: direct and indirect (Schnepf, 2004). Direct energy consumption in this sector includes energy used in agricultural activities such as harvesting, irrigation, drying, storage, greenhouse heating, and marketing, as well as energy used in infrastructure, agricultural machinery, tractors, and trucks (Martinho, 2021). Indirect energy consumption is typically found in the industrial sector and refers to the energy used in the production and processing of mineral fertilizers, chemical pesticides, insecticides, herbicides, and other agricultural chemicals (Schnepf, 2004).

Tractors, harvesters, lights, pumps, and heaters all consume considerable amounts of fuel and electricity, and the production of chemical inputs is also an energy-intensive process. Energy consumption is especially high in developed and heavily farmed agricultural areas, affecting both the economy and the environment. Although the use of fossil fuels harms the environment by increasing greenhouse gas emissions, the high energy dependence of agricultural production increases production costs (Vijayakumar et al., 2023).

ENERGY EFFICIENCY AND AGRICULTURAL PRACTICES

Enhancing agricultural energy efficiency is essential for reducing production costs and maintaining environmental sustainability. Efficient energy use has positive effects on the economy and the environment by lowering energy consumption per unit of product. Therefore, energy efficiency is crucial for agricultural advancement.

In a crop and livestock farm, daily operations include agricultural machinery, greenhouses, dryers, buildings, irrigation systems, and farm equipment, all of which lead to high energy consumption (Stephen Herbert, 2021). Increased agricultural energy efficiency by implementing day-to-day structural and technological measures.

Energy management requires the reduction of the amount of fuel used in field applications. Field applications must maximize the use of tractors and other tools and machinery

to achieve this (Stephen Herbert, 2021). It is important to change the filters, oil, tires, and balance, calculate the power requirements, and determine the optimal operating conditions for vehicles used in the field. Excess vehicle weight should be minimized to reduce fuel consumption (Carbon Trust, 2019; Mutlu, 2020). If there are fuel tanks on the farm, keep them cool to reduce fuel evaporation and regularly check for leaks. Vehicles running at idle can consume up to one-fifth of the total fuel consumption; therefore, machines should be turned off when they are not in use (Stephen Herbert, 2021; Susser, 2018).

Lighting is also required in agricultural buildings, greenhouses, poultry houses, and barns. Transparent panels, such as light tubes, are used to provide natural lighting (Can, 2023). Additional light sources are employed in areas where natural light is insufficient. LED bulbs should be used to provide energy-efficient lighting (Singh et al., 2015). Furthermore, an automatic lighting control system offers the most efficient lighting with the smallest number of lights (Carbon Trust, 2019).

Identifying and implementing ventilation system improvements can reduce energy consumption by up to 20%. Every farm requires effective ventilation; however, windows and doors that allow natural ventilation should be used whenever possible. Replacing old ventilation fans with more efficient ones reduces energy consumption. Therefore, the fan blades and broils should be ventilated regularly (Carbon Trust, 2019).

Irrigation and animal watering systems can be designed to save energy. Proper pump selection, low-pressure irrigation, drip irrigation, and distribution system optimization are required to water crops. Wherever possible, rainwater should be used to construct irrigation systems. Replacement of diesel pumps with electric pumps for irrigation improves efficiency, reduces operating costs, and increases reliability. Smart sensors can help irrigation systems run more efficiently. Using thermal insulation and automatic heating systems, animal waterers can save energy by preventing water from freezing (Iliev et al., 2012; Stephen Herbert, 2021).

In some cases, drying a product requires more energy than growing it. A moisture meter should be used to ensure that the product is dry. If mechanical drying is necessary, a natural air or low-temperature drying system can be used (Stephen Herbert, 2021).

The use of renewable energy sources in agriculture is an important strategy for promoting energy efficiency. Successful applications in this field include solar-powered irrigation systems, wind-powered water pumps, and biogas systems derived from agricultural waste (Atılgan Türkmen, 2020).

CONCLUSIONS

Enhancing the energy efficiency of agricultural practices is essential for environmental protection and sustainable economic growth. Agriculture can become more economical, ecologically friendly, and efficient through the integration of cutting-edge technologies and renewable energy sources. Regarding these issues, farmers require education and assistance. Achieving energy efficiency in agriculture supports efforts to fight climate change, conserves natural resources, and lowers greenhouse gas emissions. Consequently, agricultural policies and practices should incorporate EE as a fundamental component.

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APPLICATION OF LIPOSOMAL ENCAPSULATED ANTIMICROBIAL BIOACTIVE COMPONENTS IN FOOD PRODUCTS AS NATURAL PRESERVATION

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ABSTRACT

Encapsulation technology is needed to make more durable and effective of alternative natural preservatives and nutritional components. In recent years, liposomal structures have attracted attention and liposomes ensure the preservation of the encapsulated material until the appropriate place and time thanks to controlled or delayed release capability. Liposomal structures prevent the conversion into harmful components during storage and increase the bioavailability. The liposomal encapsulation process provides to be more stable and more durable bioactive compounds in the food and the digestive system. The slowly release of antimicrobial components during storage against microbiological contaminations can be realized without allowing mold contamination and mycelium formation in food products. In addition, which will be carried out on non-chemical "hurdle" technologies in order to control the development of food-borne microorganisms and increase antioxidant activity in order to respond to consumer expectations, aims to produce product formulations suitable for the concept of 'Clean Label'. In addition, in order to respond to consumer expectations, it is possible to control the development of food-borne microorganisms and to produce product formulations in accordance with the concept of 'Clean Label' with liposomal systems suitable for "hurdle" technologies without chemical content.

Keywords: Liposom, natural preservation, bioactive compounds, Clean Label

INTRODUCTION

Bioactive components have important antioxidant and antimicrobial effects and also effective on human health. Encapsulation procedures have implemented to increase the mechanism of bioactive components' action. Liposome encapsulation is an important application thanks to provide the development of controlled release of bioactive components in food and increased stability. One of the biggest advantages of liposomes is to made from natural components. Liposomes can be included in food formulations without the need for any legal regulation due to natural structure. Liposomes are no usage limit compared to chemical origin substances, and so excessive limits lead to no health problems. This feature removes the obstacles to the use of liposome structures in foods.

Liposomes are used to improve the water dispersibility of hydrophobic components, to increase bioavailability and to protect the encapsulated components from adverse conditions such as light, heat, pH, oxidation, hydrolysis or chemical reactions, to enable the delivery of an encapsulated agent to a specific location, to reduce negative effects and particle toxicity. Liposome systems provide to control the circulation in the body by modulation of their size and regulating the release profiles with surface modifications of the bioactive components (Alavi et

al., 2017; Lila and Ishida, 2017). Unlike other encapsulation methods, liposomal structures have no negative effect on product rheology properties thanks to very low phosphorylcholine-based lecithin concentration. In addition, the encapsulated components are more resistant to processes such as cooking and pasteurization with the controlled release of bioactive substances in the liposomal structure.

ENCAPSULATION TECHNIQUES

Encapsulation is an excellent method for the preservation of bioactive, volatile and readily degradable compounds and additives in food applications. The purpose of encapsulation is to protected active ingredient from external factors, to ensure stable in the digestive system and to release slowly, to increase bioavailability, to mask the negative taste and odor, and to prevent the active ingredient from reacting with other ingredients (Delshadi et al., 2020). In the encapsulation process consists of the active components as the core material and the appropriate wall material. The coating agent plays a key role and an ideal coating material should have low hygroscopicity, high solubility, low viscosity, low cost, ability to produce a stable emulsion and provide high protection (Gomez et al., 2018). Lipids, proteins and carbohydrates are widely used as coating material in encapsulation systems. The coating materials are desirable to be inexpensive, plentiful, non-toxic, and compatible with the food matrix (Jafari et al., 2008; Delshadi et al., 2020).

Lipid-based coating agents have excellent functionality in emulsification, film formation and encapsulation of active compounds. These coating materials are less toxic and have many potential uses in industrial applications (Fathi et al., 2012). The lipid-based coating materials are polar lipids (eg monoglycerides, phospholipids) and non-polar lipids (eg triacylglycerol, cholesterol) (Đorđević et al., 2016). Polar lipids such as phospholipids have some properties as biocompatible, suitable for stabilization, preservation and controlled release of active compounds and good surfactants (Đorđević et al., 2016; Shishir et al., 2018). Encapsulation contains microcapsules, submicron capsules, and nanocapsules sizes. Micro and nano encapsulation techniques include high pressure homogenization (HPH), micro fluidization, ultrasonic technique, spray drying, spray cooling/cooling, freeze drying, spray freeze drying, complex coacervation, emulsification (spontaneous, phase inversion, miscellaneous), anti-solvent precipitation, extrusion, electro-spinning and electro-spraying, layer deposition, solid dispersion, fluid bed coating, molecular inclusion in cyclodextrins. Different forms of micro and nano encapsulation systems are reservoir and matrix, emulsions (multilayer emulsions, nano emulsions), lipid nanoparticles (solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs), lipid vesicular carriers (liposomes, niosomes, phytosomes, bilosomes), hydrogel particles, molecular inclusion complexes, nanofibers, nanotubes, micelles.

LIPOSOMAL ENCAPSULATION LIPOSOMES

Liposomes are basically amphipathic vesicles in phospholipid structure, similar in structure to the cell membrane, with polar and nonpolar heads and double lipid layer structure. Liposomes are versatile, biocompatible and biodegradable structures that can be used as carrier systems for unstable components due to their amphipathic properties (Subramani and Ganapathyswamy, 2020). Liposomes were first described in 1965 by Bangham et al. (1965) are small intracellular shaped structures consisting of a closed membrane storing or transporting lipid-based substances. Phospholipids are one of the main groups providing liposome formation. Phospholipids are mainly composed of three-carbon alcohol, glycerol or sphingosine. Common alcohol components of glycerol-derived phospholipids are called phosphatidylserine (PS), phosphatidylcholine (PC), phosphatidylethanolamine (PE),

phosphatidylglycerol (PG), and phosphatidylinositol (PI). Phospholipids are formed by esterification of the primary hydroxyl group of glycerol with phosphoric acid. The remaining two hydroxyl groups of the glycerol backbone are esterified to fatty acids (saturated or unsaturated) and form the nonpolar tails of the lipid (Segota and Tezak, 2006). Liposomes consist of two layers of molecules with nonpolar groups. In the liposomal structure, polar head groups are directed outward, while non-polar parts are directed inward. Hydrophobic interactions and Vander Walls bonds that hold long hydrocarbon tails together play an important role in bilayer formation (Bozzuto and Molinari, 2015).

Liposomes are divided into different categories based on their structural properties and composition. Liposomes differ from each other in size and physical morphology, depending on lipid composition and preparation method, and may consist of one or more lipid bilayers. The phospholipid type influences the dimensions and physicochemical properties of the liposomes (Singh et al., 2012). Liposomes according to the composition and mechanism of intracellular delivery as follows: pH sensitive liposomes, conventional liposomes immuno-liposomes, cationic liposomes and long-circulating liposomes (Sharma and Sharma, 1997). Generally, the size change from 20 nm to 5000 nm and consist of one or more lipid bilayers. Liposomes according to lipid composition; preparation method and diameter as follows: multilamellar vesicles-MLV (>500 nm), small unilamellar vesicles-SUV (<50 nm), large unilamellar vesicles-LUV (100-1000 nm), giant unilamellar vesicles-GUV (>1000 nm), Multiple vesicles-MLV (>5000 nm), Oligomellar vesicles-OLV (100–1000 nm) and intermediate unilamellar vesicles-IUV (40-100 nm) (Lasic, 1998; Storm and Crommelin, 1998).

LIPOSOME PRODUCTION METHODS

Bangham method (thin film hydration method); One of the simplest methods for liposome formation in multilamellar vesicles is the thin-film hydration procedure. Thin-film hydration method is the most widely used technique to prepare liposomes (Bangham et al., 1965). The thin-film hydration method consists of sequentially dissolving phospholipids in an organic solvent (mostly chloroform), evaporating the solvent to form a thin film, and then dispersing the dry lipid film in an aqueous phase. In this method, sonication is used to reduce the size of large-sized liposomes (Maja et al., 2020). Apart from this, the methods applied are as follows, solvent (ether or ethanol) injection method, reverse phase evaporation (REV), dialysis, extrusion, spray drying, heating, freeze drying, cross flow injection, microfluidization, membrane contactor, supercritical reverse phase evaporation (SCRPE), improved SCRPE method (ISCRPE), supercritical antisolvent (SAS), depressurization of an expanded liquid organic solution-suspension (DELOS) and ultrasonication method. Each of these methods has different advantages and disadvantages.

LIPOSOMAL ENCAPSULATION METHOD PROPERTIES

Liposomes are preferred in the encapsulation process thanks to biocompatible, biodegradable, no show toxic effects, and high ratio protect of coated material (Laye et al., 2008; Gibis et al., 2012; Chun et al., 2013). One of the most important features of liposomes is that can be obtained from nature components. The natural structure of liposomes enables the usage in food systems without the need for any legal regulation (Taylor et al., 2005). In food science, the liposomal encapsulation method is used to encapsulate antioxidant components, antimicrobial components, enzymes and additives. The liposomal system is used in the encapsulation of many bioactive components, including fatty acids such as gambogenic acid (Tang et al., 2018), resveratrol (Caddeo et al., 2008), tea catechins (Zou et al., 2014) and linolenic acid (Vélez et al., 2019), omega-3 and protein hydrolysates (Li et al., 2015).

Liposomal encapsulation offers a versatile approach in terms of preservation and controlled release of sensitive bioactive ingredients, delaying food spoilage, protecting bioactive ingredients from degradation after consumption, and increasing the bioavailability of ingredients during adsorption (Liu et al., 2020).

Liposome structures improve the solubility of lipophilic compounds in aqueous solutions or hydrophilic compounds in hydrophobic systems. Thanks to high dispersion in water, liposomes can be used to produce low-calorie and fat-reduced products. In addition, liposomes have an important effect in preventing oxidation, removing negative flavors and reducing the energy density of food products (Farrokh et al., 2017). The structural similarity to the cell membrane provides distribution and release some bioactive components to specific areas in the body (Gabizon et al., 2004; Laye et al., 2008). This unique structure allows liposomal nanoparticles to enter the intercellular space in the body. Liposomes have no adverse effects on health and also many health benefits such as liver protection, memory enhancement and inhibition of cholesterol absorption is revealed in studies.

STUDIES ON LIPOSOMAL ENCAPSULATED INGREDIENTS IN FOOD PRODUCTS AS ANTIMICROBIAL AGENTS

The antibacterial activities of clove oil and liposome-encapsulated clove oil were investigated by Cui et al. (2015) and stated that liposome-encapsulated clove oil can be use efficiently as an antimicrobial agent for S. aureus in tofu. In a study by Pinilla and Brandelli (2016) determined the antimicrobial activity efficiency of liposome lysine and garlic extract encapsulated with phosphatidylcholine. Nanoliposome-encapsulated nisin-GE has potential as an antimicrobial formulation for food use. According to results, the use of natural antimicrobial nanoliposomes in dairy products is an important alternative way to improve food quality and shelf life. Lopes et al. (2017) carried out the encapsulation of nisin by nanoliposomes obtained using soybean phosphatidylcholine (PC), pectin or polygalacturonic acid. Antimicrobial activities of liposomes were observed against five different strains of Listeria, and showed the highest activity against L. innocua. In-vitro release studies have indicated that the nisin release rate of PC-pectin and PC-polygalacturonic acid liposomes is lower than that of PC liposomes.

Ghorbanzade et al. (2017) stated that fish oil has important benefits in the daily diet, but applications in food formulations are limited due to strong odor and rapid deterioration. So, fish oil encapsulated with nano-liposomal process and usage in the yogurt formulation. It has been stated that nano-liposome fish oil capsules provide a significant reduction in acidity, syneresis and peroxide values of yogurt. In terms of sensory properties, the addition of nano-encapsulated fish oil in yogurt shows similar properties with the control sample enriched with free fish oil. Pabast et al. (2018) investigated the effects of lamb meat in capsules containing free or chitosan-nano-liposomal encapsulated Satureja khuzestanica essential oil on chemical, microbial and sensory properties of lamb at 4°C for 20 days. As a result of the study, the chitosan-liposom encapsulated essential oil of Satureja khuzestanica could be a promising active packaging material to extend the shelf life of lamb. Lopes et al. (2019), lysozyme and nisin were lipozomal encapsulated with phosphatidylcholine (PC) and pectin or polygalacturonic acid. The co-encapsulation of lysozyme and nisin with liposome has a synergistic antimicrobial effect on L. *monocytogenes* and *S. enteritidis*, but provides greater inhibition against *L. monocytogenes*. The PC-pectin liposomes used in full-fat and skim milk medium reduced the *L. monocytogenes* population by 2 log cfu/ml in whole milk and 5 log cfu/ml in skim milk at 37°C. The L. monocytogenes population remained below the detection limit in milk stored for 25 days under refrigeration temperature. This shows that liposomes can be a promising technology to provide controlled release and stability in complex food systems.

Pinilla et al. (2019) used garlic extract encapsulated with liposome process with phosphatidylcholine and oleic acid as an antifungal agent in bread formulation. They reported that bread samples containing encapsulated garlic extract and free garlic extract (0.65ml/100g dough) were more microbiologically stable and showed mold inhibition for five days compared to control samples. As a result of the study oleic acid and liposomal garlic extract can be used as natural antifungal agents to improve the microbiological stability of cooked food products due to their thermal properties. In a study made by Lin et al. (2022), a bio-responsive composite liposome with silk fibroin, L-fucose and *Litsea cubeba* essential oil were designed for chicken preservation as antibacterial agent and as results indicated that 20% (v/v) of the composite liposomes could inactivate 99% *Campylobacter jejuni* (C. jejuni).

CONCLUSION

Food safety is an important issue for people in the food production process and consumption. In this respect, food production is faced with many technological challenges due to the increasing demand for naturally additive foods. The natural preservative components are significantly affected by environmental conditions and therefore components must be protected by encapsulation. In the food industry, liposomes have been investigated to deliver proteins, enzymes, vitamins, antioxidants and flavors. Many studies indicate that the efficacy of antimicrobial components is increased with liposome encapsulation. The great advantage of liposomes over other encapsulation technologies is their high stability. As a result, it has been demonstrated that there is a significant potential for use of liposome-encapsulated antimicrobials to improve the quality and healthiness of a wide variety of food products.

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REASONS FOR THE UNDERDEVELOPMENT OF CANOLA IN TÜRKİYE

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ABSTRACT

Rapeseed (*Brassica napus* L.), a member of the Brassicaceae family, is a strategic oilseed crop with a high oil content (35-50%) and low saturated fat content. Globally, rapeseed is considered one of the primary raw materials for biodiesel production. In addition, it contributes significantly to agricultural ecosystems as animal feed, a forage plant for bees, and a crop used in rotation systems. Despite Türkiye's favourable climate and soil conditions for rapeseed cultivation, its production has not reached the desired levels. Currently, approximately 50% of Türkiye's vegetable oil deficit is met through imports, highlighting the urgency of expanding rapeseed production as it has the potential to help close this gap. In the past, the cultivation of rapeseed was prohibited due to its high erucic acid and glucosinolate content, which led to a persistent negative perception among farmers. Although double-zero (canola) varieties have since been developed, this negative perception remains. In irrigated areas, alternative crops tend to offer higher returns, whereas in rainfed areas, farmers' adherence to traditional practices reduces rapeseed preference. Moreover, insufficient support premiums and rising raw material costs have diminished the economic appeal of biodiesel. The lack of purchase guarantees, insufficient contract farming practices, and challenges in storage further exacerbate marketing problems. Farmers' lack of knowledge about rapeseed and loyalty to conventional crops hinder the adoption of this alternative crop. Technical challenges such as bird damage, non-uniform ripening at harvest, and the additional cost associated with the use of hybrid seeds also limit production. These issues prevent rapeseed from fully realizing its potential in Türkiye's agricultural sector.

Keywords: Rapeseed, Canola, Agricultural ecosystems, Biodiesel, Erucic acid, Alternative crops.

Canola, a member of the Brassicaceae (Cruciferae) family, is a plant derived from the natural hybridization of *Brassica rapa* and *Brassica oleracea* species (Doğru, 2020). Genetically, it is an amphidiploid species with a chromosome number of 2n=38 (İşler, 2011). Canola is a preferred vegetable oil source by health-conscious consumers due to its high oil content (35-50%) and low saturated fat ratio (Gizlenci & Acar, 2021). Canola is typically grown as a winter crop, though summer varieties also exist. Depending on the region, it is harvested with a combine harvester in May and June, and its early harvest allows for the cultivation of a second crop, making it a preferred oilseed crop among farmers (Karasalan, 1999). Additionally, its advantages include being one of the earliest flowering plants, thus providing a valuable pasture for bees, its meal serving as high-quality animal feed, enabling oil mills to utilize idle periods due to early harvesting, being processed into oil without requiring dehulling, and serving as an effective crop for rotation.

The canola plant was first cultivated around 2000 BCE in India, later spreading to China and Japan. Its production surged with the onset of World War II, and it is currently the oilseed crop with the highest growth rate. Canola is extensively cultivated in Europe, Canada, and the United States, and it can be easily grown in almost every region of Türkiye (Baran, 2010). There is no definitive information on when canola began to be used as an oil source in Europe. However, Appelqvist (1972) suggested that canola was first cultivated for oil production in the Netherlands in the 17th century. Schröder-Lembke (1989), citing a church tax record from 1421, indicated that canola cultivation in the Netherlands began even earlier. It has been reported that canola was intensively grown in the Rheinland region of Germany, and the oil, being cheaper than olive oil, was used both as lamp oil for lighting and as a frying oil by the poorer segments of the population (Kimber & McGregor, 1995). Consequently, canola oil was used until the mid-19th century primarily for lighting in households and railways, as well as in soap production (Appelqvist, 1972). Due to its slow and odorless burning properties, it is still traditionally used in the sanctuaries of some churches today. However, the golden age of canola oil ended in the second half of the 19th century with the emergence of cheaper mineral oils. Following the invention of steam engines, canola oil, due to its high adhesion to moist metal surfaces, was used particularly during World War II to lubricate machine parts in contact with water on warships. To meet this demand, the first canola cultivation in Canada began in 1943 (Anonymous, 1992).

Canola was introduced to Türkiye during World War II through immigrants from Bulgaria and Romania (Öğütçü & Kolsarıcı, 1979; Özgüven, 1990). Prior to 1980, it was cultivated in various regions, particularly in Thrace, but its cultivation was banned for two years by the Turkish Ministry of Health due to the identification of risk factors such as erucic acid and glucosinolates (İpkin et al., 1990). This ban was lifted with the development of double-zero varieties, and the Turkish Ministry of Health's report dated April 15, 1987, permitted the use of canola oil in human food. To eliminate the negative perception associated with erucic acid and glucosinolates, the newly developed double-zero varieties (with erucic acid and glucosinolate levels reduced below specific thresholds) have been popularly referred to as canola.

In the early 2000s, biodiesel use experienced a rapid upward trend globally. In parallel, biodiesel consumption in Türkiye also increased. Globally, 86% of biodiesel is produced from canola (Gizlenci et al., 2011). As in the rest of the world, vegetable oils are used as raw materials for biodiesel production in Türkiye. Since the 2000s, canola cultivation areas in Türkiye have shown a rapid increase, leading to a corresponding rise in production volumes. Canola seed production, which was 187 tons in 2000, reached 650 tons in 2001 and 1,500 tons in 2002 (Gizlenci et al., 2007). The growing popularity of biodiesel was a significant factor in this increase. During this period, despite not meeting standards, biodiesel met a substantial portion of the diesel needs of construction machinery due to its cost-effectiveness. However, rising vegetable oil prices in subsequent years increased biodiesel unit costs, causing it to lose its price advantage over diesel. The economic appeal of biodiesel, which had driven the expansion of canola cultivation areas between 2000 and 2005, waned, leading to a reduction in cultivation areas. In later years, legal regulations regarding biodiesel were introduced, and mandatory blending requirements were implemented. In 2011, the Energy Market Regulatory Authority (EPDK) mandated biodiesel blending with diesel at 1% in 2014, 2% in 2015, and 3% in 2016, using domestically produced raw materials (Acar, 2014). However, this mandate was repealed due to challenges in raw material supply. Currently, a mandatory blending requirement of 0.5% biodiesel, produced from domestic raw materials, is applied to diesel.

According to 2022 global oilseed crop cultivation data, soybeans lead with 133.7 million hectares, followed by canola (39.9 million hectares), cottonseed (31.4 million hectares), peanuts (30.5 million hectares), palm (30.0 million hectares), and sunflower (29.2 million hectares) (FAOSTAT, 2024). In terms of 2023 regional data, 67.76% of canola cultivation areas in Türkiye are located in the Marmara Region, which accounts for 59.27% of canola production due to its cultivation area (TUIK, 2024). Outside the Marmara Region, Konya province has gained a significant share in canola cultivation and production in recent years.

In Türkiye, canola production is concentrated in the Thrace, Central Anatolia, and Çukurova regions. As of 2021, the canola cultivation area in Türkiye is below 50,000 hectares, with a total production of approximately 100,000 tons (Gizlenci & Acar, 2021). Based on information from the sector, it is reported that in the 2024-2025 production season, the cultivation area reached approximately 600,000 decares; however, due to irregular rainfall and subsequent drought in the autumn of 2024, approximately 20,000 decares of the cultivation area were adversely affected. In Samsun province, the cultivation area has increased, exceeding 10,000 decares in the 2024-2025 season. The primary factor contributing to this increase in Samsun is the distribution of canola seeds to producers under a project implemented by the Samsun Provincial Directorate of Agriculture and Forestry. Approximately 15-20% of Türkiye's total arable land remains uncultivated each year due to fallowing practices. As is known, the adoption of advanced agricultural techniques can eliminate the need for fallowing. In particular, the use of oilseed crops (such as canola, safflower, etc.) in crop rotation systems can help reduce fallow areas.

Globally, canola ranks second in terms of cultivation area and has a wide range of applications; however, several factors prevent it from reaching the desired cultivation levels in Türkiye. This issue is not unique to canola but applies to oilseed crops in general. Türkiye has long been a net importer of oilseeds and their derivatives. Considering that the annual vegetable oil requirement is approximately 1.5 million tons, while domestic production has not exceeded 800,000 tons, the situation becomes clearer. The reasons for the failure to achieve the desired canola cultivation areas can be outlined as follows:

- Due to its historical ban caused by erucic acid and glucosinolate content, canola has a negative perception among producers. This negative image leads farmers to approach canola cultivation cautiously. One characteristic feature of Turkish farmers is their tendency to remember negative experiences for many years, and even when these issues are resolved, their prejudices often persist unchanged.
- One of the most critical parameters in canola cultivation is ensuring that plants reach the 4-6 leaf rosette stage by the time the first autumn frosts begin. Particularly in the 2000s, late-season canola sowings were carried out by the private sector without considering this factor. In these areas, plants were caught by the first autumn frosts at the cotyledon stage before reaching the rosette stage, resulting in complete crop loss. This negative experience has remained etched in producers' memories for many years. To prevent frost damage due to failure to reach the rosette stage, sowing timing must be carefully managed. Depending on the region, sowing should generally be completed between late September and October 20. However, even if sowing is done on time, delayed rainfall or insufficient soil moisture for germination can delay sprouting, potentially leading to frost damage. To mitigate this, sowing should be done in moist soil after rainfall, or, in cases of delayed rainfall, sprinkler irrigation should be used to provide the necessary moisture for germination.

- When planning production, farmers primarily consider the net profit per decare. It is recommended that canola be cultivated in irrigated areas to achieve satisfactory yields and income. Higher yields and profits can be obtained when canola is grown in irrigated fields. However, since alternative crops grown in irrigated areas are perceived to offer higher returns, farmers do not prioritize canola. Consequently, canola is typically cultivated in non-irrigated areas, where its deep root system allows it to provide higher returns compared to alternative crops. Additionally, many farmers are reluctant to abandon their established habits, including their choice of crop types. They are hesitant to shift away from crops they have cultivated for many years. There are valid reasons for this reluctance, such as their thorough knowledge of the cultivation practices for these crops, their ability to quickly and effectively address potential issues, the suitability of their equipment for these crops, the existence of an established market system for their regular crops, and their general aversion to risk, which makes them cautious about adopting new crop types. Another reason for the limited adoption of canola is the lack of sufficient knowledge among farmers about the crop. Additionally, oilseed buyers in the market do not show enough enthusiasm for canola, which prevents them from raising awareness or encouraging producers to cultivate it.
- In the early 2000s, producers viewed canola as an alternative oilseed crop and cultivated it due to its suitability as a crop rotation option. However, during this period, they faced significant marketing challenges. The absence of purchase guarantees and the lack of contract farming arrangements prevented producers from successfully marketing their products. Due to canola's high oil content, long-term storage is not feasible, which further exacerbated difficulties for producers. For instance, during a field day event in Taşova, Amasya province, a remarkably high yield of 430 kg per decare was achieved, but the product remained unsold due to marketing issues. Although producer cooperatives such as Karadeniz Birlik and Trakya Birlik later included canola among crops with purchase guarantees, canola production in the region has not reached desired levels.
- For many years, canola did not receive support premiums, resulting in lower per-unit-area returns compared to alternative crops. Subsequently, canola was included in the support premium program; however, the premium amounts have not been updated to account for inflation for an extended period, rendering them insufficient.
- One of the primary criteria influencing crop choice is marketing challenges. The most effective solution to this issue is contract farming, which is preferred by farmers. For example, sugar beet producers continue cultivation despite undetermined product prices at the time of sowing and the need to meet numerous production requirements. In contract farming, beyond sales guarantees, producers receive support in terms of technical assistance, fertilizers, seeds, and pesticides, positively influencing their preferences. Regarding canola, contract farming is primarily practiced by biodiesel producers for use as a biodiesel raw material. Expanding contract farming could significantly contribute to increasing canola cultivation areas.
- All canola varieties cultivated in Türkiye are hybrids. Canola is approximately 35% cross-pollinated. Due to its hybrid nature and cross-pollination, certified seeds must be used annually, representing an additional cost for farmers accustomed to self-pollinating crops and producing without purchasing certified seeds each year. Seed costs are a critical consideration in production planning. Additionally, canola's one-third cross-pollination rate, combined with the presence of wild mustard—a close relative—in natural environments, can lead to unintended crossbreeding. This may result in erucic acid levels exceeding permissible limits, which is a concern for farmers.

- Vegetable oil processors in Türkiye design their facilities primarily for sunflower seed processing and are reluctant to adapt their systems for other oilseeds. They generally show little interest in oilseeds other than sunflower.
- One of the risks in canola cultivation is bird damage, particularly in small-scale cultivation areas where the damage rate can increase. During the green pod stage, birds squeeze the pods with their beaks to drink the sap, rendering the pods unproductive. Although birds also consume canola seeds, the primary damage occurs during the green pod stage. To mitigate this, cultivation should occur in larger areas or involve multiple farmers to reduce the crop's vulnerability to birds, thereby minimizing and making bird damage more tolerable.
- Canola, highly suitable for mechanized agriculture, is harvested using combine harvesters. However, its flowering period, lasting approximately 45 days, and the sequential ripening of pods from bottom to top complicate uniform maturation and harvesting. While lower pods may be mature, upper pods may not be fully ready for harvest, creating an undesirable situation. Waiting for all pods to mature can lead to pod shattering during harvesting, especially in lower pods, resulting in yield losses. To address this, harvesting is recommended during early morning or evening hours when humidity is relatively high, and pod shattering is minimized, rather than during hot daytime hours.
- Due to its high oil content, canola is difficult and labor-intensive to store. Unlike cereals or legumes, long-term storage is not easily feasible. Even short-term storage requires pile heights not exceeding 20 cm and frequent mixing to prevent heating and molding. Consequently, producers must market their product immediately and lack the option to store it for sale at a higher price later, unlike other storable crops.

The aforementioned reasons discourage producers from cultivating not only canola but also other oilseed crops. However, none of these challenges are insurmountable. With appropriate measures, all these issues can be addressed. Given the strategic importance of oilseed crops, increasing their production is critical to reducing Türkiye's dependence on imported vegetable oils, maximizing the efficient use of national land resources, boosting the national economy, and improving farmers' incomes. Canola is a prime candidate due to its suitability to Türkiye's climate, soil conditions, and crop rotation systems. Therefore, necessary regulations and incentives must be implemented to promote the widespread adoption of canola cultivation.

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ANTI-NUTRITIONAL SUBSTANCES CONTAINED IN RAPESEED

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ABSTRACT

Rapeseed (Brassica napus L.) is a significant oilseed crop due to its high oil content (33–50%) and low saturated fat levels. It is widely used in biodiesel production, the food industry, and animal feed, but must be handled carefully because of its content of anti-nutritional compounds. Historically, rapeseed oil was used for lighting, soap production, and machinery lubrication. However, due to the health risks associated with compounds such as erucic acid and glucosinolates, its cultivation and use were temporarily banned in Türkiye in the 1980s. The development of double-zero (low erucic acid and low glucosinolate) varieties led to the lifting of the ban, and these varieties - commonly referred to as "canola"- became suitable for food production. Erucic acid, when consumed in high quantities, can lead to serious health issues including accumulation in cardiac muscle, myocardial lipidosis, anemia, constipation, vision impairment, and an increased risk of lung cancer. Glucosinolates, sulfur- and nitrogen-containing metabolites, play a role in plant defense however it can cause thyroid, liver and kidney problems in animals if present in high amounts in the rapeseed meal. Phytate (phytic acid), the storage form of phosphorus, reduces mineral absorption in non-ruminant animals and may lead to nutritional deficiencies. Nitrate accumulation, which increases with excessive nitrogen fertilization, is associated with health risks in humans such as cancer, dementia, and goiter. Although rapeseed is a strategic crop with low input costs and rotation advantages, the management of its antinutrients factor is critical in production and consumption.

Keywords: Rapeseed, Anti-nutritional, Erucid acid, Glucosinolate, Phytate, Nitrate

INTRODUCTION

The rapeseed plant is an oilseed and forage crop derived from rapeseed varieties. A member of the Brassicaceae family, rapeseed (Brassica napus) is an annual plant with a chromosome number of 2n=38. It is reported that rapeseed is an amphidiploid species resulting from the spontaneous hybridization of Brassica rapa and Brassica oleracea (cabbage) in nature. Due to cabbage's Mediterranean origin, the origin of rapeseed is also considered to be Southern Europe. The oil content in mature seeds varies between 33–50% depending on the variety (Gizlenci & Acar, 2021). With the lowest saturated fat content among all vegetable oils, it is particularly preferred by health-conscious consumers.

There is no definitive information regarding when rapeseed began to be used as an oil source in Europe. However, Appelqvist (1972) suggests that rapeseed was first cultivated for oil production in the Netherlands during the 17th century. Schröder-Lembke (1989), citing a 1421 church tax record, indicates that rapeseed cultivation in the Netherlands began even earlier. It is reported that rapeseed was extensively grown in Germany's Rhineland region, and the oil, being more affordable than olive oil, was used both as lamp oil for lighting and as a frying oil

by lower-income communities (Kimber & McGregor, 1995). Consequently, rapeseed oil was widely used until the mid-19th century, particularly for lighting in households and railways, as well as in soap production (Appelqvist, 1972). Its slow and odorless burning properties still make it a traditional choice in the sanctuaries of some churches today. However, the golden age of rapeseed oil ended in the second half of the 19th century with the emergence of cheaper mineral oils. Following the invention of steam engines, rapeseed oil's high adhesion to wet metal surfaces led to its use in lubricating machinery exposed to water, particularly in warships during World War II, with Canada initiating rapeseed cultivation in 1943 to meet this demand (Anonim, 1992).

Rapeseed oil has been partially consumed by humans since the Middle Ages. However, speculations later emerged that erucic acid, present in high amounts in the oil's composition, and glucosinolates, a sulfur-rich compound group found in the meal used as animal feed after seed processing, could be harmful to human and animal health. Additionally, the phytic acid (phytin) content cannot be utilized by monogastric animals.

Breeding efforts aimed at eliminating these two risk factors yielded positive results, and the first rapeseed variety, named "Tower," was developed in Canada with reduced levels of both erucic acid and glucosinolates. These new varieties, with erucic acid content in the oil below 2% and glucosinolate content in the meal below 30 µmol/g, are generally referred to as "canola." The term "canola" has also entered the Turkish language as "kanola." The word canola is a commercial name derived from the first three letters of "Canada" combined with the initial letters of "oil," "low," and "acid." Additionally, these varieties are commonly known as "double-zero" or "çift sıfır" varieties due to their low levels of both erucic acid and glucosinolates (Sovero, 1993). In Europe, the first double-zero rapeseed varieties, "Eurol" and "Samourai," were developed between 1980 and 1990 (Despeghel, 1997).

Rapeseed was introduced to Turkey during World War II through immigrants from Bulgaria and Romania (Öğütçü & Kolsarıcı, 1979; Özgüven, 1990). Prior to 1980, it was cultivated in various regions, particularly Thrace, but its cultivation was banned for two years by the Turkish Ministry of Health due to the identification of risk factors such as erucic acid and glucosinolates (İpkin et al., 1990). The ban was lifted with the development of double-zero varieties, and on April 15, 1987, the Turkish Ministry of Health issued a report permitting the use of rapeseed oil in human food.

Rapeseed oils contain palmitic acid ranging from 0.0–0.36%, stearic acid from 0.05–0.35%, oleic acid from 72.30–83.42%, linoleic acid from 10.18–21.11%, linolenic acid from 1.23–4.07%, eicosenoic acid from 0.0–5.52%, and erucic acid from 0.0–3.01%.

Due to its high boiling point (238°C), it is an excellent frying oil. The meal remaining after rapeseed oil extraction, with its high protein content (approximately 39–40%), is incorporated into animal feed rations. Rapeseed can also be used as green fodder and silage. It is the first cultivated crop to flower in spring.

In the oil production process, sunflower and cotton seeds are first separated from their hulls, whereas rapeseed seeds are directly ground and processed. While the protein and cellulose content in sunflower seed meal and cottonseed meal vary, these ratios remain consistent in rapeseed meal. Additionally, rapeseed cultivation has lower input costs compared to many other crops.

According to 2022 global oilseed crop data, soybean leads with 133.7 million hectares, followed by rapeseed (39.9 million ha), cottonseed (31.4 million ha), peanut (30.5 million ha), palm (30.0 million ha), and sunflower (29.2 million ha) (FAOSTAT, 2024).

Based on 2023 regional data, 67.76% of rapeseed cultivation areas are located in the Marmara Region, with 59.27% of rapeseed production occurring in this region due to its cultivation area (TUIK, 2024). In recent years, Konya province has held a significant share in rapeseed cultivation and production outside the Marmara Region.

GLUCOSINOLATES

Glucosinolates are secondary plant metabolites commonly found in plants of the Brassica (Cruciferae) family, containing sulfur (S) and nitrogen (N). They are non-nutritive compounds present in rapeseed oil. These compounds consist of a β-D-thioglucose group, a sulfonated oxime group, and a side chain derived from methionine, tryptophan, or phenylalanine. Glucosinolates are water-soluble, anionic, non-volatile, and heat-resistant, exhibiting no significant biological activity unless they undergo enzymatic degradation (Fenwick et al., 1983; Fahey et al., 2001). To reduce glucosinolate hydrolysis and the solubility of sulfur in the oil, the activity of the myrosinase enzyme must be carefully controlled during heat treatment. The myrosinase enzyme becomes active within a temperature range of 50–70°C (Shahidi, 2005).

When plant tissue is cut, chopped, chewed, or otherwise damaged, glucosinolates are hydrolyzed by the endogenous myrosinase enzyme (thioglucoside glucohydrolase, E.C. 3:2:3:1) naturally present in the plant. This process results in the release of various aroma compounds, such as thiols, depending on environmental conditions (e.g., pH, temperature, humidity, and storage conditions).

Sulfur-containing glucosinolates in rapeseed oil are degradation products and are organic compounds. These sulfur components also enhance the stability of the oil. Certain components exhibit antioxidant behavior, protecting the oil from oxidation. Other beneficial effects of these components include inactivating substances, such as metals, that act as catalysts during oxidation (Shahidi, 2005).

The degradation products of glucosinolates are soluble in oil, volatile, highly reactive, and possess a sharp odor and taste profile. Additionally, some exhibit fungicidal, bactericidal, antiprotozoal, nematicidal, and allelopathic effects, playing significant roles in biological processes (Chew, 1988; Lazzeri et al., 1993; Palada, 1996; Charron & Sams, 1999). To date, approximately 120 different glucosinolates have been identified in about 450 species across 16 plant families (Cole, 1976; Fenwick et al., 1983; McGregor et al., 1983; Mithen et al., 2000; Fahey et al., 2001).

Environmental factors are among the key determinants of glucosinolate content. It has been found that glucosinolate levels in black mustard and rapeseed decrease with increased nitrate application (Fenwick et al., 1983). Irrigation regimes also influence glucosinolate content. Glucosinolates are reported to provide significant protection against diseases and pests (Harbone, 1998). Furthermore, sulfur and nitrogen fertilization not only increase yield and protein content but also lead to an increase in glucosinolate levels (Cramer, 1990; Türk et al., 2008).

The glucosinolate content in rapeseed meal is desired to be below 30 μmol/g. Numerous studies based on feeding experiments with rapeseed have investigated the physiological effects of glucosinolates and their hydrolysis products. These studies have observed enlargement of the thyroid, adrenal glands, kidneys, and liver, and liver hemorrhage (Liver Hemorrhage Syndrome - LHS) has been identified in poultry fed with rapeseed meal. Nishie and Daxenbichler investigated the toxicological effects of three glucosinolates and nine degradation products on mice. None of these compounds were found to be teratogenic, but 2-propenyl isothiocyanate,

3-methylsulfinylpropyl isothiocyanate, and 1-cyano-3,4-epithiobutane were observed to be embryotoxic. It has been established that many naturally occurring isothiocyanates are cytotoxic. Isothiocyanates formed from glucosinolates are highly unstable and spontaneously degrade into various molecules. One such degradation product, oxazolidine-2-thione, has been reported to cause thyroid gland enlargement and goiter in rabbits (Fahey et al., 2001).

Despite being an oil-rich seed, rapeseed is also a significant source of protein, and its glucosinolate content is a critical factor in its use for animal feed. These compounds can indirectly transfer to humans through the milk of animals fed with plants from this group. The total glucosinolate content in canola meal is approximately 7.2 μmol g^{-1} (Newkirk et al., 2003). Canola hay, silage, and straw can accumulate sulfur (S) at levels as high as 0.5–1.3% of dry matter (DM). To prevent hemolytic anemia caused by S-methyl-L-cysteine sulfoxide, which manifests as symptoms such as blood in urine and weakness; to avoid polioencephalomalacia (PEM) caused by reduced thiamine (vitamin B1) absorption leading to brain necrosis; to prevent elevated hydrogen sulfide levels in the rumen; to ensure proper absorption of copper (Cu) and selenium (Se); and to avoid goiter caused by isothiocyanates, the sulfur content in daily ruminant rations should generally not exceed 0.4% of DM. It is recommended that canola hay does not exceed 50–60% of the dry matter in Total Mixed Rations (TMR) for dairy cows and feedlot cattle. For example, if 14 kg/day of roughage is used in a standard diet, 6 kg could be canola hay and 8 kg could be alfalfa hay (Lardy et al., 2015; Llewellyn, 2018; Vitti, 2018). The most distinctive characteristic of sulfur-containing compounds is their strong and characteristic aroma, which can be detected even at very low concentrations.

To prevent excessive formation of sulfur and other anti-nutritional compounds in canola, it is recommended to avoid over-application of nitrogen (N) and sulfur (S) fertilizers, maintain an N:S ratio of 5:1 in the plant, allow at least 45 days for silage maturation, dilute high levels of N and S with other roughages after analysis, and, where possible, practice intercropping with peas or produce mixed silage (Llewellyn, 2018).

ERUCIC ACID

Eruic acid (cis13-docosenoic acid) is a long-chain monounsaturated fatty acid (C22:1) with 22 carbon atoms and no branching, and it has a double bond in the omega-9 position. It is found in the seeds of plants belonging to the Cruciferae and Tropaeoleaceae families, and its proportion in rapeseed oil varies between 30% and 60% (Roine et al., 1960).

The first low-erucic acid rapeseed (DEAK) of the B. napus species was developed in 1968, and the first low-erucic acid rapeseed of the B. rapa species was developed in 1971 in Canada. Rapeseed is classified based on its erucic acid content: High Erucic Acid Rapeseed (HEAR) contains 45-60% erucic acid (HEAR: High Erucic Acid Rapeseed), varieties with medium levels of erucic acid (35-40%), and varieties with erucic acid reduced to as low as 0.03%, classified as Low Erucic Acid Rapeseed (LEAR: Low Erucic Acid Rapeseed). Rapeseed should contain less than 2% erucic acid. Rapeseed oil produced in Asia contains 22-60% erucic acid.

The decrease in erucic acid content in rapeseed oil has led to a significant increase in oleic acid content, as well as a slight increase in linoleic and linolenic acid content.

The idea that erucic acid might have toxic effects was first suggested by Roine et al. (1960). Subsequent studies in mice showed that triglycerides, largely composed of erucic acid and accumulating in heart muscles, somehow negatively affect the oxidation of fatty acids in mitochondria and ATP production, reducing the contraction strength of heart muscles and causing a disease called myocardial lipidosis (Abdellatif and Vles, 1970; Houtsmuller et al., 1970; Sauer and Kramer, 1983; Anonymous, 2025a). Although no data has been found to date showing that erucic acid is harmful to human health, it is still considered a risk factor. Rapeseed oil, which has negative effects on heart diseases, can cause side effects such as anemia,

constipation, vision damage, and hypersensitivity when consumed excessively. Permanent damage to the kidneys and adrenal glands is also among the harms of rapeseed oil.
Due to the erucic acid it contains, rapeseed oil has negative effects on the immune system, and excessive consumption can cause damage to the circulatory and nervous systems. One of its greatest dangers is that it increases the risk of lung cancer.
One of the underlying reasons for the harms of rapeseed oil is the amount of trans fat it contains. The use of rapeseed oil in margarines further increases the risks associated with trans fat.

PHYTIN (PHYTIC ACID-PHYTATE)

Rapeseed contains phytic acid. Phytic acid is the storage form of phosphorus in plants. Phytates are the Ca, Mg, K, and Fe salts of phytic acid. They affect the bioavailability of minerals. Non-ruminant animals cannot separate phosphorus from phytic acid due to the absence of phytase enzyme. 50-85% of phosphorus in plants is in the form of phytic acid. They are commonly found at varying levels (0.1-6%) in plant seeds, grain feeds, roots, and tubers. In ruminants, due to microbial phytase, ruminants can utilize phosphorus from phytate. A large portion of poultry compound feeds consists of grain feeds and meals, and most of the phosphorus in these feed materials is in the form of phytin phosphorus. Since enzymes that break down phytic acid are produced in very small amounts or not at all, phosphorus from phytate cannot be utilized, and 70% of phosphorus in feeds is excreted in feces. Additionally, phytate can indirectly bind Ca, Zn, and Mn, causing deficiencies of these minerals. As a solution, phytase enzyme should be added to the ration (Yalçın, 2002). In rapeseed, total P content is 12.4 g/kg, Phytin-P content is 2.1 g/kg, and Phytin-P percentage is 16% (Sarıfakıoğulları and Önol, 1998).

NITRATE

Nitrogen (N) is one of the essential macro nutrients that affects crop growth, development, yield, and quality (Koch et al., 2020; Kumar et al., 2021). Plants utilize various N forms in soils. Nitrate (NO_3^-) and ammonium (NH_4^+) ions are the N forms preferentially absorbed by plants from the growing medium (O'Brien et al., 2016; Cui et al., 2017). Nitrate, due to its wide distribution in soil and high solubility in water, is most likely one of the most widespread pollutant ions worldwide and has a negative impact on the quality of water resources and agricultural products (Zendehbad et al., 2022).
Many nutrients such as chloride, calcium, potassium, sulfate, and phosphorus are involved in the NO_3^- accumulation process in plants. Nitrate content varies according to different parts of the plant and the age of the plant (Santamaria et al., 1999; Anjana et al., 2006). NO_3^- uptake mostly occurs in roots, but the contribution of leaves should not be overlooked (Anjana and Iqbal, 2007). NO_3^- absorbed through leaves is initially stored in vacuoles and then transported from old leaves to young leaves depending on the NO_3^- requirement of the leaves (Le Deunff et al., 2019). Maynard et al. (1976) reported that NO_3^- concentration is at the lowest levels in the flowering parts of the plant; in contrast, nitrate is found in increasing concentrations in fruits or grains, leaves, roots, petioles, or stems.
Nitrate is necessary for plant growth and development. However, very high NO_3^- concentrations in cell sap can be harmful to plants (Yosoff et al., 2015). As a result of nitrogen fertilizer application without considering the actual needs of the plant and the amount of N available in the soil, NO_3^- accumulation may occur due to excessive N uptake by the plant (Özenç and Şenlikoğlu, 2017).
NO_3^- accumulation is generally dependent on the amount and type of nutrients present in the soil, but is also closely related to application timing, the amount and composition of applied fertilizers (Zhou et al., 2000). Nitrate accumulation is one of the major disadvantages of high-

level fertilizer application in rapeseed (Chakwizira et al., 2015). The use of fertilizers based on ammonia or nitrate-ammonium mixtures can reduce NO_3^- content in plants (İnal and Tarakçıoğlu, 2001; Santamaria et al., 2001). Increased potassium application rates facilitate nitrate uptake and transport to above-ground parts of the plant, as well as promote nitrate metabolism and utilization, consequently reducing NO_3^- accumulation in some vegetable plants (Ahmed et al., 2000; Ruiz and Romero, 2002). However, some studies have shown that potassium sources in soil do not affect NO_3^- accumulation (Drlik and Rogl, 1992).

In a study conducted by Grzebelus and Baranski (2001), the effect of climate on NO_3^- accumulation was examined, and it was determined that NO_3^- content was lower in years with high precipitation. In hot and rainy years, increased NO_3^- accumulation is possible regardless of whether nitrogen comes from organic or mineral sources (Custic et al., 2003). However, NO_3^- accumulation also varies according to season (Vieira et al., 1998). NO_3^- accumulation levels are higher in autumn-winter months compared to spring (Santamaria et al., 1999). It is thought that in winter, plants cannot utilize all the nitrogen available in the soil due to lower light and temperature conditions (Anjana and Iqbal, 2007).

When saliva is swallowed and encounters acidic gastric juice, it converts to nitrosating species (N_2O_3 and NOSCN) and reacts with ascorbic acid in gastric juice to form nitric oxide (NO), which is potentially mutagenic and carcinogenic (Moriya et al., 2002; Iijima et al., 2003; McColl, 2005). Excessive local production of nitric oxide has been reported to lead to functional abnormalities associated with reflux diseases (McColl, 2005). Additionally, high nitrite oxide levels have been suggested to be associated with various skin problems such as psoriasis, dermatitis, and even skin cancers (Karwowska, 2020).

High NO_3^- intake has been reported to increase the risks of bladder (Michaud et al., 2004), esophageal, nasopharyngeal, and prostate cancers (Eicholzer and Gutzwiller, 1990). Other health problems associated with nitrate toxicity include oral cancer (Badawi et al., 1998), colon, rectum, or other gastrointestinal region cancers (Knekt et al., 1999; Türkdoğan et al., 2003), Alzheimer's disease, vascular Biswaner type or multiple small infarct type dementia (Tohgi et al., 1998), multiple sclerosis (Giovannoni et al., 1997), spontaneous abortion or congenital defects (Fewtrell, 2004), anencephaly (Croen et al., 2001), non-Hodgkin lymphoma (Michal, 1998), and cardiovascular disorders (Morton, 1971).

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DETERMINATION OF THE STABILITY OF POLYPHENOL OXIDASE FROM BLACK MULBERRY TOWARDS DIFFERENT ORGANIC SOLVENTS AND SURFACTANTS

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ABSTRACT

Black mulberry (Morus nigra L.) was used in this study as a natural source of polyphenol oxidase (PPO). It is known for its high lycopene content and antioxidant activity. The enzyme was extracted by mixing the fruit with a solution containing 0.1 M sodium phosphate buffer (pH 7.0), 0.5% PEG 6000, 10 mM ascorbic acid and 50 mM K2HPO4. This resulted in a PPO activity of 3753 U/mL. We treated the enzyme after extraction with different organic solvents (DMSO, ethanol, isopropanol and methanol) and surfactants (SDS, Triton X-100, Tween-20 and Tween-80) to see how stable and active it was. Isopropanol and methanol were the best solvents for restoring activity, while Tween-80 had the same effect as the surfactants. These results show that PPO from the black mulberry tree can handle many organic solvents and surfactants, making it a good candidate for use as a strong biocatalyst in industry, especially in systems that do not contain water or surfactants.

Keywords: Morus nigra L., polyphenol oxidase, organic solvent, surfactant, enzyme activity

INTRODUCTION

The black mulberry is a plant that belongs to the mulberry family (Moraceae). The mulberry is native to India and Japan. It grows in temperate and subtropical climate regions (Özelçi and Yiğit 2022). It has an oval-cylindrical shape with flattened ends and its fruits are black-purple, fleshy-juicy and have a sour taste (Özkaya Erkaleli and Dalkılıç, 2016). Anthocyanins, phenolic compounds and potassium are found in large quantities in black mulberries (Aşkın et al., 2022). As it contains flavonoids, it has anti-cancer, anti-tumor, anti-radiation, anti-inflammatory and antimicrobial effects. It is also a medicinal plant that can help with coughs, urinary urgency, high blood sugar and vomiting (Feng et al., 2015). According to studies, black mulberry medicines relieve tonsillitis and are good for mouth and tooth wounds (Aşkın et al., 2022). It is eaten fresh and dried. It is used in the production of foods such as molasses, fruit juice, jam and pestle (Sernikli and Kadakal, 2020).

Polyphenol oxidase (PPO; EC 1.14.18.1) is a metalloenzyme that converts phenolic compounds into o-quinone derivatives that cause enzymatic browning of fruits and vegetables. In the food industry, PPO is considered an important biocatalyst for quality control, the development of biosensors and the management of enzymatic browning in the processing of fruits and vegetables (Tilley et al., 2023; Almeida and Nogueira, 1995). However, as the

stability and activity of PPO are sensitive to environmental conditions, its use in industry may be limited (Zou et al., 2025).

The effects of organic solvents and surfactants on PPO activity are important for industrial applications. Organic solvents can increase catalytic activity by facilitating the enzyme's access to the substrate. However, at high concentrations, they can also disrupt the conformation of the enzyme and lead to inhibition (Salehi et al., 2021). Similarly, ionic and non-ionic surfactants can modulate the activity of PPO by interfering with hydrogen bonds, electrostatic interactions or hydrophobic regions on the enzyme surface (Yang et al., 2007). These effects depend on factors such as the type of surfactant, its concentration and the pH of the medium (Aguirre-Ramírez, 2021).

In this study, the effects of different organic solvents (isopropanol, methanol, ethanol, dimethyl sulfoxide) and commonly used surfactants (SDS, Triton X-100, Tween-20, Tween-80) on PPO activity were comparatively analyzed; the data obtained were evaluated regarding the stability of the enzyme. The knowledge gained will support the use of PPO in durable systems for industrial applications such as food, cosmetics and pharmaceuticals.

MATERIAL AND METHOD

The samples of black mulberries were bought at markets in the province of Kocaeli. The commonly used parts of the black mulberry samples were washed and stored at -80 °C. Fresh black mulberry samples were weighed to 200 g, homogenized and pureed with a blender. 15 g of the pureed fruits were weighed and placed separately in Falcon tubes. 15 mL of 0.1 M sodium phosphate buffer (pH 7.0) was added. The extraction process was then carried out using separate extraction media.

The chemicals used to increase the extraction efficiency were polyvinylpolypyrrolidone (PVPP) (%1, w/v), ascorbic acid (10 mM), Triton X-100 (%1, v/v), phenylmethylsulfonyl fluoride (PMSF) (2 mM), polyethylene glycol (PEG6000, 0.5%, w/v) and K_2HPO_4 (50 mM). PVPP and ascorbic acid were used to remove or reduce phenolic compounds in the medium, while Triton X-100 was added to the mixture during extraction to solubilize the enzyme bound to the membrane. PMSF was preferred to increase enzyme activity, while PEG 6000 was used to remove phenols that could inhibit PPO (Erzengin, 2009; Gauillard and Rich-Forget 1997; Palma-Orozco et al., 2011). The prepared solution was shaken for 1 min. The resulting homogenate was kept at 4 °C for 20 min and then centrifuged at 10000 x g for 30 min in the cold. After centrifugation, the mixture was filtered and the pellet at the bottom was removed, while the crude enzyme extract was recovered from the upper aqueous phase (Navarro et al., 2014).

To detect the presence of the PPO enzyme, the increase in absorbance was measured at 420 nm (24 °C) using a spectrophotometer (Cary 60, Agilent). To measure the enzyme activity, a sample cuvette was prepared by adding 200 µL of the enzyme suspension to the substrate solution (catechol) prepared in 2800 µL sodium phosphate buffer (100 mM, pH 7.0). The control cuvette was prepared in the same way, but without enzyme. The enzyme solution was prepared with 100 mM sodium phosphate buffer solution (pH 7.0) (Erzengin, 2009). One enzyme unit is the amount of enzyme that catalyzes the formation of 1 µmole of quinone in one minute at room temperature. The Bradford method was used to determine the amount of protein in the samples. The amount of protein in the samples was measured at 595 nm using the spectrophotometric method (Bradford, 1976).

In stability tests performed to investigate the effects of organic solvents and surfactants on PPO activity, the activity was determined using chloroform, isopropanol, ethanol and dimethyl sulfoxide as organic solvents and SDS, Triton X-100, Tween-20 and Tween-80 as surfactants. The organic solvents were analyzed in the concentration ranges of 5-10-30-60%

and the surfactants in the value ranges of 0.1 and 1 mM. After 30 min of pre-incubation, the PPO activity was measured at a temperature value and the remaining activity was calculated according to the following formula (İllensanmi et al., 2023; Birge et al., 2022; Šalić et al. 2018).

% Residual activity = [100 − (polyphenol oxidase activity after treatment) (polyphenol oxidase activity before treatment)] *100

RESULTS AND DISCUSSION

The polyphenol oxidase (PPO) activity of mulberry fruit was analyzed in a crude extract prepared with an extraction medium containing 50 mM K_2HPO_4, 0.5% PEG 6000, 10 mM ascorbic acid and 0.1 M sodium phosphate buffer (pH 7.0). The PPO activity under these conditions was determined to be 3753 U/mL. Comparable extraction techniques and activity values have been documented in the literature for different plant species. For example, Erzengin (2002) used 0.5 M sodium phosphate buffer (pH 7.3), 0.5% PEG 6000, 10 mM ascorbic acid and 50 mM K_2HPO_4 to analyze white mulberry (*Morus alba* L.). An extraction medium containing 0.5 M sodium phosphate buffer (pH 7.3) and 0.5% PEG yielded a PPO activity of 567 U/mL in another study on Iğdır apricots (*Prunus armeniaca* L.) (Demir et al., 2012). Ziyan and Pekyardımcı (2003) used 50 mM ascorbic acid, 10% PEG and 0.2 M phosphate buffer (pH 7.2) for Jerusalem artichoke (*Helianthus tuberosus*). Similarly, the extraction medium for the borago (*Trachystemon orientalis*) study by Alici and Arabacı (2016) contained 0.1 M phosphate buffer (pH 7.0), 1 mM ascorbic acid, 0.5% PVPP and 2% Triton X-100. Ascorbic acid and PVPP are commonly used in most extraction procedures to remove phenolic compounds from the medium. PEG 6000 helps to maintain enzyme stability by binding phenolic compounds that could inactivate PPO, Triton X-100 helps to release membrane-bound enzymes and PMSF helps to maintain enzyme activity (Gauillard and Richard-Forget, 1997; Erzengin, 2009; Palma-Orozco et al., 2011).

To determine the effects of organic solvents and surfactants on the activity of polyphenol oxidase (PPO) extracted from mulberries, experiments were carried out at different concentrations. The organic solvents were used at concentrations of 5%, 10% and 30%, including DMSO, ethyl alcohol, isopropanol and methanol. The surfactants used were SDS, Triton X-100, Tween-20 and Tween-80 at concentrations of 0.1 mM and 1 mM.

The effects of organic solvents on PPO activity are shown in Figure 1. The recoveries of PPO activity were as follows: in the presence of DMSO 77%, 108% and 10%; ethyl alcohol 52%, 114% and 82%; isopropanol 158%, 151% and 124%; and methanol 93%, 161% and 104% at a concentration of 5%, 10% and 30%, respectively. It is noteworthy that all solvents led to a significant increase in PPO activity at certain concentrations. The highest activity recovery was observed at 10% for DMSO, ethyl alcohol and methanol and at 5% for isopropanol. These results indicate that PPO extracted from mulberries has a remarkably high resistance to organic solvents.

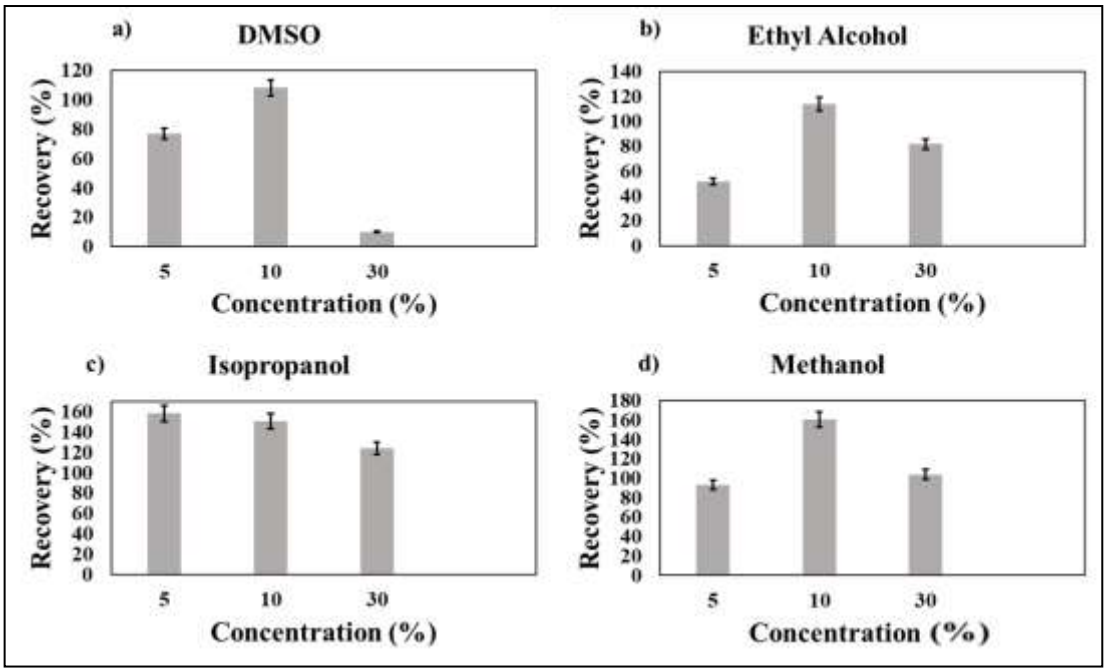

Figure 1. Effect of organic solvents (a-d) on black mulberry polyphenol oxidase enzyme activity

The effects of SDS, Triton X-100, Tween-20 and Tween-80 on PPO activity are shown in Figure 2. At concentrations of 0.1 mM and 1 mM, the recoveries of PPO activity were 57% and 52% for SDS, 74% and 35% for Triton X-100, 78% and 74% for Tween-20, and 92% and 79% for Tween-80. Both Tween-80 and Tween-20 showed high activity recovery at both concentrations. Tween-80 showed remarkable recoveries of 92% at 0.1 mM and 79% at 1 mM. In the presence of SDS, activity recovery was above 50% at both concentrations, while the recovery of 74% observed with Triton X-100 at 0.1 mM also represents a remarkably high level of activity.

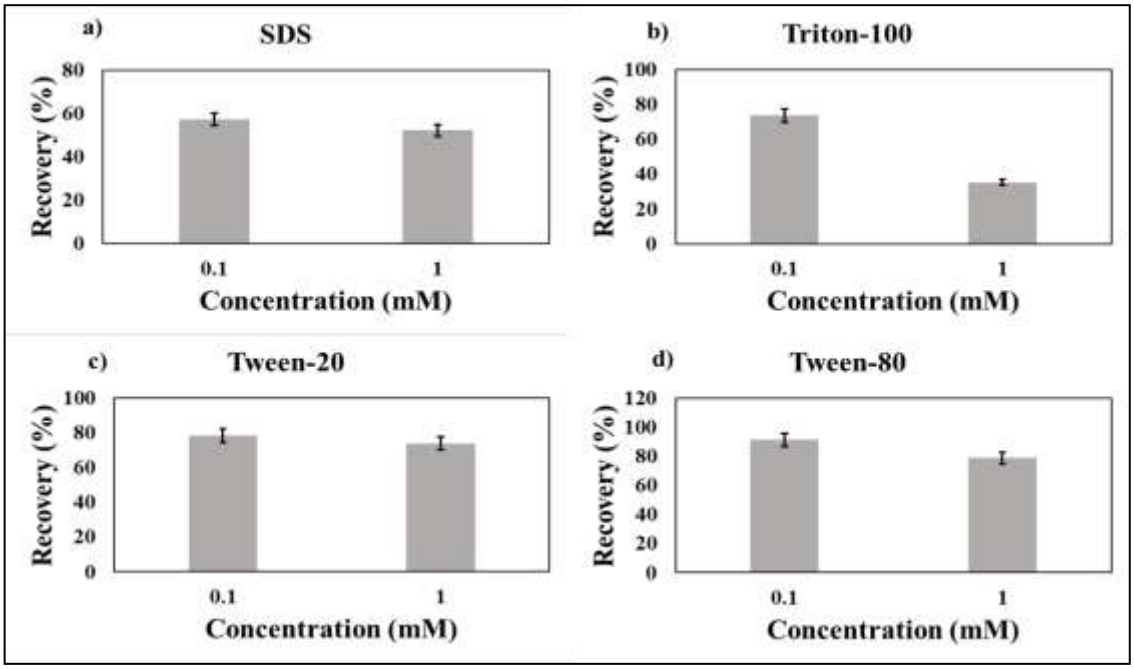

Figure 2. Effect of surfactants (a-d) on black mulberry polyphenol oxidase enzyme activity

The effect of organic solvents and surfactants on enzyme activity is a complex interaction determined by changes in the enzyme microenvironment, substrate solubility and structural integrity of the protein. Organic solvents can increase enzyme activity by increasing the solubility of hydrophobic substrates and shifting the reaction equilibrium from hydrolysis to synthesis (Samiey et al., 2014). However, they often lead to reduced enzyme dynamics, changes in active site polarity and even partial denaturation, especially with prolonged exposure, resulting in reduced catalytic efficiency and stability (Zhao, 2020). On the other hand, surfactants can modulate enzyme activity through direct interactions with the enzyme surface. Especially with hydrophobic substrates, they can cause limited conformational changes that expose the active site or improve the accessibility of the substrate and thus increase activity (Savelli et al., 2000). In some cases, surfactants such as SDS facilitate substrate access to the active site and activate the enzyme, but at higher concentrations they can destabilize the protein and cause denaturation. Crystallographic studies confirm that surfactants can have pronounced effects on enzyme conformation, which can either promote or inhibit enzymatic activity depending on the enzyme and surfactant type (Goldfeder and Fishman, 2014).

CONCLUSIONS

In this study, the PPO activity of mulberry fruit was calculated at 3753 U/mL in the crude extract obtained with an extraction medium containing 0.1 M sodium phosphate buffer (pH 7.0), 0.5% PEG 6000, 10 mM ascorbic acid and 50 mM K_2HPO_4. In the experiments performed to evaluate the effects of organic solvents on enzyme activity, the mulberry PPO enzyme showed the highest activity recovery at concentrations of 5% isopropanol, 10% methanol and 30% isopropanol. As for surfactants, the highest recovery was observed with Tween-80 at concentrations of 0.1 mM and 1 mM.

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BIOCHEMICAL AND AROMATIC CHANGES IN PERSIMMON (DIOSPYROS KAKI L.) FRUITS HARVESTED IN DIFFERENT PERIODS

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ABSTRACT

Persimmon (*Diospyros kaki* L.), originally from China and widely cultivated throughout East Asia, has recently gained popularity in several Mediterranean countries, including Türkiye. This study was conducted to determine the optimum harvest time by evaluating the biochemical and aromatic changes in persimmons at different maturity stages. The research was carried out in a commercial orchard in the Dareski hamlet of the Toroslar district, Mersin Province, focusing on the commonly grown 'Hachia' cultivar. Fruits were harvested at seven-day intervals beginning in the last week of October and analyzed at two distinct maturity stages: unripe and fully ripe. Sugar analysis revealed that glucose and fructose were the predominant sugars in both stages, with significantly higher levels in ripe fruits (8930 and 9166 mg/100g, respectively) compared to unripe ones (6561 and 7213 mg/100g). Total sugar content increased from 13,867 to 18,248 mg/100g, along with an increase in total soluble solid content (TSS) from 14.45% to 19.4%. In terms of organic acids, malic acid was dominant in unripe fruits (22.43 mg/100g), whereas succinic acid significantly increased in ripe fruits (38.27 mg/100g). Citric and ascorbic acid contents also varied notably between the two stages, and titratable acidity rose from 0.86% to 1.14% during ripening. Volatile compound profiling revealed clear maturity-dependent differences. Aldehyde compounds were more abundant in ripe fruits (37.76%), contributing to a richer and more intense aroma, while alcohols and terpenes predominated in unripe fruits (17.44% and 44.98%, respectively), with linalool, nerol, and limonene as major contributors. These findings confirm that the biochemical and aromatic profiles of persimmons are strongly influenced by the maturity stage, which has important implications for flavor development, postharvest quality, and market value. Despite increasing interest among consumers and producers, persimmon production in Türkiye is still limited due to challenges in storage and marketing. The study suggests that harvesting after the second ripening stage may improve product quality and commercial potential under the Mediterranean climate of Mersin.

Keywords: Persimmon, maturity stage, sugars, organic acids, volatile compounds.

INTRODUCTION

Fruit cultivation worldwide is generally concentrated on a limited number of species; however, persimmon (*Diospyros kaki* L.), belonging to the Ebenaceae family, has gradually expanded to many regions of the world and has gained increasing popularity in recent decades (Öz and Özelkök, 2003). Native to China, persimmon was first extensively cultivated in Japan and later established in modern orchards in countries such as the United States (California), Italy, Brazil, New Zealand, Australia, and Israel (Kaynaş & Kuzucu, 2004). The fruit, known for its attractive appearance and pleasant taste, has been given names such as “Japanese persimmon,” “Mediterranean persimmon,” and “cennet hurması” in Turkey (Yıldız Akbulut, 2021). Although the genus *Diospyros* contains numerous species, only four (*D. kaki Thunb*., *D.*

lotus L., *D. virginiana* L., and *D. oleifera* Cheng) are of commercial importance, with *D. kaki* being the most widely cultivated for fresh consumption and processing (Kitagawa & Glucina, 1984; Yıldız et al., 2012). In Turkey, persimmon production has expanded considerably in the last 20–25 years. While production levels were between 15,000–20,000 tons in the early 2000s, it increased to 127,314 tons in 2023 and reached 142,584 tons in 2024, showing a 12% growth within a year (Anonymous, 2025a). This rise reflects both consumer demand and the fruit's adaptation to different climatic regions of Türkiye, including the Mediterranean, Aegean, Marmara, and Black Sea. Persimmons are harvested between September and December, displaying wide variation in fruit shape, size, and skin color. Both astringent and non-astringent cultivars are cultivated; while astringent fruits are unpleasantly bitter when unripe due to soluble tannins, they become sweet and palatable after softening or through specific postharvest treatments (Onur, 1990).

The ripening of persimmon fruit is a complex process involving physiological, biochemical, and structural changes. These changes affect sensory attributes such as color, taste, and texture, determining overall fruit quality (Chitarra et al., 2005). During maturation, chlorophyll degradation and carotenoid accumulation cause color changes from green to yellow, orange, or reddish hues (Kluge et al., 2018; Zhao et al., 2011). Studies have shown that carotenoid content, including lycopene, increases markedly during the mid to late stages of ripening, contributing to the reddish coloration of certain cultivars (Qi et al., 2019). Texture softening is mainly attributed to pectin degradation, cell wall disintegration, and membrane breakdown, while increased intercellular spaces and water loss lead to fruit weight reduction (Salvador et al., 2007; Tessmer et al., 2016; Sapper et al., 2019). Carbohydrate metabolism plays a key role in fruit ripening and sweetness perception. Changes in sugar composition are largely influenced by cultivar-specific enzymatic activity. In general, sucrose content decreases while glucose and fructose increase during ripening due to invertase activity (Bubba et al., 2009; Giordani et al., 2011; Santos et al., 2019). Different developmental patterns have been reported, such as sucrose reduction accompanied by increases in glucose and fructose or stability in certain sugars depending on the cultivar (Santos et al., 2018). Alongside carbohydrates, organic acids, amino acids, phenolics, and choline also undergo significant changes during maturation, especially in the late stages of development (Santos et al., 2018). Nutritionally, persimmon is an important source of ascorbic acid, carotenoids, phenolic compounds, and dietary fiber, contributing to high antioxidant activity and health-promoting effects (Parseker Yönel et al., 2008). Vitamins A and E, tannins, and minerals also enhance its nutritional and functional food value. These properties have contributed to growing consumer demand worldwide. However, challenges such as cultivar identification, determination of optimum harvest time, and effective postharvest handling still cause yield and quality losses (Onur, 1990; Tuzcu & Yıldırım, 2000). A major quality determinant is astringency, resulting from soluble tannins, which decrease during ripening due to polymerization or can be artificially reduced through treatments such as ethylene exposure, CO_2 application, or storage in alkaline solutions (Itoo, 1971; Itoo, 1980; Taira et al., 1997). Considering its nutritional richness, consumer preference, and economic potential, persimmon has become an increasingly valuable fruit crop in Turkey. Nevertheless, studies on its phytochemical and volatile composition under local ecological conditions are still limited. Therefore, this study focuses on the characterization of phytochemical compounds and volatile profiles of the 'Hachiya' persimmon cultivar grown under the ecological conditions of Mersin (Toroslar, Türkiye). The results are expected to provide new insights into ripening behavior, postharvest quality, and flavor development, ultimately contributing to reducing postharvest losses and increasing consumer acceptance.

MATERIALS AND METHODS

Plant Material

Persimmon fruit samples were collected from 20-year-old Hachiya trees planted in 5x5 spacings at various stages of ripening in a producer garden in Darseki village, Toroslar district, Mersin province. The samples were collected twice in 2023, once in October (unripe period) and once in November (ripe period). Fruit samples from three distinct trees and five fruit samples from different sides of the tree (North, South, West, and East) were taken in three repetitions during each harvest period and brought to the Horticulture laboratory of Çukurova University Faculty of Agriculture in an ice box cold chain. After washing the samples in tap water, 1 gram of hand squeezed fresh fruit juice was utilized for each bichemical assay.

Method

Total Soluble Solids (TSS): The SSC of representative fruit samples was measured as °Brix using a digital refractometer (Atago, Japan). A drop of fruit juice was placed on the screen, and the displayed value was recorded as % SSC.

Titratable acidity (TA): A 1 ml sample of fruit juice was titrated with 0.1 N NaOH solution until the pH reached 8.1. The acidity was expressed as a percentage of malic acid (Cemeroğlu 2007).

Individual Sugar and Organic Acid Extraction and Determination

Sugars and organic acids were extracted individually following the procedure described by Mikulic-Petkovsek et al. (2016). Homogenized, unfrozen fruit samples were placed in a test tube containing 4 mL of bi-distilled water, at 1 g. The mixture was continuously mixed on a vortex mixer at room temperature for thirty minutes. After extraction, the samples were centrifuged at 9000 x g for ten minutes at 4°C to obtain the supernatant and then filtered into vials using cellulose membranes. The extracts were stored at -20°C for further investigation. The high-performance liquid chromatography (HPLC) system was equipped with a refractive index detector (Shimadzu RID 20A VP, Kyoto, Japan), an in-line degasser, a pump, a controller, and a 20 µL injection volume automatic injector. Chromatographic separation was performed using a reverse-phase Ultrasphere Coregel-87 C analytical column (300 mm × 7.8 mm i.d., 5 µm, Transgenomic) at a flow rate of 0.6 mL/min, heated to 70 °C, and with the mobile phase being ultrapure water. Data acquisition and processing were performed with Shimadzu Class VP chromatography management software (Kyoto, Japan). Concentrations of individual sugars are reported as percentages of fresh weight (FW) determined using original standards.

Volatile compounds were extracted by solid-phase microextraction (SPME)

Volatile compounds obtained from avocado seeds were studied using three randomly selected fruits at each stage, both unripe and commercially ripe. For each case, 1 gram of homogenized seed tissue was placed in a headspace vial, and then 1 mL of $CaCl_2$ was added. Following a 30-minute 40 °C treatment, the volatile components were extracted using a solid-phase microextraction (SPME) fiber coated with CAR/PDMS (blue fiber). Using the method detailed by Polat and colleagues (2022), the obtained aroma compounds were then analyzed and quantified using a Shimadzu GC-2010 Plus gas chromatography-mass spectrometry (GC/MS) system.).

Statistical Analyses

The experiment was performed with a completely randomised experimental block design in three replications two stages (unripe, ripe) in each replicate. Data were processed with the SPSS package program version 23.0 (SPSS Inc., Chicago, IL, USA). All data were presented as the

mean ± standard error (SE) and analyzed by one-way analysis of variance (ANOVA). (Steel, et al 1997).

RESULT AND DISCUSSION

Sugars are also related to the total soluble solids (TSS) content. In persimmon, this characteristic ranges from 12 to 22 °Brix, due to the great differences between cultivars, maturation, and postharvest treatments, which influence both the content and the profile of these compounds (Kluge et al., 2018). It is important to highlight that the presence of soluble tannins also influence the TSS content. Kim and Ko (1997) observed that astringent fruits with higher levels of soluble tannins presented more TSS than non-astringent cultivars did. When these soluble tannins are no longer present (after a precipitation treatment), the TSS content is affected and there is no clear difference between astringent and non-astringent cultivar types. Therefore, Tessmer et al (2016) suggest that TSS measurement should be used to identify the sugars level only in non-astringent cultivars.

At the ripe stage, the TSS (Total Soluble Solids) value was 19.4% and the titratable acidity was 1.14%, whereas at the unripe stage, the TSS value was 14.45% and the titratable acidity was 0.86% (Table 1; Figure 1). This characteristic positively reflects on the storage period by prolonging the fruit's shelf life. Accordingly, for fruits intended to be stored for longer durations, early harvests, especially those performed in November, are expected to provide significant advantages in terms of storage longevity. When previous studies are examined, it is evident that total soluble solids content (TSS) and titratable acidity (TA) values vary significantly depending on harvest time, cultivar, and ecological conditions. Özkan and Can (2013), in their study on the 'Hachiya' cultivar in Alaşehir (Manisa), reported TSS values of 14.47%, 18.06%, and 20.80% at the first, fourth, and sixth harvests, respectively. In the same study, TA values were determined as 0.19%, 0.16%, and 0.10% at the corresponding harvest times. Similarly, Kaynaş and Kuzucu (2004) reported SSC values of 23.84%, 23.65%, 22.65%, 22.75%, and 22.46% in persimmons harvested at five different times in the Çanakkale region. Yılmaz and Şeker (2024) found that SSC values ranged between 13.62% and 17.67%, being directly associated with harvest time. They noted that cultivars with astringent flesh generally had higher SSC values, such as 17.67% in 'Hachiya' and 15.72% in 'Rojo Brillante'. According to Mowat and Chee (2011), persimmons intended for export in New Zealand must meet industry class standards of an average SSC of 14% at harvest, with a minimum of 12%. In another study, Kılıç et al., (2022) reported SSC values between 18.05% and 20.63% in 'Hachiya', 'Rojo Brillante', 'Hana Fuyu', and 'Fuyu' cultivars grown in Denizli ecological conditions. TA values in the same study were determined to range between 0.14% and 0.28%. Üstün et al., (2017) also investigated different persimmon types and reported SSC values of 14.0–18.9%, total sugars of 12.3–17.1 mg/100 g, reducing sugars of 10.3–16.5 mg/100 g, sucrose of 0.38–1.90 mg/100 g, TA of 0.06–0.14%, and L-ascorbic acid content of 6.8–19.65 mg/100 g. In the study, the SSC was determined as 19.4% and the TA as 1.14% at the ripe stage, whereas at the unripe stage, the SSC was 14.45% and the TA was 0.86%. These findings are consistent with the results reported by Özkan and Can (2013), Yılmaz and Şeker (2013), Kaynaş and Kuzucu (2004), and Kılıç et al., (2022).

Table 1. TSS (Total Soluble Solids) value was 19.4% and the titratable acidity

	ripe persimmon	**unripe persimmon**
Total soluble solids	19.4±0.10	14.45 ±0.05
Titratable acidity	1.14±0.04	0.86±0.01

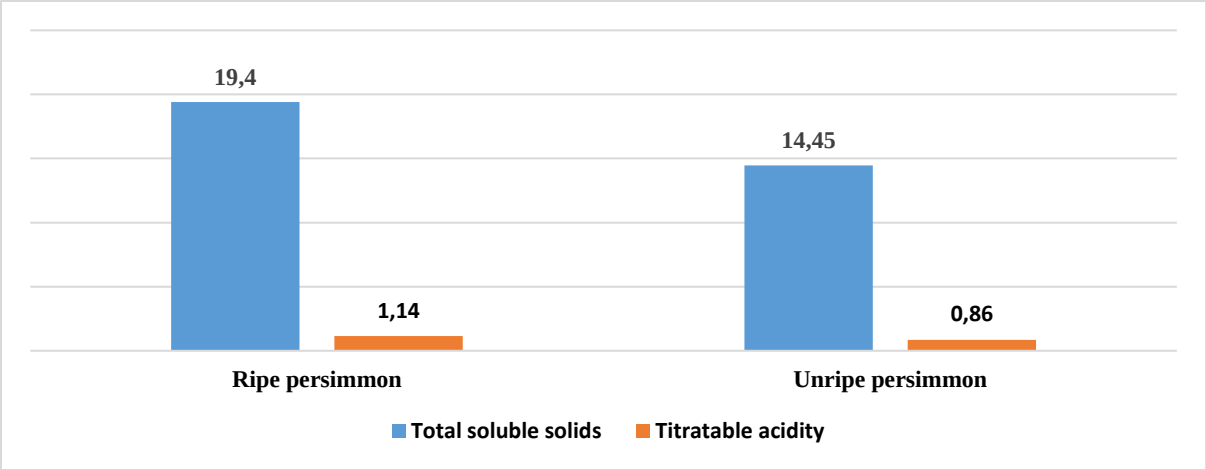

Figure 1. Contents total soluble solids (TSS) and titratable acidity (TA) of persimmon fruit

Sugars

In study, four sugars glucose, fructose, sucrose, and xylose were analyzed and measured for each cultivar (Table 2). Glucose was identified as the predominant sugar, followed by fructose, sucrose, and xylose. As the fruit ripened, starch was enzymatically hydrolyzed into simple sugars such as glucose and fructose, leading to a noticeable increase in sugar content (Yılmaz, and Bostan 2024).

At the ripe stage, the highest level was observed in glucose (95.67 ± 0.15 mg/100 g), followed by fructose (91.66 ± 3.33 mg/100 g), sucrose (89.30 ± 2.30 mg/100 g), and xylose (55.40 ± 8.35 mg/100 g). The total sugar content at this stage was determined as 182.48 ± 7.17 mg/100 g.

At the unripe stage, glucose again showed the highest concentration (72.13 ± 0.08 mg/100 g), followed by fructose (53.42 ± 18.69 mg/100 g), sucrose (65.61 ± 0.76 mg/100 g), and xylose (38.96 ± 7.61 mg/100 g). The total sugar content in the unripe stage was found to be 138.67 ± 27.13 mg/100 g (Table 2;Figure 2). The results of the study suggest that when determining the harvest date of persimmon, it is important to consider when the fruit will be marketed after harvest. If the harvested fruits are intended for storage, the harvest should be carried out during the early stages of fruit development, which corresponds to the second week of November for the Toros region. If the fruits are to be marketed without storage, the harvest should be delayed to later stages; however, in this case, as the fruit approaches the end of the ripening process on the tree, it becomes more attractive in terms of color and taste, but also more susceptible to environmental factors, particularly bird damage. On the other hand, early autumn frosts that may occur in the region during this period can cause chilling injury in the fruits. Fruits harvested in the early stages of development (the first week of November) were found not to reach high-quality edible ripeness, and the astringency caused by tannins was not eliminated in fruits at the edible stage. Robert et al. (2010) reported glucose contents in persimmon ranging from 4.78 to 8.79 mg/100g, fructose from 3.80 to 7.78 mg/100g, and sucrose from 0.91 to 1.22 mg/100g. Similarly, Candir et al., (2009) found that, considering both individual and total sugar concentrations, the cultivar 'Tone Wase' had the highest glucose (8.79 mg/100g), fructose (7.78 mg/100g), and total sugar (17.8 mg/100g) contents. In a study on harvest maturity of persimmon, Gasanov et al., (1973) reported that sugar accumulation during ripening was higher in Khiakume and Tamopan cultivars compared to Tsuronoko and Zendzhimaru. They further noted that the total sugar content varied among cultivars depending on harvest stage, with the highest level found in Emon and the lowest in Tamopan. In the same study, tannin content was determined as 0.164 mg/100g in Khiakume and Zendzhimaru, and

0.083 mg/100g in Tsuronoko, Emon, and Tamopan cultivars. Glew et al., (2005) demonstrated that the major sugars accumulated in persimmon juice were fructose and glucose, which increased up to 43.55 mg/100g FW and 35.45 mg/100g FW, respectively, during ripening. Sucrose remained relatively low and declined throughout ripening. These values were slightly lower than those reported by Daood et al. (1992) for an unidentified *D. kaki* L. cultivar, which contained higher glucose (11.0 mg/100g) and fructose (10.1 mg/100g) levels. The lowest total sugar content was measured in fully ripe 'O'Gosho', 'Cal Fuyu', and 'Hana Fuyu' cultivars, averaging 10.7 mg/100g. Sweetness is clearly one of the most important internal quality attributes and a key breeding objective in persimmon cultivation. Therefore, promoting cultivars with higher sugar contents is strongly emphasized. The total sugar content in persimmon is comparable to that in apples, which range between 11.5 and 18.3 mg/100g (Hofer et al., 2005). However, in apples, the predominant sugar is fructose, followed by glucose and sucrose (Veberic, Zadravec and Stampar, 2007). In persimmon cultivars with the highest total sugar concentrations, levels can reach 15–23 mg/100g, with glucose being the dominant sugar, followed by fructose and sucrose (Usenik, Fabcic and Stampar, 2008).

Table 2. Sugar content in the persimmon fruit (mg/100 g)

Sugars	Ripe persimmon	Unripe persimmon
Xylose	55.40±8.35	38.96±7.61
Sucrose	89.30±2.30	65.61±0.76
Glucose	95.67±0.15	72.13±0.08
Fruktose	91.66±3.33	53.42±18.69
Total Sugar	182.48±7.17	138.67±27.13

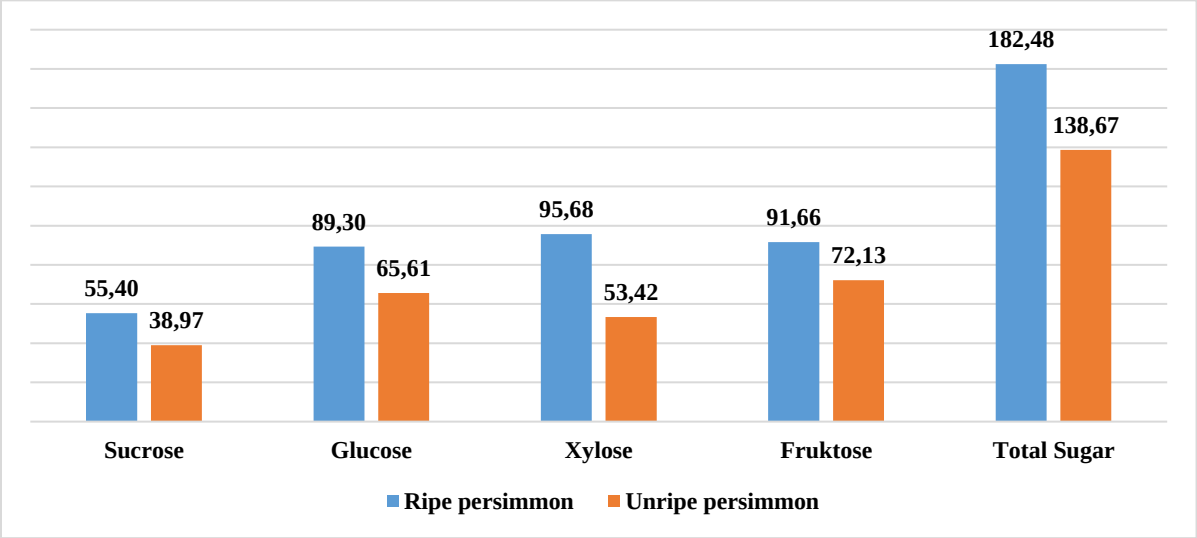

Figure 2. Sugar content in the persimmon fruit

The glucose and fructose levels determined in our study, when compared with the ranges reported by Robert et al., (2010) and Candır et al., (2009) (glucose: 4.78–8.79 mg/100g; fructose: 3.80–7.78 mg/100g), were found. This indicates that varietal/genotypic differences and ecological conditions play a decisive role in sugar accumulation. Our findings, compared with the 15–23 mg/100g levels reported by Gasanov et al., (1973) and Usenik et al., (2008), were at high levels. This result demonstrates that fruit ripening stage and growing conditions

have a strong effect on total sugar content The sucrose levels obtained in our study were consistent with / deviated from the findings of Glew et al., (2005), who reported that sucrose remains relatively low and decreases during ripening. Our findings show that, in terms of sweetness and internal quality characteristics, persimmon exhibits trends similar to those reported in the literature, although differences arise depending on cultivar, growing conditions, and ripening stage. It can be concluded that genotypes with higher sugar accumulation should be preferred in cultivation.

Organic acids

Organic acids play a critical role in defining fruit taste and regulating various physiological processes, with their composition largely dependent on the cultivar. The sugar–acid balance is one of the main determinants of flavor in fruits. In addition, organic acids contribute to nutrient digestion, stimulate blood circulation, and serve as important quality indicators. Their type and concentration vary among fruit species and generally decrease as ripening progresses (Kıralan and Gündoğdu, 2021).

In study, a natural decrease in organic acid content was observed in persimmon cultivars as ripening advanced. The contents of **L-ascorbic acid, citric acid, succinic acid, and malic acid** were determined in the cultivars, and the results are summarized in Table 5. Among these, **succinic acid and malic acid were identified as the predominant organic acids.**
The sweet taste of a fruit is important for its quality and sensorial acceptance, and both primary metabolites, sugars and organic acids, play an important role. It is estimated that a higher proportion between sugars and organic acids leads to a better perception of sweet taste. (Veberic et al., 2009; Santos et al., 2017). Malic acid exerts an important influence on the sensory characteristics of persimmon (Kluge,et al 2018) and, as identified by some authors, it is one of the main organic acids in this fruit, followed by citric acid and fumaric acids (Veberic et al., 2009; Santos et al., 2017; Ryu et al., 2016).

In ripe fruits, succinic acid content was 38.27 ± 3.34 mg/100 g FW, malic acid 18.23 ± 0.50 mg/100 g FW, citric acid 11.29 ± 0.77 mg/100 g FW, and L-ascorbic acid 8.09 ± 0.45 mg/100 g FW. The total organic acid content was determined as 75.89 ± 5.35 mg/100 g (Table 3;Figure 3). In unripe fruits, higher values were recorded: succinic acid 21.37 ± 3.04 mg/100 g FW, citric acid 10.02 ± 0.16 mg/100 g FW, malic acid 22.43 ± 0.79 mg/100 g FW, and L-ascorbic acid 6.94 ± 0.67 mg/100 g FW (Table 3;Figure 3). The total organic acid content of unripe fruits was calculated as 60.77 ± 3.37 mg/100 g. The observed reduction in total acidity during fruit ripening reflects the typical metabolic changes associated with maturation (Patel et al., 2019).

Robert et al., (2010) reported that the total organic acid content was 68.1 mg/100g in the cultivar ‘Jiro’, while in the cultivars ‘Triumph’ and ‘Tipo’ it was more than twice as high, averaging 14.39 mg/100 g of organic acids. Among the organic acids analyzed, the highest malic acid content was measured in the cultivar ‘Triumph’ (10.44 mg/100 g FW), whereas the lowest value was found in ‘Jiro’ (4.01 mg/100 g FW). The malic acid levels in all analyzed cultivars were considerably lower than those reported by Daood et al., (1992), who measured 35.45 mg/100 g FW in ripe fruit of an unidentified persimmon cultivar. Compared to sweet cherry, which contains 35.30–81.20 mg/100 g FW of malic acid (Usenik et al., 2008), the fully ripe fruits of the analyzed persimmon cultivars had much lower malic acid levels. The cultivars contained between 1.96 and 7.01 mg/100 g FW of citric acid, values comparable to those obtained by Daood et al., (1992), who reported 6.57 mg/100 g citric acid in ripe persimmon fruit. In comparison with apple, which contains between 5.20 and 70 mg/100 g FW of citric acid, persimmon is lower in citric acid content. Glew et al., (2005) demonstrated that the major organic acids in date plum fruit were citric and malic acids, which increased during the immature and mid-ripe stages and then decreased in overripe fruit. According to Usenik et al.,

(2008), ripe persimmon fruit is also rich in these organic acids. However, compared with elderberry, which contains on average 30.80–48.10 mg/100 g of citric acid (Veberic, Jakopic, Stampar and Schmitzer, 2008), persimmon has a significantly lower citric acid content. In other tropical fruits, such as papaya and pineapple, the concentration of citric acid in ripe fruit is much higher (Hernández, Lobo, & González, 2008). This result is consistent with the low organic acid contents in persimmon reported by Robert et al., (2010) and Daood et al., (1992).

Table 3. Organic acids content in the persimmon fruit (mg/100 g)

	Ripe persimmon	Unripe persimmon
Citric Acid	11.29±0.77	10.02±0.16
Malic Acid	18.23±0.50	22.43±0.79
Ascorbic Acid	8.09±0.45	6.94±0.67
Succinic Acid	38.27±3.34	21.37±3.04
Total acid	75.89±5.35	60.77±3.37

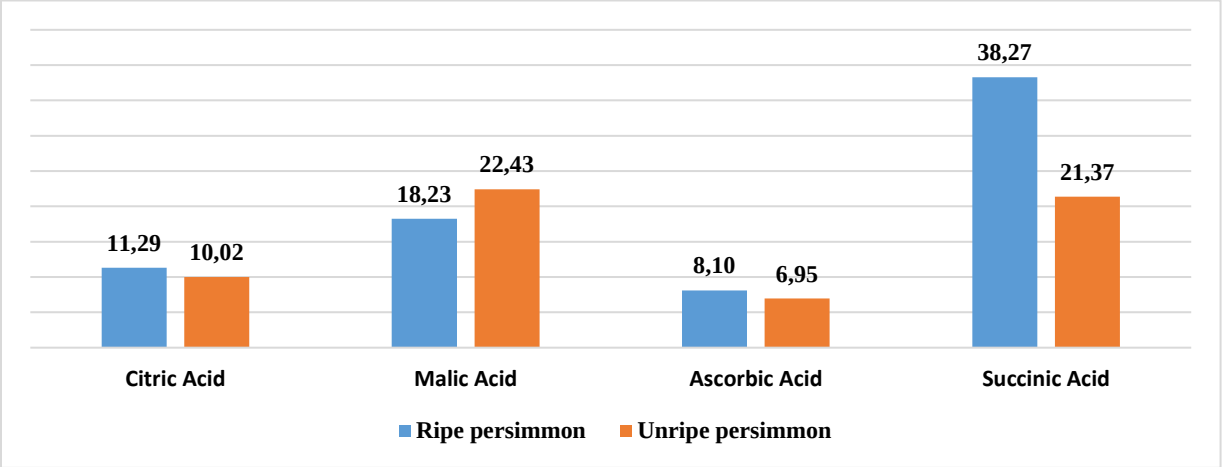

Figure 3. Organic acids content in the persimmon fruit

Volatile Compounds

The volatile compounds identified in persimmon fruits are summarized in Figure 1. Using blue SPME, at the ripe stage the main volatile groups detected were total terpenes (10.39%), total esters (9.37%), total aldehydes (37.76%), total acids (37.08%), total ketones (2.32%), and total alcohols (3.38%)(Figure 4). A total of 31 individual components were identified in the juice, comprising four alcohols, nine aldehydes, one ester, nine acids, three ketones, and five terpenes. Among the extraction methods tested, blue SPME proved to be the most suitable for isolating volatile compounds from persimmon juice.

Aldehydes and acids were found to be the predominant volatile groups in persimmon fruits (Table 4). Key aldehydes detected included hexanal (25.16%), (E)-2-hexenal (2.69%), and trans-2,4-dodecadienal (2.63%), with notably higher levels in ripe fruits. Among acids, acetic acid (22.82%), nonanoic acid (2.62%), and hexanoic acid (2.07%) were the most abundant, also with higher levels in ripe fruits. Within the alcohol group, menthol (1.27%) and 1-octanol (0.82%) were prominent, particularly in ripe fruits. Esters such as 1,2-benzenedicarboxylic acid, diethyl ester (7.26%) were identified as important contributors to aroma and were highly abundant in ripe fruits. Esters are known to impart fruity and floral notes that play a critical role in cultivar differentiation. Regarding terpenes, benzene (isothiocyanatomethyl), benzene, 1-methyl-2-(1-methylethyl)-, and naphthalene, 1,6-dimethyl-

4-(1-methylethyl)- were the major compounds identified. Among them, benzene (isothiocyanatomethyl) was particularly abundant (6.52%) in ripe fruits (Table 4).

At the unripe stage, the main volatile groups detected were total terpenes (44.98%), total esters (1.39%), total aldehydes (10.23%), total acids (17.56%), total ketones (8.40%), and total alcohols (17.44%). A total of 27 individual compounds were identified in the juice, consisting of five alcohols, seven aldehydes, two esters, eight acids, three ketones, and four terpenes. Aldehydes and acids were again found to be the predominant volatile groups in persimmon fruits (Table 3). The key aldehydes detected included hydroxymethyl furfural (6.10%) and 2-furancarboxaldehyde (1.84%), both showing notably higher levels in unripe fruits. Among acids, acetic acid (5.29%), (E)-9-octadecenoic acid (5.10%), and pentadecanoic acid (4.81%) were prominent, also with higher abundance at the unripe stage. Within the alcohol group, linalool (7.10%), geraniol (3.76%), and nerol (3.48%) were prominent, particularly in ripe fruits. The major ketone identified was 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (8.40%). Regarding terpenes, (+)-limonene (42.93%) was the dominant compound identified in unripe persimmon fruits. In the study, it was determined that with increasing ripeness, the amounts of aldehydes, esters, and acids increased, while the amounts of alcohols, ketones, and terpenes decreased. The results of the study revealed that the main factor responsible for changes in the aroma compounds of persimmon fruit was the progression of ripening over time. As the maturity index increased, the composition of aroma compounds also changed. Terpenes were found to increase up to a certain stage of ripening and then decrease in the later stages, with a higher number of terpene compounds identified in the unripe stage. Limonene was identified as the common terpene component in both cultivars.

In persimmon, the total alcohol content within the volatile compound profile decreased during ripening, whereas the proportion of ester compounds increased. In contrast, ketone levels showed a decreasing trend as ripening progressed. As fruits ripen, the quantity and quality of volatile compounds responsible for aroma increase, reaching their maximum at full maturity. The synthesis of aroma compounds is influenced not only by the genetic makeup but also by ecological factors, rootstock, cultivar, altitude, cumulative temperature during the growing season, ripening stage, and storage conditions. The source of aroma compounds largely comes from sugars, proteins, and fats metabolized during ripening. Therefore, aroma formation during ripening occurs not only through enzymatic synthesis but also through the breakdown and transformation of these metabolites. In other words, as the fruit ripens, volatile aroma compounds are primarily produced via metabolic degradation processes and enzyme activity (Perez et al., 1996; Kaynaş et al., 2022).

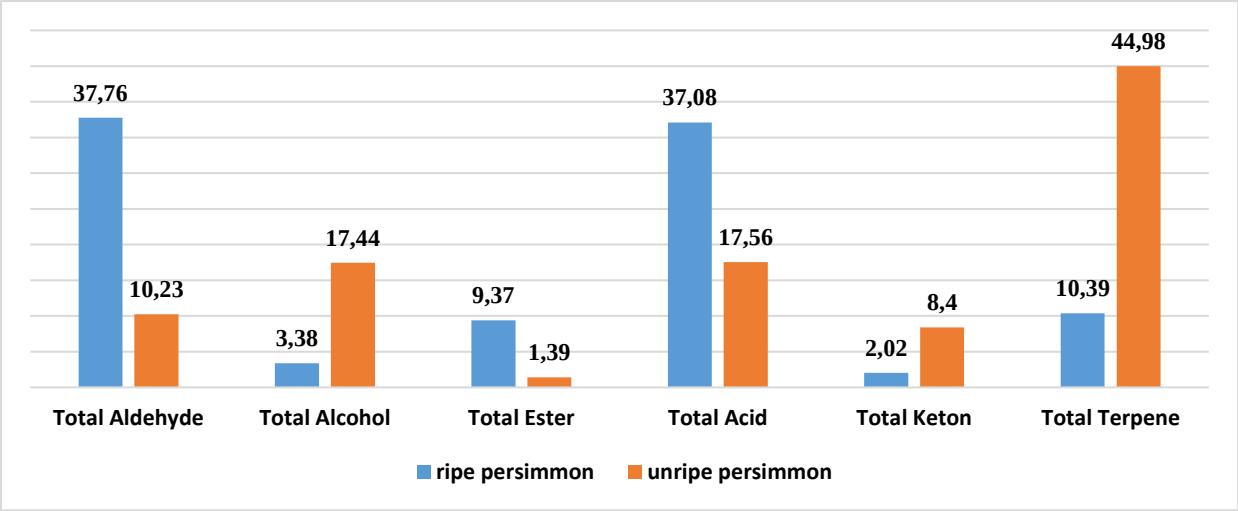

Figure 4. Volatile composition in the persimmon fruit (%)

Table 4. Volatile composition in the persimmon fruit (%)

R.time	Compounds	ripe persimmon	unripe persimmon
	Aldehyde(-al)		
3.061	Hexanal (CAS)	25.16	
5.015	Heptanal (CAS)	0.49	
5.704	2-Hexenal, (E)- (CAS)	2.69	
7.435	Octanal (CAS)	1.19	
10.057	Nonanal (CAS)	1.59	0.5
13.2	Benzaldehyde (CAS)	1.6	
16.091	2-Decenal, (E)- (CAS)	1.39	
18.586	Trans-2-dodecenal	1.02	
26.187	Trans, trans-2,4-dodecadıenal	2.63	
10.735	2-Furancarboxaldehyde (CAS)		1.84
19.286	Hydroxy methyl furfural		6.1
19.649	Neral		0.32
19.974	Dec-2(E)-enal		0.43
20.161	Citral		0.53
21.784	Undec-8-enal <cis->		0.51
	Total	**37.76**	**10.23**
	Alcohol(-ol)		
10.429	2H-Pyranmethanol, tetrahydro-2,5-dimethyl- (CAS)	0.54	
14.342	1-Octanol (CAS)	0.82	0.97
16.145	Menthol	1.27	
30.271	Phenol, 2,4-bıs(1,1-dımethylethyl)-	0.75	
16.8	Linalool		7.14
19.368	Nerol (CAS)		3.48
19.802	Geraniol		3.79
20.478	Phenol, 5-methyl-2-(1-methylethyl)- (CAS)		2.06
	Total Alcohol	**3.38**	**17.44**
	Ester(-ate)		
1.275	Acetic acid, ethyl ester (CAS)	2.11	
30.912	1,2-Benzenedicarboxylic acid, diethyl ester (CAS)	7.26	

Table 4. Volatile composition in the persimmon fruit (%)

R.time	Compounds	ripe persimmon	unripe persimmon
	Ester(-ate)		
16.568	2-Furancarboxylic acid, methyl ester (CAS)		0.87
27.531	Tricosyl heptafluorobutyrate		0.52
	Total Ester	**9.37**	**1.39**
	Acid		
11.971	Acetic acid (CAS)	28.82	5.29
16.26	Butanoic acid (CAS)	0.49	
21.116	Hexanoic acid (CAS)	2.07	0.37
25.494	Octanoic acid (CAS)	1.12	0.57
27.535	Nonanoic acid (CAS)	2.62	
32.158	Benzoic acid (CAS)	0.4	
5.181	Formic acid	1.14	
21.554	Decanoic acid (CAS)	0.42	0.42
24.765	Dodecanoic acid (CAS)		0.38
30.113	(E)-9-Octadecenoic acid,		5.1
30.333	Pentadecanoic acid (CAS)		4.81
33.413	Octadecanoıc acıd		0.62
	Total Acid	**37.08**	**17.56**
	Keton(-one)		
3.902	1-Methoxy-2-propoxyethane	0.46	
7.834	4-Butoxy-2-butanone	1.56	
7.078	2-Propanone, 1-hydroxy- (CAS)	2.48	
17.822	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one		8.4
	Total Keton	**2.02**	**8.4**
	Terpene (-ene)		
5.135	(+)-LIMONEN	0.45	42.93
6.859	Benzene, 1-methyl-2-(1-methylethyl)- (CAS)	1.34	
8.075	Diethylene glycol tert-butyl ether methyl ether	0.79	
25.849	Benzene, (isothiocyanatomethyl)-	6.52	
27.991	Naphthalene, 1,6-dimethyl-4-(1-methylethyl)- (CAS)	1.29	
14.455	beta.-Myrcene		0.78
16.038	gamma.-Terpinene		0.72
26.639	Hexadecane, 1-chloro- (CAS)		0.55
	Total Terpene	**10.39**	**44.98**

Besada et al., (2013) reported a high-resolution volatile profiling of fruits from 12 persimmon cultivars, identifying 67 compounds. This profiling was used to characterize the different astringency types of persimmon fruit before and after deastringency treatment. Fruits that do not naturally accumulate astringent compounds (PCNA type) exhibited high levels of **3(2H)-benzofuranone**, whereas this compound was not detected in any astringent-type fruits (non-PCNA). In addition, at harvest, PCNA cultivars showed higher accumulation of **benzeneacetaldehyde** and lipid-derived aldehydes (hexanal, heptanal, octanal, and decanal) compared to non-PCNA fruits. Wang et al., (2012) reported that gas chromatography–mass spectrometry (GC-MS) analysis of the extract led to the positive identification of 50 compounds, among which aldehydes emerged as the most important class of volatile compounds. Gas chromatography–olfactometry (GC-O) analysis of the aroma extract revealed that 32 compounds had an aroma impact. The six most intense aroma-impact compounds were methional, (E)-2-Hexenal, Phenylacetaldehyde, (E,Z)-2,6-Nonadienal, Hexanal, and Furaneol. The aroma qualities of these compounds largely explain the aroma profile of persimmon fruit, which was described as fresh peach, a touch of orange, sweet, woody, floral, green, and potato-like. The unambiguous identification of trace aroma-impact compounds – (E,E,Z)-2,4,6-Nonatrienal and (E,Z,Z)-2,4,7-Decatrienal – was achieved by applying a solid-phase extraction procedure for the selective isolation of carbonyl compounds using GC-MS.

CONCLUSION

The study observed a significant increase in TSS values and a decrease in titratable acidity (TEA) throughout the ripening process. Harvesting: For optimum quality, high TSS (18–20%) and low TEA (0.1–0.2%) values should be targeted at harvest. Therefore, producers are advised to pay attention not only to rind color but also to the sugar/acid balance. In terms of sugar composition, glucose and fructose levels increase with ripening, while sucrose levels gradually decrease. This can be explained by the high activity of the invertase enzyme (Bubba et al., 2009; Santos et al., 2019). Sugar-acid balance: Sweetness (high glucose + fructose) and low acidity (reduction of malic and citric acid) play important roles in consumer taste. Therefore, post-harvest ripening methods (CO_2, ethylene, limewater) should be used to improve flavor balance. In terms of organic acids, it has been observed that malic and citric acid levels gradually decrease during the ripening process, resulting in a decrease in acidity and an increase in sweetness perception. In terms of volatile compounds, aroma compounds, particularly aldehydes, esters, and furaneol, have been found to vary with ripening and contribute to the fruit's characteristic aroma (Wang et al., 2012; Besada et al., 2013). Storage conditions: Because persimmon is climacteric, low-temperature and controlled-atmosphere storage conditions are important for maintaining the sugar-acid balance in the fruit and preventing aroma loss. The Hachiya variety, with its high sugar and aroma profile, can be utilized in drying, juice, and functional food products to create value-added products. The TSS, TA individual sugars, organic acids, and aroma values obtained from the analyses were found to be at sufficient levels in the final harvest period in terms of fruit edibility. Detailed examination of sugar and organic acid metabolism in different genotypes, correlation with consumer taste, and monitoring of changes during storage are recommended.

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EFFECTS OF AQUAFABA AS ALTERNATIVE PLANT ADDITIVE ON PHYSICAL, TEXTURAL AND SENSORY CHARACTERISTICS OF EGGLESS TURKISH PASTA (ERIŞTE)

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ABSTRACT

Consumers experience health problems with egg consumption and also preference of vegan and vegetarian nutrition leads to the search for alternative egg substitutes in new product development. This research determined the quality and acceptability of Turkish noodle (Erişte) substituted with 25%, 50%, 75% and 100% chickpea aquafaba instead of egg. Erişte were analyzed for physical, textural, and sensory properties compared with sample containing egg. An increase in substitution led to a rise of 12.76% in water uptake, 12.94% in volume increase and 40.19% in the cooking loss. The addition of up to 75% aquafaba increased the firmness of .erişte significantly compared to the control sample. Erişte sample containing 100% aquafaba showed significantly $p < 0.05$ higher values in L* (75.11) and hue angle (94.85), while lower values in a* (-2.13), b* (24.30) and saturation index (24.39). The odor (7.00), taste (7.00), appearance (7.00), chewiness (7.00) and overall acceptability (6.88) of samples containing aquafaba were found more acceptable than control sample (4.00, 5.50, 5.90, 5.95 and 5.47, respectively). Based on our results, possible to produce erişte with acceptable sensory properties, and good physical quality product by adding up to 50% aquafaba.

Keywords: Aquafaba, egg-less noodle, physical properties, textural properties

INTRODUCTION

Turkish pasta (Erişte) is one of the traditional Turkish foods was generally produced from wheat flour, salt and egg. Milk, whey powder and some other additives can also be added in different regions of Turkey (Özkaya et al., 2004). The high rate of egg component used in pasta products can reduce production and consumption due to both the cost of the product and various reasons. So, many issues such as changing consumer preferences, increasing allergens, improving food safety, improving nutritional balance, reducing price variability and supporting environmental sustainability have increased the interest in researching egg substitutes and alternatives (Grizio and Specht, 2018).

Plant-based proteins are one of the most important food components to use as egg substitution in product development. Recently, the viscous liquid obtained from cooked chickpeas or other legumes and pulses called ‘aquafaba’ according to the Latin origin of water (aqua) and beans (faba) has been recently used as eggs replacement in many foods (Erem et al., 2021). Some studies in the literature revealed that aquafaba has many functional properties such as water and oil holding capacity, emulsion stabilizer, foaming, gelling and thickening in various formulations (Mustafa and Reaney 2020) and can be used as an egg substitute in vegan products (Raikos et al., 2020).

Aquafaba has used in meringue (Stantiall et al. 2018), sponge cake (Mustafa et al. 2018) and vegan mayonnaise (Raikos et al., 2020) as an emulsifier instead of egg. To our knowledge, no studies have been conducted on the inclusion of aquafaba in the formulation of egg-less Turkish pasta (Erişte). The objective of this study was to investigate egg substitutes for Turkish pasta using aquafaba. The effects of aquafaba on physical, textural and sensory properties of eggless pasta were evaluated.

MATERIAL AND METHOD

Materials

Commercial chickpea, wheat flour, whole egg and salt were purchased from local markets in Konya, Turkey.

Methods

Production of Aquafaba

Aquafaba was prepared according to the method described by Baik and Han (2012) with some modifications. Firstly, chickpeas were washed and were cleared from dirt, dust and foreign matter. Aquafaba was obtained by cooking 100 g chickpea in 500 mL water (1:5 chickpea/water ratio) for 30 min in boiling water at 98 °C. After the cooking, water and cooked chickpeas waited for 12 hr in a refrigerator at 4 °C. Finally, aquafaba was obtained by removing cooked chickpeas.

Production of Turkish noodle (Erişte)

For production of control Turkish pasta sample, firstly wheat flour (100 g), whole egg (40 g), salt (1 g) and water were mixed and kneaded in a laboratory-type mixer (Hobart N50, Offenburg, Germany) for 8 min. The kneaded dough rested for 20 min and 2 mm thickness/5 mm wide long strips were obtained (1 time in section 6 and 7) by a pasta machine (Shule Pasta Machine; Jiangsu, China). Then, strip-shaped dough were cut to a length of 4 cm. The drying of samples took place in a laboratory-type oven (Nüve KD 200, Ankara, Turkey) at 50 °C for 18 h. In the other samples, aquafaba were replaced at levels of 25, 50, 75 and 100% on the basis of whole egg. The samples were preserved in sealed polyethylene bags at 4 °C.

Cooking properties

Volume increase, weight increase and cooking loss values of pasta were determined according to Bilgiçli et. al. (2011). The weight increase and volume increase were found by differences of dry and cooked erişte samples weights and by the volume difference of water overflow, respectively. For cooking loss determination, cooking water was evaporated to constant weight in an oven and the weight of total solids expressed as a percentage (AACC, 2000).

Texture analysis

The firmness values of erişte samples were measured by TAXT Plus Texture Analyser (Stable Microsystems, Surrey, UK) and A/LKB-F was used as a probe (AACC, 1990). Firstly, 10 g samples were cooked in 200 mL distilled water for optimum cooking time and drained. Then, three strips of erişte sample were placed onto the stand and analyzed.

Color analysis

Color measurement was made by measuring L* value [(0) black- (100) white], a* value [(+) red- (−) green] and b* value [(+) yellow - (−) blue] using the Hunter Lab Color Quest II

Minolta CR-400 (Konica Minolta Sensing, Inc., Osaka, Japan) (Francis, 1998). Three measurements were taken on each sample. The hue angle and the saturation index value were determined with arctan(b*/a*) and $(a^{*2}+b^{*2})^{1/2}$ equations, respectively.

Sensory analysis

Sensory analysis was carried out for cooked Turkish pasta samples. Turkish pasta samples were evaluated in terms of color, odor, taste, appearance, chewiness and general acceptability properties by 12 panelists (aged 20–50).Sensory properties of samples were scored using a 7-point hedonic scale in which a score of 7 = like extremely, 1 = dislike extremely.

Statistical analysis

The results were expressed as mean ± standard deviation and were analyzed using the Statistical software JMP 5.0.1 (SAS Institute). The averages of the main variation sources were compared at $p < .05$ level. All measurements were performed in duplicate for each sample.

RESULTS AND DISCUSSION

The data on the effect of different levels of aquafaba on the cooking quality and firmness properties are shown in Table 1. The water uptake values of Turkish pasta samples increased with the use of more than 50%. Compared to control, addition of aquafaba increased volume increase value of Turkish pasta samples from 219.69 to 248.11%. Similar behavior in terms of cooking loss properties was observed Turkish pasta samples produced with different aquafaba. While Turkish pasta prepared with 50-100% legume flour revealed the highest cooking loss, the addition of 25% aquafaba showed similar cooking loss with control sample. The findings of this study demonstrated that the addition of aquafaba increased 1.82-fold with 100% aquafaba the firmness values of pasta samples compared to the control sample (Table 1).

Table 1. Physical properties of Turkish pasta[1]

	Water uptake (%)	Volume increase (%)	Cooking loss (%)	Firmness (g)
Control	215.59±1.89b	219.69±6.03b	4.23±0.11b	642.12±23.35c
25% Aquafaba	220.06±3.44b	229.72±4.81ab	4.75±0.25b	698.98±61.62c
50% Aquafaba	236.70±3.03a	244.86±6.52a	5.43±0.10a	809.39±90.10bc
75% Aquafaba	238.14±1.25a	245.15±5.82a	5.74±0.16a	988.47±35.67ab
100% Aquafaba	243.12±3.16a	248.11±3.95a	5.93±0.13a	1166.62±92.43a

[1]Means followed by the different letters within a column are significantly ($P < 0.05$) different.

Color values of Turkish pasta samples are demonstrated in Table 2. Color L*, a*, and b* results of Turkish pasta samples varied in the range of 65.24-75.11, 0.26-(-2.13), and 24.30-30.56, respectively. Compared to the control sample, the addition of aquafaba into the formulation of Turkish pasta significantly ($p < .05$) increased L*, but decreased a* and b*values. The findings are associated with the natural color properties of eggs. The reason of this result was lower lightness and higher yellowness values of egg compared with aquafaba. The SI value of Turkish pasta sample incorporated with 100% aquafaba were lower than the control and other samples. The hue angle values demonstrated an increase with high aquafaba usage ratio.

Table 2. Color properties of Turkish pasta[1]

	L*	a*	b*	Saturation Index	Hue angle
Control	65.24±1.58c	0.26±0.07a	30.56±0.83a	30.56±0.77a	89.51±0.58b
25% Aquafaba	67.72±1.98bc	-0.37±0.10b	29.94±0.69a	29.94±0.10a	90.71±0.99b
50% Aquafaba	70.52±1.37abc	-0.92±0.14c	29.73±0.59a	29.74±0.84a	91.77±1.02ab
75% Aquafaba	73.12±1.87ab	-2.06±0.03d	28.50±1.92ab	28.58±0.68a	94.27±0.35a
100% Aquafaba	75.11±0.66a	-2.13±0.04d	24.30±1.00b	24.39±0.52b	94.85±0.91a

[1]Means followed by the different letters within a column are significantly ($P < 0.05$) different

Sensory properties of Turkish pasta samples are presented in Figure 1. According to the results; color score shown no any change with incorporation of aquafaba in pasta formulation. When compared with the control sample, the odor, taste, appearance, chewiness and overall acceptability scores of the Turkish pasta samples were found higher with high aquafaba addition levels, statistically. As a result, 50% and more aquafaba usage improved the sensory properties and overall acceptability of Turkish pasta. Mustafa et al. (2018) prepared sponge cake with egg white and aquafaba. The color and texture of sponge cake made with egg white or aquafaba was similar and acceptable, but cakes made with aquafaba were less pliable and less sticky than cakes made with egg whites.

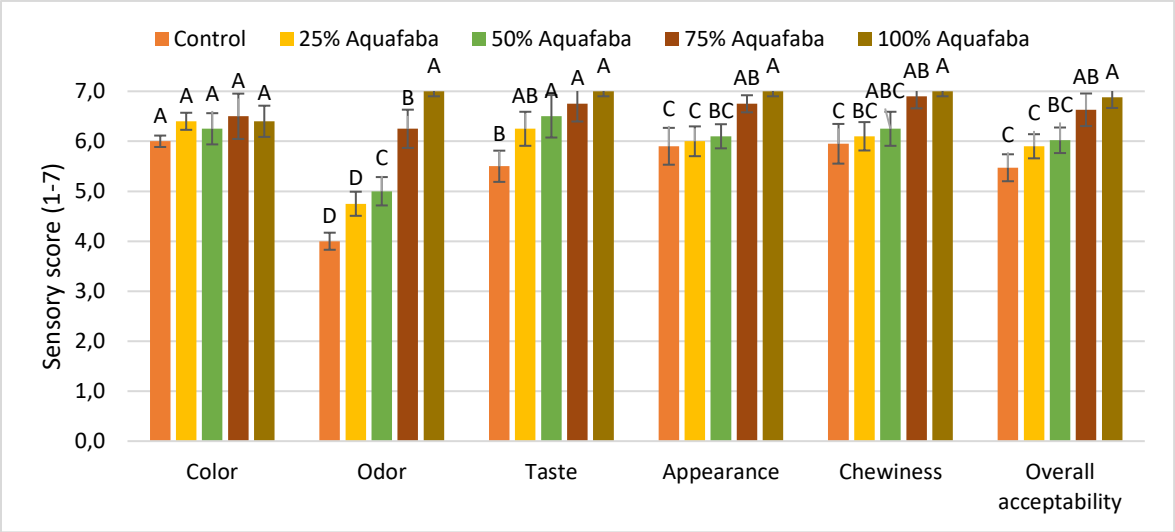

Figure 1. Sensory properties of Turkish pasta

Aslan and Ertaş (2020) used aquafaba as an egg substitute in the cake formulation. According to the results of sensory evaluation, they reported that samples containing 25% aquafaba were preferred more by the panelists.

CONCLUSIONS

In this study, the usability of aquafaba as egg substitute in Turkish pasta (Erişte) production was investigated. According to results, the use of aquafaba as an egg substitute was concluded with an increase in the water uptake, volume increase and cooking loss of pasta samples and so, in terms of cooking quality properties considerably not caused a negative effect

up to 50% ratio. Also, the use of aquafaba increased the firmness values compared to the control samples. The incorporation of aquafaba in the pasta samples positively affected the sensory profile in terms of all sensory scores except to color scores.

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PHYTOCHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITIES OF GINGER (Zingiber officinale)

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ABSTRACT

Secondary plant metabolites are rich sources of bioactive substances, which have shown to possess several beneficial health effects including antioxidant, antihemolytic and anti inflammatory activities. This study aimed to evaluate the phytochemical composition and antioxidant activities of plants extracts from the ginger (*Zingiber officinale*). Polyphenols were estimated by the Folin-Ciocalteu method and total flavonoids by the AlCl3 method in the aqueous and hydro-methanolic extracts. The antioxidant capacity was determined by the DPPH and FRAP tests. The results showed that the ginger extract in maceration, by methanol, is very rich in triterpenes, steroids, and the decoction, by distilled water, is rich in starch. The antioxidant activity of our extracts indicated that the polyphenols were more present in the maceration extract (1.46 ± 0.006 µg EAG/g) compared to the decoction (1.40 ± 0.003 µg EAG/g), as well as flavonoids in decoction (0.72 ±0.000) and maceration (3.57 ±0.422 mg EAG/g). Methanolic extract reveals a very great antioxidant activity compared to the decoction. The analysis of the toxicity of *Zingiber officinale* demonstrated an average rate which does not exceed a percentage of hemolysis of 58% for the two extracts. The study of anti-inflammatory activity of human red blood cells has been proven by the calculation of the percentage of inhibition of hemolysis very high in all extracts.

The findings suggest that Zingiber officinale possess large antioxidant properties, a lower percentage of cytotoxicity and a very good anti-inflammatory activity and could serve to protect human from oxidative damage caused by free radicals.

Keywords: Zingiber officinale, phytochemical screening, DPPH, TFC, FRAP

INTRODUCTION

Medicinal plants are still the main source of new drugs, and are considered an essential raw material for the discovery of new molecules needed to develop future medicines. In addition, these plants have played a vital role in the development of human culture around the world (Khan et al., 2019).

In particular, many aromatic plants and spices have attracted the interest of numerous research groups (Bellik et al., 2013). Ginger, the rhizome of *Zingiber officinale*, has been used as a food, spice, supplement and flavouring agent for over 3000 years in countries such as Burma, China, Congo, Germany, Greece, India, Indonesia, Japan, Sri Lanka, Tibet, and the United States due to its beneficial properties such as pungency and aroma (Kubra and Rao, 2012 ; Kiyama, 2020). It is also used in traditional medicine to treat nausea, indigestion, abdominal pain, colic, and constipation. The rhizomes of *Z.officinale* are often chewed raw and the juice swallowed to relieve generalised abdominal pain. *Z.officinale* is also used as a respiratory aid to relieve coughs (Amir et al., 2011).

This plant material contains a large number of molecules with multiple uses in the food industry, cosmetology and pharmaceuticals. These include steroids, alkaloids, phenolic acids, tannins, terpenes and flavonoids (Bahorun et al., 1996).

Thus, the evaluation of phytopharmaceutical, anti-inflammatory and anti-haemolytic properties remains a very interesting and useful task, particularly for a plant whose use is less frequent or unknown in traditional medicine. Secondary plant metabolites are rich sources of bioactive substances, which have shown to possess several beneficial health effects including antioxidant, anti inflammatory and antihemolytic activities.

Given its characteristics, ginger has been extensively studied for its biological activity. The free radical scavenging properties of its compounds have been well established as antioxidants (Bellik et al., 2014).

Therfore, this study aimed to evaluate the phytochemical composition and biological activities for two ginger extracts obtained by maceration and decoction. Polyphenols, total flavonoids, and total tanins were estimated in the aqueous and hydro-methanolic extracts. We also determined the antioxidant capacity by DPPH and FRAP tests, as well as the in vitro anti inflammatory and anti hemolytic activities of extracts of ginger.

MATERIAL AND METHOD

The plant material used in this study corresponds to the *Zingiber* species under the name *Zingiber officinale*, that was obtained from a herbalist in the wilaya of Tlemcen (Fig 1).

Preparation of ginger extracts:

First of all, we began by washing the ginger rhizome several times, then peeling it and cutting it into small pieces. Next, we ground the pieces in a blender until we obtained a ginger homogenate. Finally, the ginger homogenate is put in a box and stored in the refrigerator at a temperature (3 to 4°C) until use.

Different extracts of ginger were prepared by decoction in distilled water, by maceration method in water-methanol (20/80, v/v) according the protocole described by Tuma et al. (2004). The decoction was prepared with 10g of ginger homogenate that is placed in 100 mL of distilled water, bring to the boil (100°C) in a beaker for 15 minutes, then the mixture is filtered and recovered**.**

10g of ginger homogenate is macerated in 100 mL of water-methanol (20/80; V/V) for 24 hours at room temperature. This operation is repeated three times, renewing the solvent each time. The macerates are filtered through filter paper each time and the three filtrates are combined. Most of the solvent is removed from the filtrate under vacuum at 40°C using a rotary evaporator, and the remainder is removed using an oven at 40°C.

Phytochemical screening

This qualitative test was carried out for the presence of various phytochemical constituents like saponins, tannins, flavonoids, steroids, terpenoids, alkaloids, glycosides, as per standard methods.

Saponin Analysis

2 ml of extract is shaken vertically for 30 seconds until a stable foam forms (height greater than 1 cm), and let stand for 15 min.

Tannin Analysis

By adding 1 mL of aqueous extract, 1 mL of water and 1-2 drops of diluted FeCl3 (1%), tannins can be detected. After incubation, the presence of catechin or gallic tannins is indicated by a dark green or blue-green colour, respectively.

Figure 1. Zingiber officinale Rhizome

Test for Proanthocyanidols

A positive reaction occurs when a purple colour appears after adding 2 mL of infused water to 2 mL of concentrated hydrochloric acid and allowing the mixture to stand in a boiling water bath for 5 min.

Test for Flavonoids

1 mL of the extract is mixed with 1 mL of concentrated HCl and 3 turns of magnesium. The appearance of a pink, red or yellow colour indicates the presence of flavonoids.

Analysis of Steroids

Tilting the tube at a 45-degree angle while adding 1 mL of sulphuric acid to the ginger extract, the mixture turn to red, and indicated the presence of unsaturated sterols.

Analysis of Anthocyanes

2 mL of hydrochloric acid are added to 2 mL of ginger extract, where a pink-red colour appears, then ammonia is added, which gives a blue-purple colour indicating the presence of anthocyanins.

Test for Alkaloids

1 mL of crude extract was mixed with 5 mL of hydrochloric acid, then add 5 drops of Meyer's reagent. A white or brown precipitate will form, indicating the presence of alkaloids.

Phenolic compounds

Total phenolic compounds

Polyphenol determination was performed by spectroscopic method using the Folin-Ciocalteu assay according to the method described by Singleton and Rossi (1965). 200 µL of each extract were mixed with 0.8mL of sodium carbonate solution (7.5%), and 1mL of Folin-Ciocalteu reagent diluted 10 times. The mixture is shaken and incubated in dark for 30 min at room temperature. The absorbance was measured at 765 nm and compared to a standard curve with gallic acid. The polyphenol content was expressed as mg of Gallic acid equivalents per gram of dry weight (mg GAE/g DW). All tests are performed in triplicate for each sample.

Total Flavonoid's compounds

The favonoids of the extracts was performed by the aluminum chloride colorometric method (Neupane and Lamichlane, 2020). A very stable complex was formed between aluminum chloride and the oxygen atoms on the carbon 4 and 5. At 430 nm, the absorbance was measured against a blank and the calibration curve was performed under the same conditions by quercetin

(0-1000µg mL^{-1}). The results were expressed as mg of quercetin equivalents per g of dry weight (mg EQ/g DW).

Antioxidant activities

DPPH radical scavenging activity

The antioxidant activity of ginger extracts was evaluated by the DPPH method as described by Sanchez-Moreno et al. (1998). In this assay, 50 µL of the extract in methanol was mixed with 1,95 mL of methanol DPPH solution (0.025 g/L).

Antioxidants reduce DPPH, which has a purple colour into a yellow compound, the absorbance reading was taken at 515 nm against a blank containing the DPPH solution. The activities of the different extracts were compared to ascorbic acid used as the standard antioxidant. The results were expressed as a percentage of inhibition (%AA).

$\%AA = (Abs_{control}-Abs_{test} / Abs_{control}) x100$

50% of DPPH radical inhibition (IC50) was calculated graphically by linear regression of the plotted graphs between the percentages of inhibition and different concentrations of the fractions tested. It should be noted that the lower the IC50 value, the greater the activity of the extract tested (Ondua et al. 2019).

Ferric reducing antioxidant power (FRAP)

The concept of this method is to evaluate the reducing activity in ferric iron Fe(III) molecules extracts of the FRAP complex to ferrous iron to Fe(II), by measuring the blue colour of the compound (Prussian blue) which absorbs at 700 nm. High absorbance indicates that the sample has high reducing power.

1 mL of different extracts at different concentrations were combined with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide complex K3[Fe (CN)6]. After, 20 min of incubation at 50 °C, the reaction is halted by adding of 2.5 mL of trichloroacetic acid (10%) solution. Then, tubes were centrifuged at 3000 rpm for 10 min. After that, 2.5 mL of each supernatant was mixed with 2.5 mL of distilled water and 500 µL of (FeCl3, 6H2O) (0.1%). The absorbance was measured at 700 nm against a blank, and ascorbic acid was used as a standard under the same operating conditions.

In vitro anti-inflammatory activity

In order to evaluate the inflammatory activity of plant extracts, we applied the in vitro protein denaturation inhibition test (Rajesh et al., 2019). The method consists of inhibiting the denaturation of bovine serum albumin (BSA) by heating the extracts to 72°C.

A 0.2% BSA solution was prepared in Tris-HCl buffer (0.05 M, pH 6.6). To 1 mL of this solution, 1 mL of each extract at different concentrations (100, 150, 250, 500 µg/mL) or of the standard (diclofenac sodium) was added. After incubation at 37°C for 15 min, the test tubes were heated at 72°C for 5 min to induce denaturation, and then cooled for 10 min. The absorbance of these solutions was determined at a wave length of 660 nm. Each experiment was carried out in triplicate and the mean absorbance was recorded.

The percentage of inhibition of protein denaturation inhibition was determined on a percentage basis relative to control using the formula :

Per cent inhibition of protein = 100-[{(At- Ac) / At}] x 100.

At = Absorbance of test solution (tested extract). Ac = Absorbance of control

Anti-hemolytic activity

The anti-hemolytic effect of an extract is assessed in vitro using an erythrocyte model.

The evaluation of anti-hemolytic activity is based on the ability of extracts to stabilise the membrane of human erythrocytes when subjected to osmotic stress induced by a hyposaline solution. In the presence of a protective agent (such as a plant extract with membrane-protective properties), membrane integrity is preserved, reducing the release of haemoglobin. The degree

of hemolysis is quantified by measuring the absorbance of the supernatant at 560 nm, with the inhibition efficiency expressed as a percentage. A human erythrocyte suspension at 10% was prepared from whole blood collected in heparinised tubes, then centrifuged at 3000 rpm for 10 min.

The resulting cell pellet was washed three times with 0.9% isotonic NaCl solution. For the evaluation, 0.5 mL of the extract to be tested (diluted in 0.9% NaCl) was mixed with 1.5 mL of phosphate buffer (PBS, 0.15 M, pH 7.4) and 2 mL of hyposaline solution (0.36% NaCl). This mixture was incubated at 37°C for 20 min. Next, 0.5 mL of the erythrocyte suspension was added, followed by incubation at 56°C for 30 min. After rapid cooling, the tubes were centrifuged at 3000 rpm for 5 min. The absorbance of the supernatant was measured at 560 nm to assess the hemolysis rate. Gallic acid was used as a positive control. The percentage of hemolysis was calculated using the following equation :

Hemolysis rate % = [(Ac – At) / Ac] x 100

Ac = Absorbance of control. At = Absorbance of test solution (tested extract).

RESULTS AND DISCUSSION

Phytochemical screening was performed by employing standard screening tests. According to the results, *Z.officinale* is rich in saponins, terpenoids and steroids (Table 1). It should be noted that the ginger maceration and decoction extracts studied differ in terms of their secondary chemical composition.

However, there are similarities between these two extracts, namely the total absence of tannins (hydrolysable), proanthocyanidols (condensed tannins), flavonoids, anthocyanins and alkaloids.

Contents of total polyphenols and flavonoids

The total polyphenol content of Zingiber officinale was assessed using the Folin Ciocalteu method to determine the total number of polyphenols present in the two ginger extracts.

The results of our study show that ginger maceration extract is rich in polyphenols compared to decoction extract (Table 2).

Oueslati et al. (2018) show that the 30% methanolic extract has a total polyphenol content of 51.7 mg EAG/ g of extract. According to Hammoudi and Fekiri (2021), the phenolic compound content of natural dried ginger water extract was 68.05 ± 04.02 mg EAG/g of extract. Thus, based on these results, we conclude that the extract containing a large amount of polyphenols is the aqueous extract of natural dried ginger. However, this difference in polyphenol content may also be due to species diversity, climatic conditions, maturity and different extraction processes such as the type of solvent, temperature and extraction time (Popovici et al., 2009).

Table 1. Phytochemical composition of Ginger

Phytochemical compounds	Methanolic extract	Water extract
Saponosides	+	+
Tannins	-	-
Proanthocyanidols	-	-
Flavonoids	-	-
Terpenoids and Steroids	+++	+
Anthocyanes	-	-
Alkaloids	-	-

(+++) = Prominent; (+) = very less present; (–) = Absent.

The results reveal that methanolic ginger extract is richer in flavonoids than aqueous extract. This difference could be explained by the temperature factor (Table 2).

We compared the results of the current study of ginger extract (maceration and decoction) with chloroform extract (ECh), ethyl acetate extract (EAE), n-butanol extract (ENB), hexane extract (EH) carried out by Mameri and Sadaoui (2019), and microwave-dried ginger powder by Bourai and Azzouk (2018). The results indicate that there is indeed a difference in the amount of flavonoids present in the extract in our study compared to the extracts in the other studies. In fact, the flavonoids in our two extracts are poorer than ENB, EAE, EH, and ECh, whose respective contents are 47.61, 81.55, 83.036, and 89.77 µgEQ/mg of extract. All these results show that using solvents with different polarities (methanol/water and chloroform, etc.) allows different quantities of flavonoids to be extracted.

Antioxidant activity

In this study, the antioxidant activities of Ginger extracts were evaluated by DPPH and FRAP assays using ascorbic acid as a standard.

According to table 2, methanolic extract presents a high significant DPPH activity, expressed by the lowest IC50 compared of the aqueous extract. Then, FRAP analysis revealed a powerful activity of methanolic extract compared with aqueous extract.

Phenolic compounds are responsible for the antioxidant activities of plants. DPPH and FRAP assays were used to assess the antioxidant activity of plant extracts. This study is not similar to those of Meghezzi and Dali (2018), where methanolic extract has antioxidant activity with an IC50 of 0.22 mg/mL, and Amari (2016), who showed that the extract of *Z. officinale* rhizomes has antioxidant activity with an IC50 of 5.23 mg/mL. This variability in IC50 may be due to geographical diversity, extraction methods, and whether the rhizomes were fresh or dried.

Our extracts have very low reducing power compared to the work of Beggas and Bendoukhane (2017), who found an EC50 of 1.74 mg/mL for HG. The study on dried ginger also showed that Zingiber officinale has antioxidant capacity, with an EC50 of 3.915 mg/mL (Hammoudi and Fekiri, 2021). Finally, the extract with the best reducing activity is the methanolic extract at a concentration of 0.13 mg/mL.

Table 2. Total phenolics and flavonoids content in the extracts of Ginger

Extract	Total phenolic content (µg EAG/mg of extract)	Total flavonoid content (µg EQ/g DW)	DPPH IC50 (µg/mL)	FRAP EC50 (µg/mL)
Maceration	1.46 ± 0.006	3.57 ± 0.422	32.02 ± 0.30*	130.11 ± 0.28*
Decoction	1.4 ± 0.003	0.72	52.83± 0.31**	163.27 ± 0.30**
Ascorbic acid	-	-	27.17 ± 0.42	95.6 ± 0.50

Values are means of three replicates ± SD. The values are significantly different, *P<0.05, and **P< 0.01. EAG: aquivalent galllic acid; EQ: equivalent quercetin; DW: dry weight

In vitro Anti hemolytic and anti inflammatory activities

The present results exhibited a concentration dependent inhibition of protein (albumin) denaturation by the extracts. The percentage values were obtained using various concentrations of test compounds, and readings are presented as the mean of triplicates.

The results obtained suggest a proportional relationship between the increase in concentration and the percentage inhibition of BSA denaturation, both for diclofenac and for the two ginger extracts.

Comparative analysis reveals that the percentage inhibition of BSA denaturation induced by plant extracts remains lower than that observed with diclofenac. At a concentration of 500 µg/ml, the latter showed a maximum inhibition of 94.86 ± 5.7%. In contrast, at a concentration of 500 µg/ml, the maceration extract showed an inhibition rate of 76.06 ± 4.43%, while that of the decoction extract was 68.35 ± 7.67% (Fig.2)

Maceration, which is carried out at room temperature or slightly heated, better preserves the integrity of heat-sensitive compounds such as flavonoids, polyphenols and other secondary metabolites, known for their anti-inflammatory pharmacological properties. Several studies have shown that flavonoids possess potent anti-inflammatory activity in models of acute and chronic inflammation by inhibiting lipid peroxidation, platelet aggregation, and capillary permeability.

The study of the anti-hemolytic activity of extracts obtained by maceration and decoction, compared to gallic acid, reveals marked differences depending on the extraction method and concentrations used. Analysis of the results shows a positive correlation between the concentration of the extracts and the percentage of hemolysis, reflecting a dose-dependent activity.

At low concentrations (100 µg/ml), the hemolytic activity of gallic acid is significantly higher (57%) compared to extracts obtained by maceration (12%) and decoction (23%). This result suggests that gallic acid, as a pure compound, has a more directe and effective action on red blood cell lysis than complex extracts. However, the relatively moderate activity of the extracts at this concentration could be attributed to the low amount of bioactive compounds released or the presence of antagonistic compounds (Fig. 3).

When the concentration increases to 125 µg/ml and 250 µg/ml, a gradual increase in hemolysis is observed for all types of samples. In particular, the maceration extract reaches 65% at 250 µg/ml, approaching the efficacy of gallic acid (84%), while the decoction reaches only 49%. This difference can be explained by the better preservation of heat-sensitive metabolites, such as polyphenols and flavonoids, during maceration. At maximum concentration (500 µg/ml), the maceration extract exhibits hemolytic activity almost equivalent to that of gallic acid (82% versus 80%), while the decoction peaks at 51%. This result corroborates the hypothesis that maceration is a more favourable method for extracting the active ingredients responsible for hemolytic activity.

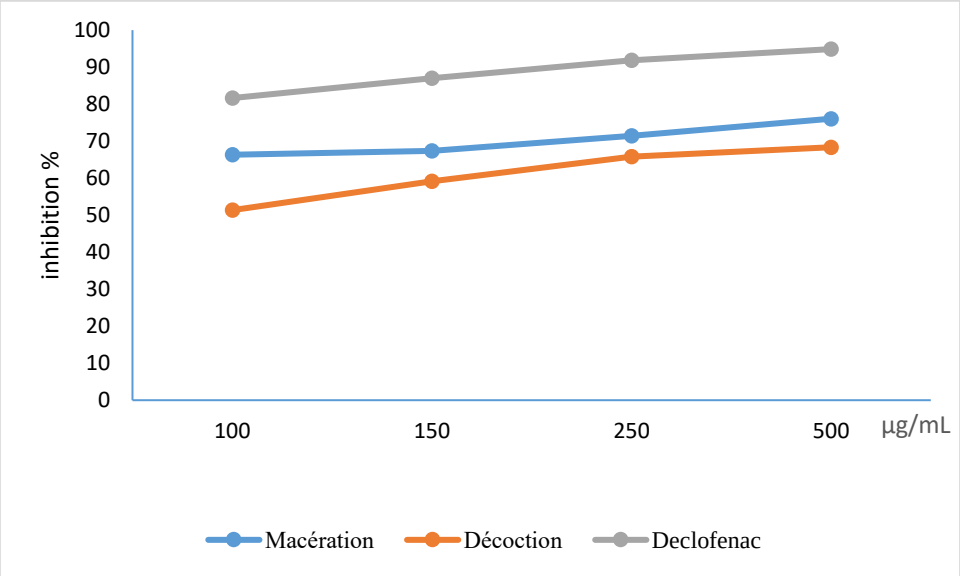

Figure 2. Percentage inhibition of BSA denaturation

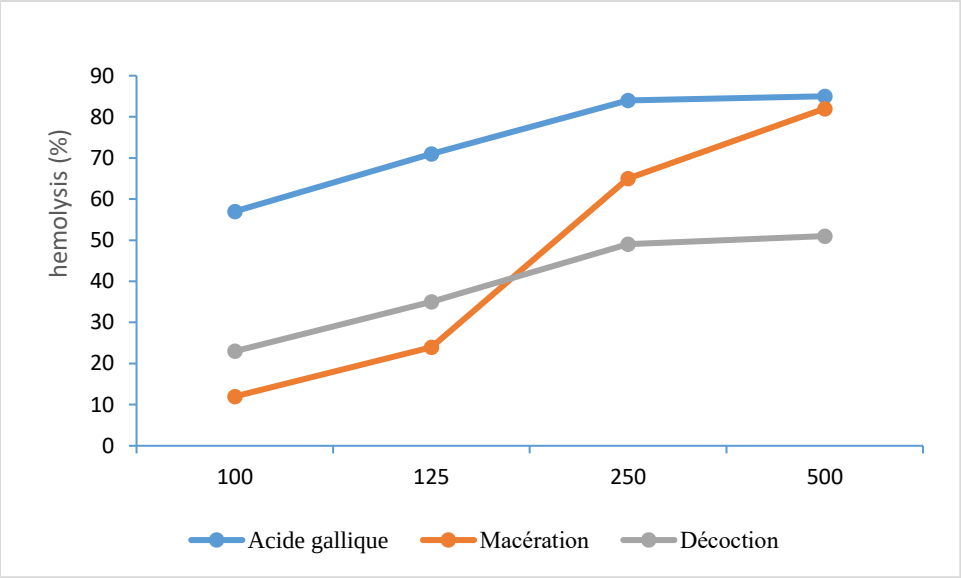

Figure 3. Percentage inhibition of hemolysis

CONCLUSIONS

The findings suggest that *Zingiber officinale* possess large antioxidant properties, a lower percentage of cytotoxicity and a very good anti-inflammatory activity and could serve to protect human from oxidative damage caused by free radicals.

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SOUS-VIDE: AN INNOVATIVE APPROACH TO COOKING AQUATIC PRODUCTS

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ABSTRACT

Cooking is a heat treatment used to make food edible, ensure microbial safety, and enhance flavor. To achieve these goals, foods are prepared using various cooking methods. Traditional techniques such as frying, baking, grilling, and boiling are widely used for cooking seafood.

Cooking transforms food into more easily edible and digestible products with improved taste and extended shelf life through various physical and chemical processes. However, depending on the cooking method and conditions, it can also result in the loss of nutritional value and sensory properties, as well as the formation of undesirable compounds. For example, frying can lead to health problems such as obesity, hypertension, and cardiovascular diseases. Grilling may result in the formation of toxic substances while baking and boiling can cause the loss of essential nutrients. Today's consumers tend to prefer healthy foods that retain their nutritional value. In recent years, sous-vide, a cooking method that addresses these preferences, has emerged as an innovative technique for preparing aquatic products. Sous-vide technology, also known as "cooking under vacuum," involves cooking vacuum-sealed products in a water bath with precise control of time and temperature. This method helps preserve the product's nutritional value and flavor, prevents undesirable changes, particularly the formation of off-flavors and odors caused by oxidation, and extends shelf life. In this presentation, information will be provided on sous-vide cooking of fish and other seafood, which are nutritionally valuable but highly perishable.

Keywords: Sous-vide, cooking, fish, seafood

INTRODUCTION

Sous-vide, which means "cooking under vacuum" in French, means cooking foods packaged under vacuum in heat-resistant bags at a precise temperature and for a certain period (Zakrzewski et al., 2023). Sous vide products, cooked in vacuum bags, rapidly cooled, and stored in the cold, are ready-to-eat foods. In recent years, as consumer habits have changed, the demand for ready-to-eat foods has increased. With increasing consumer awareness, interest in methods that will minimize nutritional losses in foods has also increased. Sous vide-cooked foods meet these demands (Bhattacharya et al., 2024). Sous-vide technology can be applied to a wide range of products, from vegetables to soups, meat and meat products, and various sauces. Products prepared with this technique are sold as ready-to-eat foods and are suitable for use in all fast-food outlets. Therefore, it is believed that increasing its commercial production and sales will greatly benefit consumers. Sous-vide cooking method emerged in the 1960s as a result of French chefs' efforts to improve cooking quality and has become a method used globally today. The vitamin and mineral losses may occur with traditional cooking methods. Sous-vide reduces these losses (Misu et al., 2024). Due to sous-vide cooking used low temperatures, food cooks evenly, ensuring the inside is properly cooked without overcooking the outside, and preserving

the food's moisture. It ensures the juiciness and tenderness of meats and enhances the flavor of foods (Przybylski et al., 2021; Modzelewska-Kapituła et al., 2022; Kowalska et al., 2023).

Except for some products eaten raw, seafood is cooked. Traditional methods for cooking seafood include frying, grilling, baking, and steaming. Cooking seafood is primarily done to make it edible, but it also increases flavor, shelf life, and food safety. Heat treatment affects color, texture, and nutritional value (Abraha, 2018). However, traditional methods can lead to losses in nutritional value and flavor. Furthermore, at high temperatures, losses in water-soluble nutrients such as vitamins and minerals reduce their nutritional value (Puertollano et al., 2011). These methods also affect textural properties because they occur at high temperatures (Hassoun et al., 2021; Yang et al., 2023). The seafood products are rich in high biological value protein, unsaturated fatty acids, vitamins, and minerals. Therefore, it is important to cook and process them using methods that preserve their nutritional value. Furthermore, due to their perishable nature, methods that extend self-life and ensure safety are needed.

SOUS-VIDE COOKING PROCESS

Sous-vide technology is based on two applications that distinguish it from traditional cooking: (1) vacuum packaging of the product in a heat-resistant plastic bag, (2) cooking at precisely controlled temperature and time (Baldwin, 2012). The process steps of sous-vide cooking are as follows:

Vacuum packaging

Foods to be cooked sous-vide are placed in bags made of heat-resistant, air- and gas-tight plastic material and then packaged under vacuum (Karki et al., 2022). Vacuum packaging provides effective heat transfer from water to food, ensures uniform heat distribution throughout the food, and thus ensures even cooking. It extends the shelf life of food by eliminating the risk of recontamination during storage. It prevents unpleasant odors that may occur due to oxidation. It prevents the loss of moisture and volatile aromatic compounds and the growth of aerobic bacteria during cooking (Abel et al., 2022).

Cooking at controlled temperature

The vacuum bags containing the food are immersed in the water bath of the sous-vide equipment. The equipment is set to the target cooking temperature and cooked under precise temperature control. Since the temperature is constant and controllable in sous-vide cooking, the food can reach the desired degree of doneness, and the same quality product can always be obtained. This precise control minimizes the risk of overcooking while preserving sensitive proteins and natural flavours (Bhattacharya et al., 2024).

Cooling

After the cooking process is completed, the bags are removed from the water bath and quickly cooled to 2-3°C. Cooling must be rapid due to the possibility of spore-forming bacteria surviving during cooking (Bhattacharya et al., 2024). This prevents the proliferation of viable bacterial cells and spore germination.

Storage

To control bacteria that can survive during the cooking process and prevent the growth of anaerobic spore-forming bacteria such as Clostridium botulinum, Clostridium perfringens, and Bacillus cereus, chilled food should be stored at <3°C (Zavadlav et al., 2020**)**.

ADVANTAGES AND DISADVANTAGES OF SOUS-VIDE COOKING

Sous-vide cooking has several advantages. It prevents moisture loss and, consequently, weight loss. The moisture loss that occurs in traditional cooking methods also leads to the loss of nutritional components. However, sous-vide cooking prevents water loss, resulting in a juicier and more tender meat, while also preserving nutrients by preventing the loss of water-soluble vitamins and minerals (Misu et al., 2024). In sous-vide cooking, lipid oxidation is significantly reduced thanks to the controlled, low temperature and vacuum packaging. This also reduces flavor and nutritional losses caused by lipid oxidation (Ayub and Ahmad, 2019). Since oxygen is removed from the environment with vacuum packaging, the risk of aerobic bacteria development is reduced, sous-vide cooking extends the shelf life of the food (Misu et al., 2024). In addition, since vacuum packaging reduces the risk of recontamination during storage, the shelf life is extended (Baldwin, 2012). Controlled temperature-time parameters ensure that the food is cooked at the desired rate and homogeneously (Baldwin, 2012; Ayub and Ahmad, 2019). Aroma losses and textural changes caused by high temperatures are prevented by sous-vide cooking (Diaz et al., 2011). The vacuum-sealing process helps retain volatile flavour compounds, preventing them from escaping during cooking (Bhattacharya et al., 2024).

Despite all these advantages, sous-vide cooking has some disadvantages.Because the sous-vide technique uses lower temperatures than traditional methods, pathogens are not always eliminated. Improper cooking, processing, and storage conditions can increase the risk of microbial contamination. Furthermore, if precise temperature control is not achieved and the products are not rapidly cooled after cooking, spore-forming bacteria can survive While vacuum packaging provides significant advantages, it also provides a suitable environment for anaerobic pathogen growth. There is also a risk of harmful chemicals leaking into the food from the plastic materials used in vacuum packaging (Bhattacharya et al., 2024).

SOUS-VIDE APPLICATIONS FOR AQUATIC PRODUCTS

The sous-vide technique is used to cook a variety of foods. Scientific research exists on its use in various foods. For example, some studies have examined its effects on the quality of foods such as beef, chicken, pork, salad, lamb, and meatballs (Vaudagna et al., 2002; Wang et al., 2004; Diaz et al., 2008; Leykane et al., 2010; Roldan et al., 2015; Can and Harun, 2015). However, the number of studies on seafood is limited.

Seafood, due to its high-water activity and neutral pH, is among the foods that spoil quickly. These characteristics provide an environment for bacterial growth. Furthermore, their high free amino acid content reduces their shelf life. However, with the application of sous vide technology, vacuum packaging allows for a longer shelf life than traditional methods (Zakrzewski et al., 2023; Lovaszova et al., 2024). This method is important for fish, as it extends the shelf life of seafood while preserving its freshness and appearance. Gonzales-Fandos et al. (2004) conducted sensory and microbiological analyses on rainbow trout (Oncorhynchus mykiss) after sous-vide cooking at 90°C for 3.3 and 1.04 minutes and at 70°C for 5.18 minutes, and storing at 2 and 10°C. They determined that heat treatment of the core at 90°C for 3.3 minutes was the most effective method for extending shelf life. Another study reported that 15 minutes at 90°C was the most effective method for extending the shelf life of salmon slices cooked sous-vide at different temperature-time combinations (5 min/65°C, 10 min/90°C, and 15 min/90°C), but sensory properties were not optimal under these conditions (Gonzales-Fandos et al., 2005). Garcia-Linares et al. (2004) investigated the effects of fish fat content on

microbial safety during sous-vide cooking and determined that the fat content of fish should be considered when creating adequate temperature/time combinations to ensure fish microbial quality and shelf life. It has been reported that seerfish steaks, for which the sous-vide application was developed by optimizing the process parameters, can be stored at 3°C for up to 65 days (Singh et al., 2016).

Cooking at high temperatures causes myofibrillar proteins to shrink and reduce their water-holding capacity. However, sous-vide cooking preserves the secondary structure of proteins better than traditional methods. Wan et al. (2019) demonstrated that sous-vide cooking in sea bass fillets was effective in preserving the secondary structure of proteins. Long-term cooking at low temperatures reduces thermal degradation of proteins and lipids (Zvadlav et al., 2020). Since myofibrillar proteins and collagen are sensitive to high temperatures, these proteins must be protected to ensure tenderness and juiciness in fish (Kathuria et al., 2022). Sous-vide processing significantly increased the structural stability, firmness, and flexibility of muscle fibers in tilapia fillets, reduced nutrient loss, enriched flavor, and mitigated the effects on taste and fishy odor (Yang et al., 2023).

Sous-vide cooking prevents microbial growth in seafood and extends shelf life. A decrease in the total viable count has been reported in seerfish steaks after sous-vide cooking (Singh et al., 2016). Hand-picked crab meat poses a risk of microbial contamination from processors and the processing environment. No microorganisms were observed to grow on crab lump meat when cooked sous-vide, regardless of the cooking regimen (Olatunde and Benjakul, 2021). Conventionally cooked mussels (90°C-10 min) have a shelf life of approximately 14 days, while mussels cooked sous-vide (85°C-10 min) and chilled have a shelf life of approximately 21 days. Since bivalve mollusks, as filter feeders, can accumulate microorganisms, including pathogens, from seawater, the possibility of producing pasteurized mussels and new mussel-based ready meals with extended shelf life is an important innovation for producers and the preservation of fresh appearance and nutritional value, as well as the prevention of product loss, is gaining its value (Bongiorno et al., 2018).

The quality of fish cooked sous-vide depends on the temperature-time combination. A study using salmon sous-vide reported that lower time-temperature parameters resulted in less nutrient loss, but it was not suitable for microbiological quality. While no microbiological limits were exceeded, this method should be used with extreme caution when using low-temperature, short-time cooking (Głuchowski et al., 2020). Temperature and duration also affect lipid oxidation in seafood. Cropotova et al. (2019) determined that lipid oxidation in Atlantic mackerel accelerated with increases in temperature and duration.

CONCLUSIONS

While seafood is a highly nutritious food, it is also highly perishable. The flesh of seafood provides a suitable environment for microorganisms to grow. Therefore, if not processed and stored appropriately after catching or harvesting, its quality can deteriorate rapidly.The sous-vide technique is a suitable method for cooking seafood, as it reduces moisture, nutrient, and flavor losses while also preventing the growth of spoilage-causing aerobic bacteria. These beneficial effects of sous-vide cooking on seafood have been demonstrated in various studies. However, further research is needed to fully understand and optimize this technique. Processing parameters significantly affect both the quality and safety of the final product. Therefore, different processing conditions should be investigated across a range of seafood products. Moreover, additional efforts are required to ensure product safety. Since spore-forming bacteria may survive the cooking process, comprehensive studies should address not only cooking parameters but also rapid cooling, cold storage, and serving stages.

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FISH SPECIES SETTLING IN AN ARTIFICIAL REEF MODEL CREATED FROM FALLEN CONCRETE PIERS OF PIPES INTAKE FROM THE SEA IN KONYAALTI, ANTALYA

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ABSTRACT

At Konyaaltı Beach, Antalya, HDP pipes were laid approximately 400 meters offshore to receive water from the sea. Concrete piers were used for weighing these pipes. Many of these piers fell due to movement and displacement of the pipe during winter storms. During our scuba dives in this region, the concrete piers that fell from the pipe were retrieved from where they fell and two artificial reef prototypes were built at a depth of 20 and 21 meters. This research aims to identify the fish species that settle in these artificial reef prototypes. The fish that settle in the artificial reef models belong to the families Holocentridae, Serranidae, Scorpaenidae, Sigaenidae, Muraenidae and Monacanthidae. Our research indicates that the Red Sea migrants predominate in terms of the number of families and species of fish occupying the artificial reefs.

INTRODUCTION

The term *"reef"* generally refers to any naturally occurring structure that lies beneath the water surface (Ahana et al., 2010). As the name suggests, *artificial reefs* are man-made structures that are typically placed on the seabed or intentionally submerged into aquatic environments. The primary aim of artificial reefs is to provide new habitats for marine organisms, enhancing and supporting marine ecosystems (Anon, 2024).

The construction of artificial reefs dates to ancient times. For instance, approximately three thousand years ago in the Mediterranean, stones discarded from tuna fishing traps known as *Tonnara* gradually accumulated and acted as fish aggregating structures—considered early forms of artificial reefs (Riggio et al., 2000). Around 250 BC, during the First Punic War, Romans reportedly constructed an artificial reef using stones and building materials to blockade Lilybaeum harbor against Carthaginians, and they installed poles in the canals using large timber and anchors. Similarly, it has been reported that the Persians built an artificial reef at the mouth of the Tigris River to block Arab pirates (Anon, 2025).

Modern artificial reefs are designed to mimic the ecological characteristics of natural reefs through careful planning and material selection. These structures provide shelter, food, and breeding grounds for various marine organisms such as fish, crustaceans, mollusks, algae, and plants thereby creating vibrant, biodiverse marine ecosystems. Materials used in artificial reef construction include concrete and steel, decommissioned ships, sunken objects, and specially designed reef modules all promoting the attachment and settlement of marine life (Anon, 2024).

The primary purposes of artificial reefs include marine habitat restoration, biodiversity enhancement, fisheries management, recreational opportunities, coastal protection, and erosion control. Strategically placed in areas where natural habitats have been degraded or lost, artificial reefs aid in the revitalization of marine ecosystems. They also facilitate the formation of various links within the food chain, triggering dynamic biodiversity development and increasing overall productivity (Anon., 2024).

These structures additionally act as *Fish Aggregating Devices (FADs)* by attracting fish to specific areas, making them easier to locate and harvest. This reduces fishing pressure on natural populations and supports sustainable fisheries management (Kasım, 2010). Artificial reefs are often positioned near shorelines to serve as natural wave barriers that reduce coastal erosion. By dissipating wave energy, they help protect coastal infrastructure from storm surges (Anon, 2024).

Beyond their ecological benefits, artificial reefs also offer recreational opportunities for scuba divers, snorkelers, and tourists. By establishing vibrant underwater ecosystems, they attract marine enthusiasts and contribute to local tourism, thereby supporting the coastal economy. They also promote and support sport fishing activities (Anon., 2024).

In a related study, live coral cover was recorded at 18.75% on artificial reefs, compared to 23.8% on nearby natural reefs. However, the abundance of fish and associated organisms was significantly higher on artificial reefs (28.13%) than on natural reefs (6.9%). This difference is attributed to the increased shelter provided by artificial structures. Furthermore, artificial reefs allow for vertical use of the water column, enabling more organisms to settle and inhabit the area (Kumar & Raghunathan, 2010).

MATERIAL AND METHOD

In this study, artificial reef material consisted of concrete weights that had fallen from an HDP pipeline, which had been previously installed to transport seawater from Konyaaltı Beach to a city aquarium located inland (Fig. 1). The pipeline extends approximately 400 meters offshore. The T-shaped open end of the pipeline had shifted roughly 50 meters west due to entanglement with fishing nets and the impact of winter storms, resulting in the dislodgement of several concrete weights.

During scuba dives conducted in April 2025, these weights were collected from the seabed and used to construct two prototype artificial reefs at depths of approximately 20–21 meters (Fig. 1, 2). No natural reefs are present in the area, and the seabed is characterized by sandy and sandy-gravel substrates.

Scuba dives were conducted at 2–3-day intervals to monitor the fish species that settled on the prototype reefs. Photographic and video documentation was carried out using GoPro Hero 8 and GoPro Hero 13 action cameras. The diversity of species on the reefs was assessed using the Shannon Diversity Index (H′), calculated as follows:

$H = -\sum[(pi) * \log(pi)]$

H = Shannon Diversity index

Pi : It corresponds to the abundance of an individual species with respect to the others.

Pi = n/N

n = No of individuals in a given species

N = The total number of individuals in a community

$Eh = H/Ln(S)$

S= Total number of unique species

Figure 1. Reef made from fallen concrete legs of HDP pipe.

Figure 2. Second artificial reef at Konyaaltı Beach

RESULTS AND DISCUSSION

In this study, no foreign materials were introduced into the marine environment, and no harm was caused to marine organisms. The prototype artificial reef was constructed using concrete weights that had previously detached from the HDP pipeline already present on the seafloor.

The first species to colonize the prototype reef was *Sargocentron rubrum*, commonly known as the Redcoat Squirrelfish and locally referred to as the "Nylon Fish" in the Antalya region. Individuals of this species settled on the reef in schools and continued to inhabit the area. Observations revealed a uniform size distribution among individuals (Fig. 3).

Sargocentron rubrum is a non-native species to the Mediterranean. Its natural distribution includes the Western Pacific, Indian Ocean, and Red Sea. It entered the Mediterranean via the Suez Canal, classifying it as a Lessepsian migrant (Kabaklı & Ergüden, 2018). This species, which prefers rocky crevices and caves at depths ranging from 1 to 84 meters, has tough scales and can reach up to 32 cm in length (usually around 27 cm). Although it has low commercial value as a food fish, it is considered valuable in the aquarium trade (Golani et al., 2021).

Five additional fish species from the Serranidae family were identified on the reef:

- *Epinephelus aeneus* (White Grouper)
- *Epinephelus costae* (Goldblotch Grouper)
- *Epinephelus marginatus* (Dusky Grouper)
- *Mycteroperca rubra* (Mottled Grouper)
- *Serranus scriba* (Painted Comber)

These species are native to the Mediterranean and typically inhabit rocky substrates (Golani et al., 2006). Among them, the Dusky Grouper (*E. marginatus*) is a protected species in Turkey. Its harvesting is completely prohibited, and it is listed as *Vulnerable (VU)* on the IUCN Red List.

Table 1. Fish species settled in artificial reefs and calculation of the Shannon Diversity Index

Family	**Species**	**Frequency**	**Pi**	***Ln* (Pi)**	***Pi (ln*pi*)***
Holocentridae	*Sargocentron rubrum*	200	0.833	-0.182	0.15
Serranidae	*Epinephelu aeneus* *Epinephelus marginatus* *Epinephelus costae* *Mycteroperca rubra* *Serranus scriba*	3 2 2 4 4	0.013 0.008 0.008 0.017 0.017	-4.342 -4.828 -4.828 -4.075 -4.075	0.06 0.04 0.04 0.07 0.07
Scorpaenidae	*Pterois miles*	7	0.029	-1.238	0.04
Siganidae	*Siganus rivulatus*	10	0.042	-3.170	0.13
Monacanthidae	*Stephanolepis diaspros*	3	0.012	-4.423	0.05
Muraenidae	*Muraena helena*	1	0.004	-5.521	0.02
				H	**0.67**
				E_H	**0.37**

Figure 3. Sargocentron rubrum settled on the prototype artificial reef

Another species observed on the artificial reef was *Siganus rivulatus*, belonging to the **Siganidae** family. Commonly known in the region as the "White Rabbitfish," this species was frequently seen on the reef in schools. Primarily herbivorous and feeding on algae, these fish were sometimes observed venturing away from the reef or exhibiting hiding behavior between the concrete blocks during scuba dives. Originating from the Red Sea, this species is also one of the Lessepsian migrants that later entered the Mediterranean (Çiftçi et al., 2022).

Another Red Sea-origin species recorded was *Stephanolepis diaspros*, commonly known as the "Filefish," belonging to the **Monacanthidae** family. Typically inhabiting rocky and sandy substrates (Metin & Akyol, 2021), this fish was among the first to settle on the artificial reef. During every scuba dive, two individuals of this species were consistently encountered on the reef.

Moray eels (*Muraena helena*) generally inhabit rocky environments, such as beneath rocks, in crevices, and inside underwater caves, and are mostly active at night (Kaya & Özen, 2022). The moray eel was also one of the species observed to have settled on the artificial reef. The same individual was frequently encountered during scuba dives, indicating residency.

CONCLUSIONS

In addition to the species listed above, other fish were observed around and on the reef. However, these species appeared to be temporary visitors rather than resident reef dwellers. Only the species that were consistently observed and identified as reef residents during the scuba dives are reported in this study (Table 1). A total of 10 fish species from 6 different families were found to have settled on the reef. Notably, 4 of these families are of Red Sea origin. The Shannon Diversity Index for the reef community was calculated as 0.67, indicating low species diversity and the dominance of a single species within the community.

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EXPLORING THE AVAILABILITY OF BULGARIAN PLANT GENETIC RESOURCES IN THE INTERNATIONAL CROP NETWORKS

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ABSTRACT

Ex situ collections are backup copy of diversity and thus its conservation is guaranteed. One of the most widely discussed topics in current ecological politics is concerning the open access to the plant genetic resources by all stakeholders. EU countries are acting together to ensure the access to the gene fund as a public resource responsible for food security and with critical importance for the sustainability of the agrifood system. IPGR-Sadovo maintains the richest genebank collection in Southeast European region and it is nominated as a national focal point in the European search catalogue for plant genetic resources – EURISCO (*http://eurisco.ecpgr.org*). The electronic network includes passport information according to FAO/Bioversity descriptors. The study aims to present the status of Bulgarian National Inventory in EURISCO. The standard of the data allows the development to be evaluated and described using passport descriptors: number of accessions, genus, species, common crop name, collecting number, geographic coordinates (latitude, longitude, elevation), collecting and acquisition date, FAO institute code, biological status of accession (wild, landraces, breeding material, advanced cultivar), collecting source (wild or cultivated habitat, research organization or genebank). The results show high diversity level between data recorded for each descriptor and crop. The published *ex situ* accessions in EURISCO from Bulgaria are 71,239 in total and belong to 532 genera and 1,927 plant species. The national collection is the 7th largest in Europe with a share of 3.4% after Great Britain, Russia, Germany, Ukraine, Poland and Spain. The Information System for documentation of Bulgarian genebank implements an export from the database to EURISCO. A convenient and flexible architecture of the application has been developed, which includes intelligent modules to facilitate the data transfer process. Through EURISCO, the data for Bulgarian collection is presented in the other international information networks such as, AEGIS, FAO WIEWS and GENESYS. The research work was carried out under the IS-PGR-SADOVO project, supported from the Bulgarian National Science Fund.

Keywords: Accession, Plant databases, Passport descriptors, European collaboration, *Open access*.

INTRODUCTION

One of the biggest challenges in the modern world is ensuring food security. An important step in this direction is increasing the productivity of crops in conditions of climate change and reduced inputs, while ensuring sustainable development and protection of natural resources. National genebanks offer diverse collections of locally adapted crops which can support farmers' climate resilience, nutritional security and economic innovations, yet are often overlooked in crop adaptation strategies. Across much of the world, national genebanks are unknown to farmers, or poorly connected to breeders (Heaton et al., 2025).

Ex situ collections are backup copy of diversity and thus its conservation is guaranteed. One of the most widely discussed topics in current ecological politics is concerning the *open access* of plant genetic resources by all stakeholders (Pillinget al., 2020).

EU countries are acting together to ensure the access to the gene fund as a public resource responsible for food security and with critical importance for the sustainability of the agri-food system (Brink and Hintum, 2020).

Each country is responsible for preserving its gene pool as a national treasure – a resource with biological and economical value, initial material for the creation of varieties that meet the requirements of current and future generations. Enhancing the efficiency of genebanks requires implementing a quality management system that ensures data transparency, as well as the documentation and standardization of processes (Engels and Ebert, 2021; Hintum et al., 2025).

IPGR-Sadovo maintains the richest seed genebank collection in Southeast European region and it is nominated as a National Focal Point in the European search catalogue for plant genetic resources – EURISCO (Dias et al., 2012; Weise, 2021).

The study aims to present the status of Bulgarian National Inventory in EURISCO.

MATERIAL AND METHOD

The National Genebank, located in the Institute of Plant Genetic Resources, part of the Agricultural Academy, is the main center for the long-term conservation of the plant gene pool of Bulgaria and as such is an active partner in the international system for the preservation of biodiversity. It has been nominated by the European Program on Plant Genetic Resources (ECPGR) as a responsible center for Bulgaria, which guarantees the participation of our country in the European Electronic Catalogue (EURISCO) and in the construction of the virtual European Genebank - AEGIS (Hintum et al., 2021). IPGR is committed to the implementation of the Convention of Biological Diversity; The European Strategy for Plant Genetic Resources (ECPGR, 2021) follows the EU's Environmental Policies and the Global Sustainable Development Goals. This commitment emphasizes the importance of the Institute in the conservation of plant diversity at the national and international level.

The Information Centre for Plant Genetic Resources in Sadovo was created in 1982. It was completely renovated and the National GeneBank Information System for plant genetic resources is established. The Information Centre works according to the international standard for documentation of FAO/Bioversity (2017) for *ex situ/on farm* conservation of plant genetic resources and FAO/Bioversity (2022) for *in situ* preservation of crop wild relatives. It is following the priorities for *open access* to the genebank collections in Europe, for available food plants databases as a bioresource for crop breeding and agriculture, and for maintaining a global system for free germplasm exchange between all users (Brink and Hintum, 2020; ECPGR, 2021).

The electronic database of Bulgarian genebank contains the following passport information: taxonomic description, catalogue number, date of acquisition, country of origin, donor of the sample, collection site, ecology-geographical characterization, biological status, type of storage: long-term (base collection), medium-term (exchange collection), short-term (working collection), *in vitro*, cryo-conservation, field collection, botanical garden and agroecological data for *in situ* preservation of crop wild relatives.

The Genebank Standards for Plant Genetic Resources for Food and Agriculture in the conservation process is followed (FAO, 2013).

The IS-PGR-SADOVO project supports the establishment of quality standard for *ex situ/ in situ/on farm* storage, in accordance with the Operational Guide for the Bulgarian Genebank, approved by the ECPGR Management Committee (ECPGR, 2023).

The project focuses on the development and implementation of innovative technologies for the management and storage of plant genetic resources in the Bulgarian genebank. The main goal is to optimize the quality and efficiency of the processes in the National Genebank by using intelligent tools. The project significantly improves the management, storage and access to collections, thereby supporting the long-term conservation of the plant gene pool as a public resource used by the scientific community, breeding companies and other users.

RESULTS AND DISCUSSION

EURISCO provides information about more than 2 million accessions of plants and their crop wild relatives, preserved *ex situ* by about 400 institutes. It is based on a network of National Inventories of 43 member countries and represents an important effort for the preservation of world's agrobiological diversity by providing information about the large genetic diversity kept by the collaborating institutions (Fig. 1, 2).

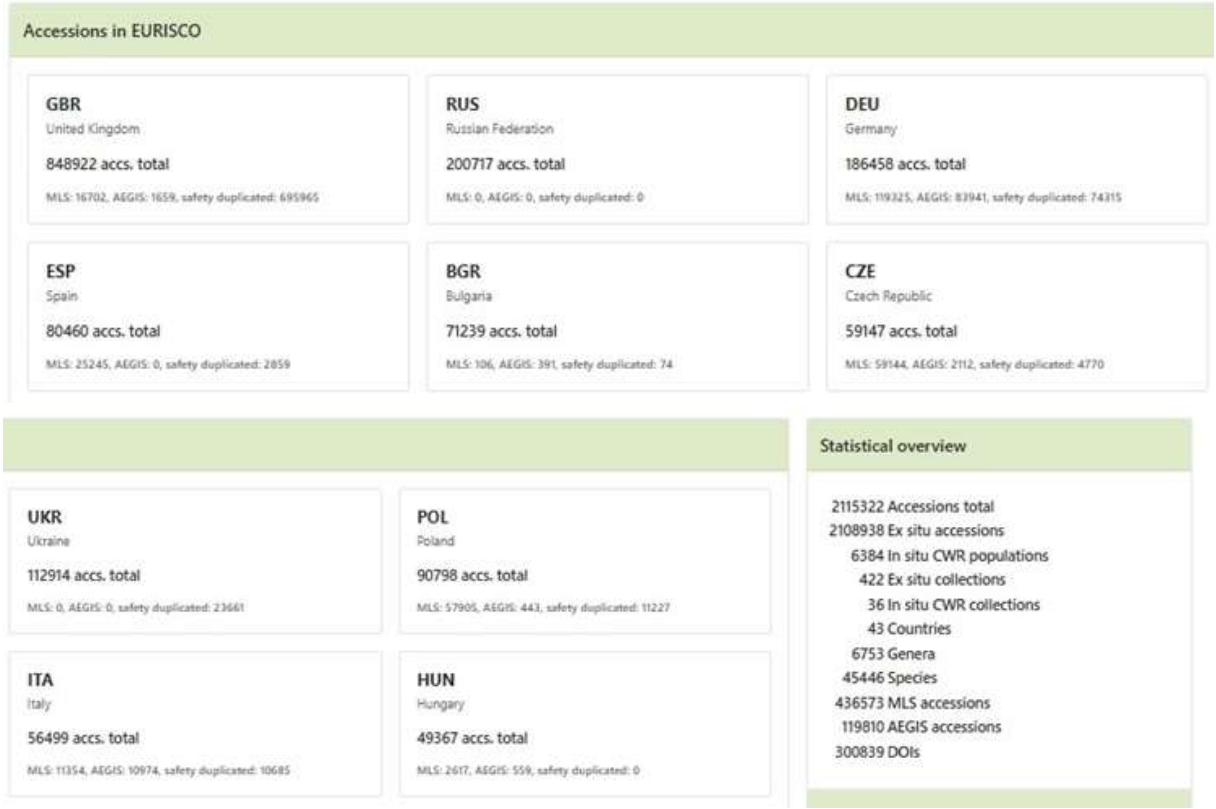

Figure 1, 2. Statistical overview in EURISCO

The analysis shows that Bulgaria maintains the richest ex situ collection in the South-Eastern Europe as the 7th largest in Europe with a share of 3,40% after The Great Britain, Russia, Germany, Ukraine, Poland and Spain.

The results show high diversity level between data recorded for each descriptor and crop. The published accessions in EURISCO from Bulgaria are 71,239 in total and belong to 532 genera and 1,927 plant species (Table 1). The highest number of accessions are from genera *Triticum, Hordeum, Zea, Phaseolus, Secale, Avena, Vicia, Capsicum, Pisum* (Fig. 3).

Table 1. Holding institutes of the Bulgarian National Inventory

FAO CODE	Holding institute name	Number of accessions	Accessions of Bulgarian origin
BGR001	Institute for Plant Genetic Resources, Sadovo	66,798	17,316
BGR005	Institute of Rose and Essential-oil Plants, Kazanlak	563	4
BGR007	Institute of Maize, Knega	19	19
BGR015	Institute of Forage Crops, Pleven	2	2
BGR029	Dobrudja Agricultural Institute, General Toshevo	3,857	1,829
	Total number	71,239	19,170

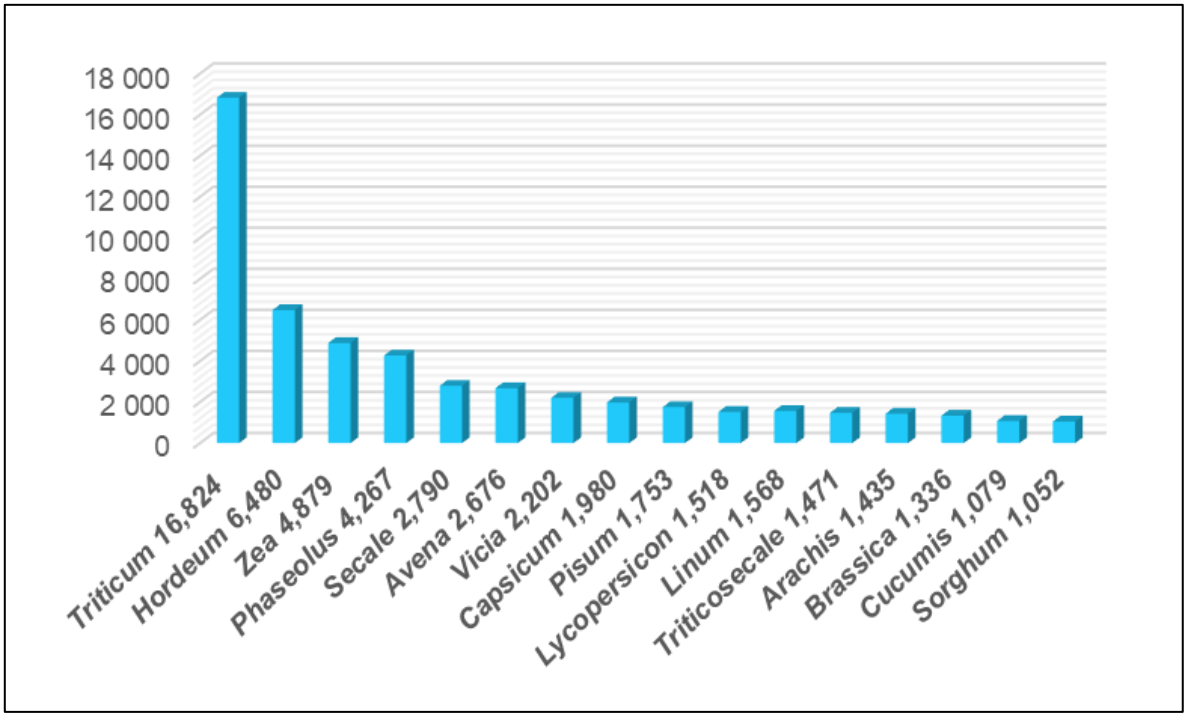

Figure 3. Genera with the highest number of accessions in the Bulgarian Collection

Background crop wild relatives represent a valuable resource for ensuring food security. Although they are negatively affected by the loss of habitats due to climate change and land use change, they are underrepresented in conservation programmes. The establishment of genetic reserve networks has been put forward as an approach to protect crop wild relatives (Bönisch et al., 2025).

In addition to *ex situ* data the EURISCO infrastructure has recently been extended for the management of *in situ* data. Bulgaria is from the pilot countries which have already provided data on *in situ* crop wild relatives populations. The status includes passport data for 243 wild populations (20 populations uploaded to EURISCO in 2023 and 223 populations in 2024) as a result from monitoring of local sites (Fig. 4).

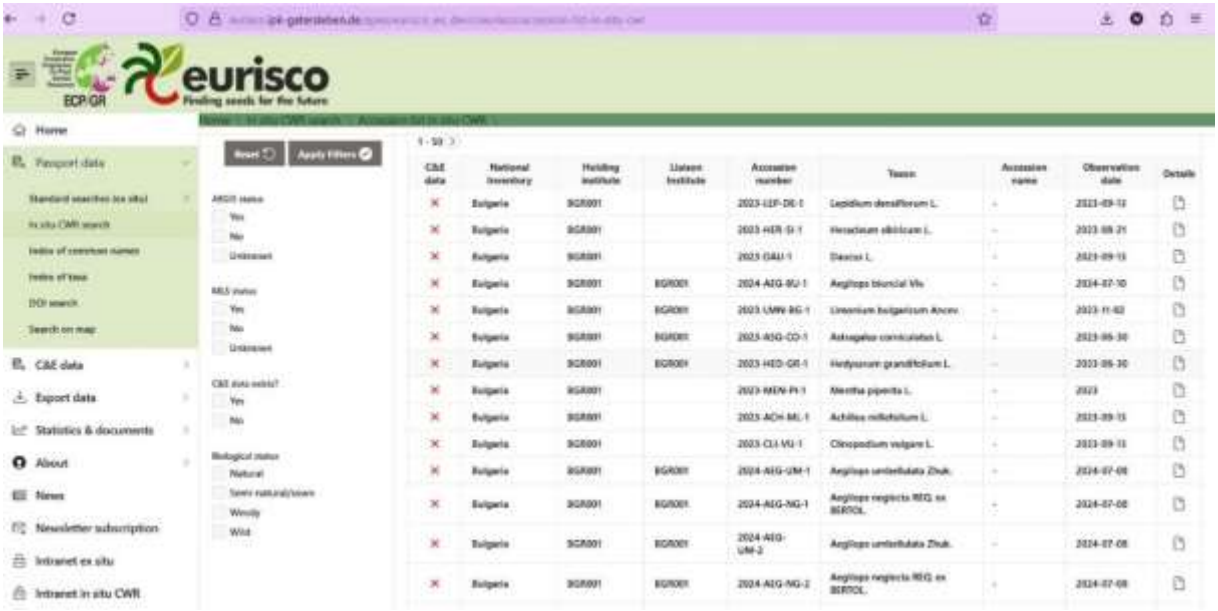

Figure 4. Bulgarian crop wild relatives in EURISCO

The National Genebank Information System is following the EU descriptors for documentation of plant genetic resources. Through the created module the data is exporting according to the standard and it is uploading to the EURISCO *ex situ/in situ* intranet. A convenient and flexible architecture of the application has been developed, which includes intelligent tools to facilitate the staff and to improve the genebank management (Fig. 5).

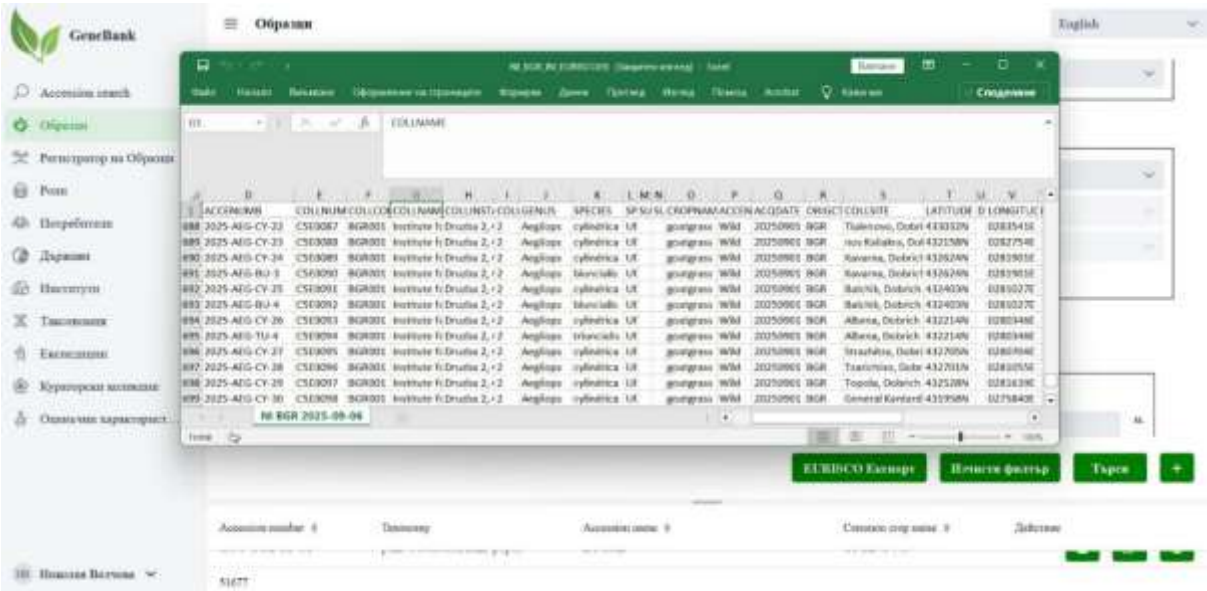

Figure 5. EURISCO standard data export in the GeneBank Information System

The IS-PGR-SADOVO project activities optimize the EURISCO transfers and increases the quality of data for conserved plant genetic resources. The Genebank Information System is guaranteeing the functionality of documentation as well as the open access to the plant gene pool to all stakeholders, in accordance with internationally signed documents, such as the International Treaty (ITPGRFA, 2009) and the Nagoya Protocol (CBD, 2011). The automatic EURISCO transfer module has a scientific and social impact with its functions to serve a large number of consumers at regional, national and international level. One of the most

important impacts is that the National Seed Genebank is recognized as one of the richest collections in Europe.

Through EURISCO the information about the Bulgarian National Inventory is accessible in other international databases, such as FAO WIEWS (2020) and GENESYS (2015).

The exploring the availability of Bulgarian plant genetic resources in the international crop networks shows that the enrichment of collection and all information activities are directed to increasing the open access to genebank accessions assisting the priority topics:

- Close interaction with scientific and educational centers in Bulgaria and abroad;
- Collection of existing old varieties and on farm conservation;
- Study and monitoring of new territories for in situ conservation of crop wild relatives;
- Creation of demonstration collections with educational and practical purposes (with wild species and traditional varieties);
- Preparation of a plan for better interaction between biodiversity conservation, crop breeding and agriculture to establish good practices.

CONCLUSIONS

International crop networks provide *open access* to the potential users of conserved genotypes according to the principles of the International Treaty on Plant Genetic Resources for Food and Agriculture and the implementation of the Nagoya Protocol on equitable distribution of their benefits.

Electronic portals eliminate the restrictions associated with distant locations of genebanks and improve coordination between researchers in the area of plant genetic resources. The European cooperation allows for better coordination of activities regarding germplasm registration, conservation, characterization, evaluation, documentation and use.

The National Genebank Information System is following the FAO/Bioversity descriptors for documentation of plant genetic resources. Through the created module the data is exporting according to the standard and it is uploading to the EURISCO *ex situ / in situ* intranet. A convenient and flexible architecture of the application has been developed, which includes intelligent tools to facilitate the staff and to improve the genebank management.

Bulgaria with its high ecogeographic diversity and extreme production conditions is a valuable international partner in climate change mitigation research projects.

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SOME RADICAL SCAVENGING ACTIVITIES OF OLIGOMERIC PROANTHOCYANIDINS OBTAINED FROM Pinus brutia Ten. BARKS

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ABSTRACT

Pinus brutia Ten. (Turkish pine), widely distributed along the eastern Mediterranean and Aegean coasts, plays a vital ecological role in afforestation projects due to its windbreaking and soil-stabilizing properties. Beyond its ecological functions, *P. brutia* also holds significant economic value through its resin, timber, and seeds, which are used in various industries such as construction, furniture, pastry production, and traditional medicine. In recent years, the global emphasis on sustainability and waste valorization has directed attention to the potential applications of forestry by-products. Among these, the bark of Turkish pine—typically considered a waste material has emerged as a promising source of oligomeric proanthocyanidins (OPCs), polyphenolic compounds known for their potent antioxidant activity.

In this study, OPC-rich extracts were obtained from Turkish pine bark through ethanol (PBET) and ethanol-water (PBAS) based extractions. The radical scavenging capacity was evaluated using DPPH and ABTS assays. The results revealed that the OPC extract exhibited strong antioxidant potential, with IC_{50} values of 6.92±0.39 - 8.17±0.56 µg /mL (DPPH) and 6.15±0.54 - 7.20±0.32 µg/mL (ABTS), comparable as better than standard antioxidants such as BHA (19.70±0.25 µg/mL DPPH and 12.80±0.50 µg/mL ABTS) and α-tocopherol (38.70±0.32 µg/mL DPPH and 33.20±0.25 µg/mL ABTS) These findings highlight the significant radical scavenging ability of *P. brutia* bark extracts, which is attributed to the presence of hydroxyl-rich flavan-3-ol oligomers.

This study suggests that Turkish pine bark, an abundant and underutilized forestry residue, may serve as a valuable bioresource in nutraceutical, cosmetic, or pharmaceutical applications. Future research may focus on isolating specific OPC fractions and evaluating their biological activities in vivo. The integration of such natural waste-derived antioxidants into commercial products may contribute to both environmental sustainability and human health.

Keywords: *Pinus brutia* Ten., Turkish Pine, Bark, Radical scavenging activity

INTRODUCTION

Pinus brutia Ten. which known as Turkish pine is the one of the most common tree species in Mediterranean basin (Sheffer et al., 2012). *Pinus brutia* Ten. (Turkish pine), a characteristic tree of the Eastern Mediterranean ecosystem, holds great ecological and economic significance. In the furniture sector it's preferred due to good quality of its timber, and in the construction sector also Turkish pine's importance is remarkable (Pantelas, 1986). Its resin and seeds also have its places at pastry and adhesive industry (Pizzi, 1982; Gönültaş et al., 2018; Dunky, 2021). *Pinus brutia*'s resin and essential oils from its different parts were studied and showed important in vitro results in some known bioactivity determiner (Loizzo et al., 2008; Ulukanli et al. 2014; Chammam et al., 2024).

Its bark, generally considered as a forestry by-product, is rich in oligomeric proanthocyanidins (OPCs), a class of polyphenolic compounds formed mainly from catechin and epicatechin subunits (Hemingway, 1989; Ucar et al., 2013). These compounds are known for their potent antioxidant activities, attributed to their hydroxyl-rich structure capable of scavenging free radicals (Pedan et al., 2017; Luca et al., 2020).

Previous studies on *Pinus pinaster* (French maritime pine) bark extracts, such as the commercial Pycnogenol®, have revealed diverse health benefits including antioxidant, anti-inflammatory, and cardioprotective properties (Nattagh-Eshtivani et al., 2022; Park et al., 2023) While research on *P. pinaster* is extensive, investigations focusing on *P. brutia* bark remain comparatively limited. Recent *in vitro* studies demonstrated that *P. brutia* bark extracts possess high phenolic content and strong radical scavenging activity, suggesting a similar or even greater potential compared to other well-studied species (Taş-Küçükaydın, 2023).

This study aims to evaluate the radical scavenging activities of ethanol and ethanol-water extracts of *P. brutia* bark, using DPPH and ABTS assays. Results are compared with standard antioxidants (BHA, α-tocopherol) and discussed in the context of literature data on other natural polyphenolic sources. The radical scavenging activity on DPPH and ABTS assays was chosen for checking the effect of scavenging in lipophilic and hydrophilic environment and due to their results generally associated with anti-aging, cell wall's oxidative stress relieving effects, and suitability with cosmetic, nutraceutical, and pharmaceutical applications (Gulcin, and Alwasel, 2023).

MATERIAL AND METHOD

Plant Material

Pinus brutia bark samples were collected from natural populations in the Muğla region, Turkey. The samples were dried under shade, powdered, and stored at 4 °C until analysis.

Extraction Procedure

Two extraction systems were applied: PBET: ethanol extraction, and PBAS: ethanol-water %50-50 extraction. Powdered barks were extracted with the solvent system for 24 h under at room temperature. Filtrates were concentrated under reduced pressure and lyophilized.

Antioxidant Activity Assays

DPPH and ABTS radical scavenging assays were carried out with extract concentrations ranging from 2–50 µg/mL. Absorbances were measured at 517 nm (DPPH) and 734 nm (ABTS). Radical scavenging capacity was expressed as IC_{50} values. BHA and α-tocopherol were used as reference standards (Blois, 1958; Re et al., 1999; Tel et al., 2012; Çayan et al, 2014).

RESULTS AND DISCUSSION

The ethanol (PBET) and ethanol-water (PBAS) extracts of *P. brutia* bark exhibited strong antioxidant activities (Table 1). Results were found as respectively IC_{50}: 6.92 ± 0.39 µg/mL and 8.17 ± 0.56 µg/mL for DPPH, and IC_{50}: 6.15 ± 0.54 µg/mL and 7.20 ± 0.32 µg/mL for ABTS assays. These values indicate superior antioxidant potential compared to standard antioxidants BHA (IC_{50}: 19.70 ± 0.25 µg/mL for DPPH and IC_{50}: 12.80 ± 0.50 µg/mL for ABTS) and α-tocopherol (IC_{50}: 38.70 ± 0.32 µg/mL for DPPH and IC_{50}: 33.20 ± 0.25 µg/mL for ABTS).
The results are consistent with previous studies on *P. pinaster* bark extracts, particularly Pycnogenol®, which exhibited IC_{50} values in the range of 5–10 µg/mL. This suggests that *P. brutia* bark is comparable or even superior in radical scavenging activity. Furthermore, in the studies on grape seed proanthocyanidins (IC_{50}: 10–20 µg/mL) showed weaker activities than *P. brutia* extracts, highlighting the promising antioxidant potential of this species (Habib et al., 2022).
The high activity can be attributed to hydroxyl-rich flavan-3-ol oligomers present in *P. brutia* bark, which facilitate electron and hydrogen atom donation (Godos et al., 2024). Therefore, *P. brutia* bark, commonly regarded as forestry waste, represents a potential bioresource for nutraceutical, cosmetic, and pharmaceutical applications.

Table 1**.** Antioxidant activities of the fractions from *P. brutia*

	Antioxidant activity (IC_{50} µg/mL)	
Samples / Standards	**DPPH assay**	**ABTS assay**
PBET	6.92±0.39	6.15±0.54
PBAS	8.17±0.56	7.20±0.32
BHA	19.70±0.25	12.80±0.50
α-tocopherol	38.70±0.32	33.20±0.25

CONCLUSIONS

This study demonstrated that oligomeric proanthocyanidin rich alcohol and alcohol-water extracts of *Pinus brutia* bark exhibit strong antioxidant properties, as revealed by low IC_{50} values in DPPH and ABTS assays. These values were significantly better than synthetic (BHA) and natural (α-tocopherol) standards. The findings suggest that *P. brutia* bark, an abundant forestry residue in Turkey, can serve as a valuable bioresource. Future work should focus on advanced characterization of OPC fractions using chromatographic and spectroscopic techniques, assessment of in vivo activities, and exploration of industrial applications for nutraceuticals and pharmaceuticals.

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APPLICATION OF ADAMS-BASHFORTH-MOULTON METHOD IN PREDICTING VEGETABLE PRODUCTION IN ALBANIA

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ABSTRACT

The aim of this research is to provide information about estimating and predicting the vegetable production in Albania until the year 2029 with the hope that the government and stakeholders can design more targeted distribution and investment strategies for more effective agricultural production planning. We have applied the Adams-Bashforth-Moulton method in Verhulst growth model. This method is a multistep method which consists of Adams-Bashforth method as predictor and Adams-Moulton method as corrector. Using vegetable production data for the period 1998-2024 obtained from the Institute of Statistics in Albania, the model was initialized using the fourth-order Runge-Kutta method to generate four initial values which were then substituted into the fourth-order Adams-Bashforth predictor equation. The Adams-Moulton method can provide a fairly accurate solution and is completed iteratively. Calculations and comparisons of predicted and actual data of vegetable production are done using Matlab and Excel software packages for the Verhulst growth model and numerical method in the study. The simulation results show that every year the amount of production increases and for the period 1998-2024 using both Verhulst model and numerical method we have coefficient of determination=0.98 and correlation coefficient=0.99. These results show that Verhulst model and Adams-Bashforth-Moulton method can be used to describe and predict the vegetable production in Albania. This research can serve as a foundation for more effective agricultural production planning and supply chain optimization in Albania.

Keywords: Adams-Bashforth-Moulton-method, Vegetable production, Fourth-order Runge-Kutta method, Numerical method, Verhulst model.

INTRODUCTION

Albania is a small country with plenty of opportunities for vegetable production. Vegetables are edible parts of herbaceous plants planted in the ground such as peppers, tomatoes, potatoes, eggplants, cucumbers, onions, etc. Due to the favorable geographical position and climate conditions, in Albania can be cultivated great diversity types of vegetables. Nowadays are cultivated more than 30 types of vegetables. The coastal areas are the most favorable areas of Albania for agricultural production, with fertile soils and relatively good access to the national road system. Most of the country's vegetable production is concentrated in the western part of the country areas such as Lushnje, Fier, Vlore and the region of Korçe in the eastern part of the country. These areas are with mild and relatively short winters providing an ideal climate to produce a wide range of vegetables. Altogether, the small-scale production

structures favor vegetable production, where also greenhouses and irrigation are common to improve production conditions (Bernet and Kazazi, 2012), (Mustafaj et al., 2018).

The importance of vegetables in human nutrition has been known since ancient times, where except for food, some types of vegetables have also been used to treat various diseases. Before the 1990s, the production of vegetables in the country served as food for the population. Much of the production of vegetables was carried out in the coastal areas of Albania because of the favorable climate for vegetable cultivation. For the first time the production of vegetables in the greenhouses was carried out in Divjaka, which was the main area of vegetable cultivation. The main place of vegetables was occupied by tomatoes and cucumbers which were produced in the existing greenhouses. The cultivated areas for the main vegetables have been grown in many parts of the country. This made the production of these vegetables extend and increase. Nowadays, the production of vegetables in the country occupies an important place in the overall agricultural production in supporting the national economy, not only as an inherent culture among Albanian farmers, but also for consumption by the Albanian population. Our country's goal is to orient and secure a sustainable agricultural development. The way to do that is to help increase vegetable production in order to meet the country's needs, minimize imports and also increase export opportunities. So, the support and management of this development gains special importance (Mustafaj et al., 2018), (Xhoxhi and Kongoli, 2023). Agriculture production, in particular vegetable production, has become increasingly competitive in the last decade because of increased cultivation area (including greenhouses), increased yields and improved technologies. The number of collection points and aggregators for trade, especially for the export of vegetables, has increased (Ministry of Agriculture and Rural Development, 2024). In these conditions, accurate prediction of vegetable production is essential for effective planning and decision-making in agriculture.

To estimate the future production of vegetables in Albania, a basic understanding of differential equations is required. Differential equations are a branch of mathematics with many real-life applications. They are used to model a large number of problems in natural sciences, in engineering and in health sciences (Zheng, 2023; Nandeesha et al., 2024; Kapoor and Tothang, 2021; Wahbi et al., 2024; Braun, 2013). Numerical methods have become an effective approach to address complex problems, especially in the field of agriculture. These methods are used to solve mathematical models, providing approximate solutions.

In efforts to make more accurately estimates for vegetable production, we have applied the Adams-Bashforth-Moulton (ABM) method for solving the Verhulst model. This model, which is a nonlinear differential equation, is used in the study to estimate the vegetable production in Albania. The model provides a useful framework for understanding the growth behavior of vegetable production. Determining the solution to a nonlinear ordinary differential equation is often challenging and complex. In one hand, the Verhulst model describes the growth process that is constrained by environmental capacity, which is highly relevant for agricultural commodities such as vegetables. On the other hand, the ABM method is a predictor-corrector numerical integration technique that provides highly accurate estimates. Before applying this method, a one-step method is required to obtain initial values. The one-step method we have used is the fourth-order Runge-Kutta method, which is an early stage method in the study. After the calculation of the initial values by this method, we get the solution of the Verhulst model by ABM method.

Using ABM method, this research not only fills the gap in the use of more sophisticated prediction methods but also offers practical solutions that can be applied in agriculture sector to provide deeper and more relevant insights for stakeholders in this sector.

MATERIAL AND METHODS

THE DATA

The data about the vegetable production are taken from the database of Albanian Institute of Statistics (INSTAT) for period 1998 till 2024 (INSTAT, n.d.). Calculations and comparisons of predicted and actual data of vegetable production are done using Matlab and Excel software packages for the Verhulst growth model and numerical method in the study. Vegetables are healthy and therefore the cultivation of them has always been increasing over the study period, as can be seen from the Figure 1. This figure provides a graphic representation of the progress of the vegetable production in Albania during the period 1998 till 2024, based on the INSTAT data.

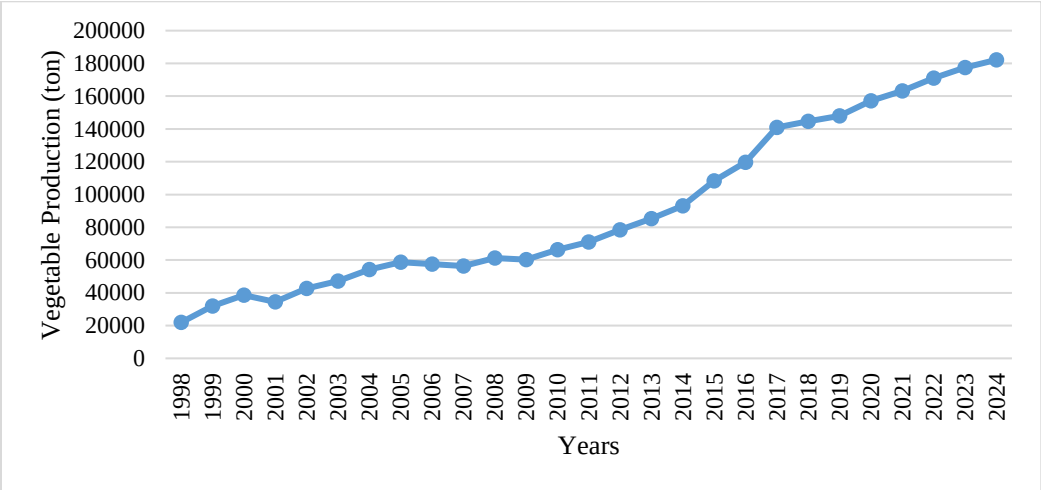

Figure 1. Vegetable production (ton) in Albania, 1998-2024
Source: Developed by the authors based on the data from INSTAT (INSTAT, n.d.)

THE VERHULST MODEL

The Verhulst model has been firstly introduced by a Belgian mathematician Pierre François Verhulst. In 1838 he published an article (Verhulst, 1838) in which he proposed a model to describe human population growth, which was called the logistic model (equation) and is presented by equation (1):

$$\frac{dy(t)}{dt} = ry(t)\left(1 - \frac{y(t)}{K}\right) \tag{1}$$

where $y(t)$ is the population size at time t, r is the growth rate and K is the carrying capacity (the maximum size of the population being studied). Nowadays this model is often applied to model the evolution of many systems in agriculture (Dishmema et al., 2023; Latif et al., 2020), in business and economics (Ramos, 2013), biology (Waltman, 1983; Kooi et al., 1998), medicine (Pelinovsky et al., 2020; Daci and Tola, 2021), and demography (Hrytsiuk et al., 2023). In this study we used the Verhulst model noted as in the equation (2):

$$\frac{dV(t)}{dt} = rV(t)\left(1 - \frac{V(t)}{K}\right) \tag{2}$$

where $V(t)$ is the vegetable production (in ton) at time t, r is the growth rate and K is the carrying capacity (the maximum size of the vegetable production). The solution of equation (2) is:

$$V(t) = \frac{KV_0e^{rt}}{K+V_0(e^{rt}-1)} \tag{3}$$

The parameters of the specific solution, which are determined using data points of equal time interval $\{(t_0, V_0), (t_1, V_1), (t_2, V_2)\}$ are given by the following formulae (4) and (5):

$$r = \frac{1}{t_1} ln \left| \frac{V_2(V_1 - V_0)}{V_0(V_2 - V_1)} \right| \tag{4}$$

$$K = \frac{V_1(V_0V_1 + V_1V_2 - 2V_0V_2)}{V_1^2 - V_0V_2} \tag{5}$$

where $t_1 \neq 0$ and $V_0, V_1, V_2 \neq 0$ (Ramos, 2013).

FOURTH-ORDER RUNGE-KUTTA METHOD

This method was invented by mathematicians Carl Runge (1856-1927) and Wilhelm Kutta (1867-1944). The fourth-order Runge-Kutta method is popular and has been used to solve ordinary differential equations problems that require high solution accuracy. This method is an early stage method in our study, because in the calculation only an initial value is needed then we get the solution by ABM method. The most popular form of the fourth-order Runge-Kutta method is presented from the equations (6) and (7) below: (Chapra S. C., 2018)

$$y_{i+1} = y_i + \frac{h}{6}(k_1 + 2k_2 + 2k_3 + k_4) \tag{6}$$

where

$$\begin{aligned} k_1 &= f(t_i, y_i) \\ k_2 &= f\left(t_i + \frac{h}{2}, y_i + \frac{h}{2}k_1\right) \\ k_3 &= f\left(t_i + \frac{h}{2}, y_i + \frac{h}{2}k_2\right) \\ k_4 &= f(t_i + h, y_i + hk_3) \end{aligned} \tag{7}$$

ADAMS-BASHFORTH-MOULTON METHOD (ABM METHOD)

The ABM method is a multi-step method widely used for solving ordinary differential equations. To obtain a solution, multi-step methods require some initial solutions that can be obtained from single-step methods (such as the Euler method, the Heun method, the Taylor series method and the Runge-Kutta method). Multi-step methods are also called predictive-corrective methods because they directly use the predictive and corrective equations in their solutions without first finding the derivatives of the function. The predictive equation, or first equation, is usually used to predict (obtain a first approximation value for y_{i+1}^P), while the corrective equation, or second equation, is usually used to obtain the corrected value (the second approximation to y_{i+1}^C). The most well-known multi-step method is the fourth-order ABM method. The truncation error of the fourth-order ABM method is smaller than that of the second and third-order ABM methods, so it can provide a fairly accurate solution (Chapra and Canale, 2021). The predictor equation of the fourth-order Adams-Bashforth method is presented by the equation (8):

$$y_{i+1}^{(0)} = y_i + \frac{h}{24}(55f_i - 59f_{i-1} + 37f_{i-2} - 9f_{i-3}) \tag{8}$$

After the calculation of $f_{i+1}^{(0)} = f\left(t_{i+1}, y_{i+1}^{(0)}\right)$, substitute it in the fourth-order Adams-Moulton method which is a corrector equation presented by the equation (9):

$$y_{i+1}^{(l)} = y_i + \frac{h}{24}\left(9f\left(t_{i+1}, y_{i+1}^{(l-1)}\right) + 19f_i - 5f_{i-1} + f_{i-2}\right) \quad (9)$$

The procedure conducted in this study for solving Verhulst model equations using the fourth-order ABM method is as follows:

1. Determine the vegetable production rate (r) and carrying capacity (K) based on the real data used in the study (INSTAT, n.d.). It is given the initial value problem of the non-linear differential equation:
$$\frac{dy(t)}{dt} = f\big(t, y(t)\big) \quad (10)$$
with initial value $y(t_0) = y_0$, with a fixed step size h and $t_{i+1} = t_i + h$.
2. Define the Verhulst model as a nonlinear differential equation:
$$\frac{dy(t)}{dt} = f\big(t, y(t)\big) = ry(t)\left(1 - \frac{y(t)}{K}\right)$$
3. Determine the initial values y_0, y_1, y_2, y_3, the step size (h), and calculate the initial solution using the fourth-order Runge-Kutta method.
4. Calculate the function values f_i, f_{i-1}, f_{i-2} and f_{i-3} with $i = 3, 4, ...$ as follows:
$$\begin{aligned} f_{i-3} &= f_0 = f(t_0, y_0) \\ f_{i-2} &= f_1 = f(t_1, y_1) \\ f_{i-1} &= f_2 = f(t_2, y_2) \\ f_i &= f_3 = f(t_3, y_3) \end{aligned} \quad (11)$$
5. Calculate the numerical solution using the fourth-order Adams-Bashforth method given by equation (8):
$$y_{i+1}^{(0)} = y_i + \frac{h}{24}(55f_i - 59f_{i-1} + 37f_{i-2} - 9f_{i-3})$$
6. Calculate $f_{i+1}^{(0)} = f\left(t_{i+1}, y_{i+1}^{(0)}\right)$ and substitute in the equation (9) to find the numerical solution using the fourth-order Adams-Moulton method.
$$y_{i+1}^{(l)} = y_i + \frac{h}{24}\left(9f\left(t_{i+1}, y_{i+1}^{(l-1)}\right) + 19f_i - 5f_{i-1} + f_{i-2}\right)$$
7. Analyze the numerical solution using the ABM method.

RESULTS AND DISCUSSION

We have calculated the parameters of the model and the predicted values from Verhulst model. In this study, the ABM method was applied by solving the problem of predicting the vegetable production in Albania. To implement this method, we followed the following steps:

Step 1. Determine growth rate (r) and carrying capacity (K).

We find the initial value by the fourth-order Runge-Kutta method using equation (2):
$$\frac{dV(t)}{dt} = f\big(t, V(t)\big) = rV(t)\left(1 - \frac{V(t)}{K}\right)$$
First we find the growth rate (r) and the carrying capacity (K). These parameters r and K were determined using data points of equal time interval $\{(t_0, V_0), (t_1, V_1), (t_2, V_2)\}$ by the formulae (4) and (5) in Excel using the analytical solution of the Verhulst model and after that they were optimized for best fitting values. The results can be seen in the Table 1.

Table 1. The parameters of the Verhulst model

Parameter estimation	r	K
Value	0.117959239816092	297741.591172142

Source: Developed by the authors

We take $r = 0.117959239816092 \approx 0.12$ and $K = 297741.591172142 \approx 297742$.
For $[a, b] = [0, 31]$ ($t \in [a, b]$, $t = 0$ is the year 1998 and $t = 31$ is the year 2029) with many iterations $n = 31$, we have $h = \frac{b-a}{n} = \frac{31-0}{31} = 1$.

Step 2. Define the Verhulst model as a nonlinear differential equation.

The Verhulst model in general form is:

$$\frac{dV(t)}{dt} = rV(t)\left(1 - \frac{V(t)}{K}\right)$$

with the initial value (12)

$$V(t_0) = V_0$$

which in our case it will be:

$$\frac{dV(t)}{dt} = 0.12\, V(t)\left(1 - \frac{V(t)}{297742}\right)$$

with the initial value (13)

$$V(t_0) = 21908$$

where $V(t_0) = 21908$ is the vegetable production (ton) at $t = 0$ (year 1998).

Step 3. The Verhults model was solved to find numerical solutions, so that solutions were obtained using the Runge-Kutta method and the ABM method.

We determine the four initial solutions V_0, V_1, V_2 and V_3 using the fourth-order Runge-Kutta method at intervals [0, 31] with step sizes $h = 1$ and $V_0 = 21908$. The results for only these values V_0, V_1, V_2 and V_3 are shown in the Table 2.

Table 2. The initial solution using Runge-Kutta method

i	t_i	$h = 1$	
		V_i	$\frac{dV(t)}{dt} = 0.12\, V(t)\left(1 - \frac{V(t)}{297742}\right)$
0	t_0	21908.0000000	2435.51985490794
1	t_1	24471.623002311	2695.23398523024
2	t_2	27305.538604083	2976.16590333825
3	t_3	30431.087901314	3278.50092888025

Source: Developed by the authors

The initial values $V_0 = 21908.0000000$, $V_1 = 24471.623002311$, $V_2 = 27305.538604083$ and $V_3 = 30431.087901314$ are obtained using Matlab and these values will serve as the initial values to be used in the ABM method.

Step 4. Calculate the values of $f_i, f_{i-1}, f_{i-2}, f_{i-3}$ with $i = 3, 4, \ldots n$ and substitute them in the Verhulst model:

$$\frac{dV(t)}{dt} = 0.12\, V(t)\left(1 - \frac{V(t)}{297742}\right)$$

For $i = 3, f_i = f_3 = f(t_3, y_3) = f(3, 30431.087901314)$

$$= 0.12 * 30431.087901314 * \left(1 - \frac{30431.087901314}{297742}\right)$$
$$= 3278.50092888025$$

$$f_{i-1} = f_2 = f(t_2, y_2) = f(2, 27305.538604083\ )$$
$$= 0.12 * 27305.538604083 * \left(1 - \frac{27305.538604083}{297742}\right)$$
$$= 2976.16590333825$$

$$f_{i-2} = f_1 = f(t_1, y_1) = f(1, 24471.623002311)$$
$$= 0.12 * 24471.623002311 * \left(1 - \frac{24471.623002311}{297742}\right)$$
$$= 2695.23398523024$$

$$f_{i-3} = f_0 = f(t_0, y_0) = f(0, 21908.0000000)$$
$$= 0.12 * 21908.0000000 * \left(1 - \frac{21908.0000000}{297742}\right)$$
$$= 2435.51985490794$$

The values of $f_i, f_{i-1}, f_{i-2}, f_{i-3}$ are shown in the Table 2 above. Next, the obtained values are substituted into the Adams-Bashforth method with a step size of $h = 1$.

Step 5. Determine numerical solutions using the Adams-Bashforth method:

$$V_{i+1}^{(0)} = V_i + \frac{h}{24}(55f_i - 59f_{i-1} + 37f_{i-2} - 9f_{i-3}) \qquad (14)$$

where V_i is the value of the initial solution to the Runge-Kutta method and f_i is the value of the initial solution using the Verhults model.

For $i = 3$, $V_3 = 30431.087901314$, $t_{i+1} = t_i + h$, so $t_4 = t_3 + h = 3 + 1 = 4$.

$$V_{3+1}^{(0)} = V_3 + \frac{h}{24}(55f_3 - 59f_2 + 37f_1 - 9f_0)$$

$$V_4^{(0)} = 30431.087901314 + \left(\frac{1}{24}\right) * (55 * 3278.50092888025 - 59 * 2976.16590333825 + 37 * 2695.23398523024 - 9 * 2435.51985490794)$$

$$V_4^{(0)} = 33869.743799264.$$

Step 6. Determine the numerical solution using the Adams-Moulton method:

$$V_{i+1}^{(l)} = V_i + \frac{h}{24}\left(9f_{i+1}^{(0)} + 19f_i - 5f_{i-1} + f_{i-2}\right) \qquad (15)$$

where $f_{i+1}^{(0)} = f\left(t_{i+1}, V_{i+1}^{(0)}\right)$.

For $i = 3$, $V_3 = 30431.087901314$, $t_{i+1} = t_i + h$, so $t_4 = t_3 + h = 3 + 1 = 4$ and $l = 0$, we have

$$f_4^{(0)} = f\left(t_4, V_4^{(0)}\right) = f(4, 33869.743799264)$$
$$= 0.12 * 33869.743799264 * \left(1 - \frac{33869.743799264}{297742}\right) = 3602.02553079620$$

For $l = 1$ we have,

$$V_{3+1}^{(1)} = V_3 + \frac{h}{24}\left(9f_4^{(0)} + 19f_3 - 5f_2 + f_1\right)$$

$$V_4^{(1)} = 30431.087901314 + \left(\frac{1}{24}\right) * (9 * 3602.02553079620 + 19 * 3278.50092888025 - 5 * 2976.16590333825 + 2695.23398523024)$$

$$V_4^{(1)} = 33869.5942302486.$$

Step 7. Now we can calculate the relative error and then compare it with termination criteria $\varepsilon = 6 * 10^{-6}$.

$$\frac{\left|V_4^{(1)} - V_4^{(0)}\right|}{\left|V_4^{(1)}\right|} = \frac{|33869.5942302486 - 33869.743799264|}{|33869.5942302486|} = 4.41602619683626e - 06$$

From the above results it can be seen that the error is relatively smaller than the termination criteria: $4.4 * 10^{-6} < 6 * 10^{-6}$. Because the error value is smaller than the stopping criterion, namely $\varepsilon = 6 * 10^{-6}$, the calculation can be continued to the steps as before for $i = 4, 5, \ldots, 31$, until the 31-st iteration. In the Figure 2 is shown the numerical solution with ABM method for the period 1998-2029.

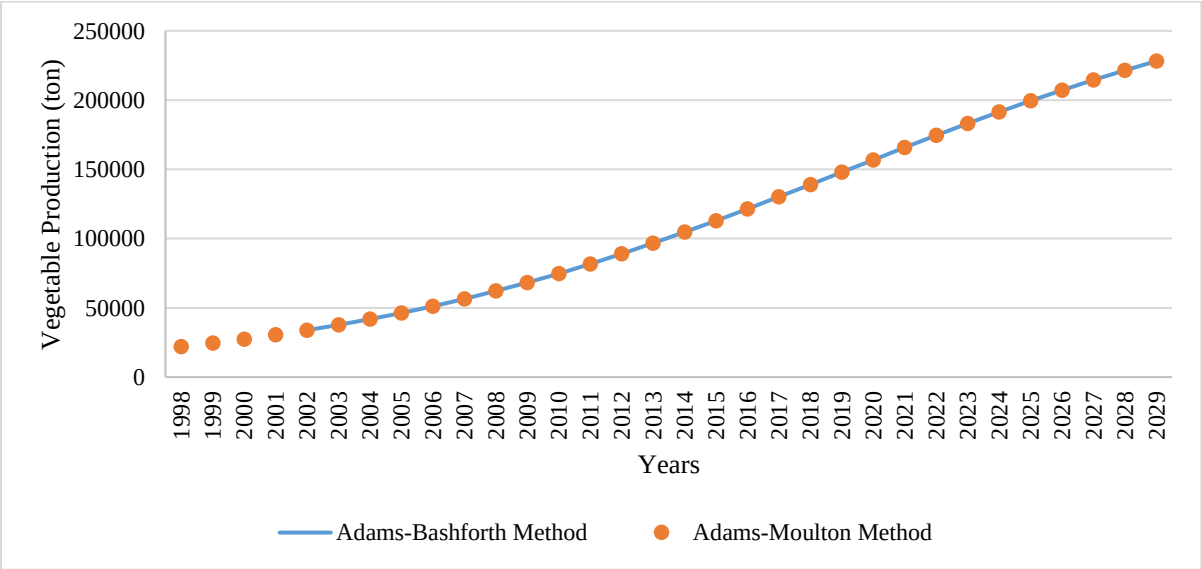

Figure 2. Numerical Solution with ABM Method
Source: Developed by the authors

This graph also provides insights into the expected changes in the future. These projections can be used to take necessary steps to ensure the enhancement of vegetable production and help farmers and local government to plan better strategies and to address factors affecting the vegetable production in the country. In the Table 3 are presented all the estimation results and the relative error using the ABM method on the Verhulst model for vegetable production in Albania.

Table 3. The estimation results using the ABM method on the Verhults model for the vegetable production in Albania

		$h = 1$		
Year	t_i	$V_i^{(0)}$	V_i	Relative Error
1998	0		21908	
1999	1		24471.623	
2000	2		27305.5386	
2001	3		30431.0879	
2002	4	33869.7437992642	33869.59423	4.42E-06
2003	5	37642.0992043624	37641.91616	4.86E-06
2004	6	41768.1033905258	41767.88509	5.23E-06
2005	7	46265.9124518255	46265.66019	5.45E-06
2006	8	51151.2751549303	51150.99224	5.53E-06
2007	9	56436.7196556296	56436.41265	5.44E-06
2008	10	62130.6917086590	62130.37084	5.16E-06
2009	11	68236.6742603895	68236.35366	4.70E-06
2010	12	74752.3332638083	74752.03089	4.05E-06
2011	13	81668.7431178582	81668.48012	3.22E-06
2012	14	88969.7520170354	88969.55157	2.25E-06
2013	15	96631.5501541705	96631.4356	1.19E-06
2014	16	104622.5006424370	104622.4932	7.13E-08
2015	17	112903.2831409270	112903.3992	1.03E-06
2016	18	121427.3831777290	121427.6316	2.05E-06
2017	19	130141.9368194820	130142.317	2.92E-06
2018	20	138988.9125635670	138989.4129	3.60E-06
2019	21	147906.5831061890	147907.1812	4.04E-06
2020	22	156831.2126111120	156831.8764	4.23E-06
2021	23	165698.8639818250	165699.5548	4.17E-06
2022	24	174447.2185100630	174447.8948	3.88E-06
2023	25	183017.2989743190	183017.9205	3.40E-06
2024	26	191354.9969982050	191355.5288	2.78E-06
2025	27	199412.3247776000	199412.7407	2.09E-06
2026	28	207148.3373063650	207148.6218	1.37E-06
2027	29	214529.7003409470	214529.8489	6.93E-07
2028	30	221530.9078139180	221530.9265	8.42E-08
2029	31	228134.1770438690	228134.0802	4.25E-07

Source: Developed by the authors

It can be seen that the vegetable production in Albania is increasing. Value V_i for $i = 4$ to 31 in the Table 3 is the corrector Adam-Moulton method value which states the prediction of the vegetable production. The simulation results show that using both Verhulst model and numerical method we have coefficient of determination=0.98 and correlation coefficient=0.99. The coefficient of determination=0.98 shows a high degree of approximation, which means that the evaluation by the regression equation covers 98% of the variation of the obtained values by

the ABM method. The correlation coefficient=0.99 indicates that the linear relationship between the variables is very strong and positive (in the same direction), which means that as the data values increase, the obtained values by the ABM method increase proportionally. These results show that Verhulst model and ABM method can be used to describe and predict the vegetable production in Albania. Based on numerical simulation results, the estimated vegetable production in Albania in 2025 is projected to be 199412.7407 tons, in 2026 is projected to be 207148.6218 tons, in 2027 is projected to be 214529.8489 tons, in 2028 is projected to be 221530.9265 tons and in 2029 is projected to be 228134.0802 tons. The following Table 4 shows the results of the prediction of vegetable production and the percentage increase in production for the period 2025-2029.

Table 4. Results of the prediction of vegetable production for the period 2025-2029

Year	Production Quantity	Percentage Increase in Production
2025	199412.7407	4.21%
2026	207148.6218	3.88%
2027	214529.8489	3.56%
2028	221530.9265	3.26%
2029	228134.0802	2.98%

Source: Developed by the authors

Table 4 shows that the vegetable production in Albania in 2025-2029 increases every year and the total vegetable production is increased with an average percentage increase of 3.58%. These findings present an optimistic perspective on the future of vegetable production in Albania. In the Figure 3 is shown a comparison of the real data of vegetable production from INSTAT with the approximation results by ABM method and analytic solution of Verhulst model for the period 1998-2029.

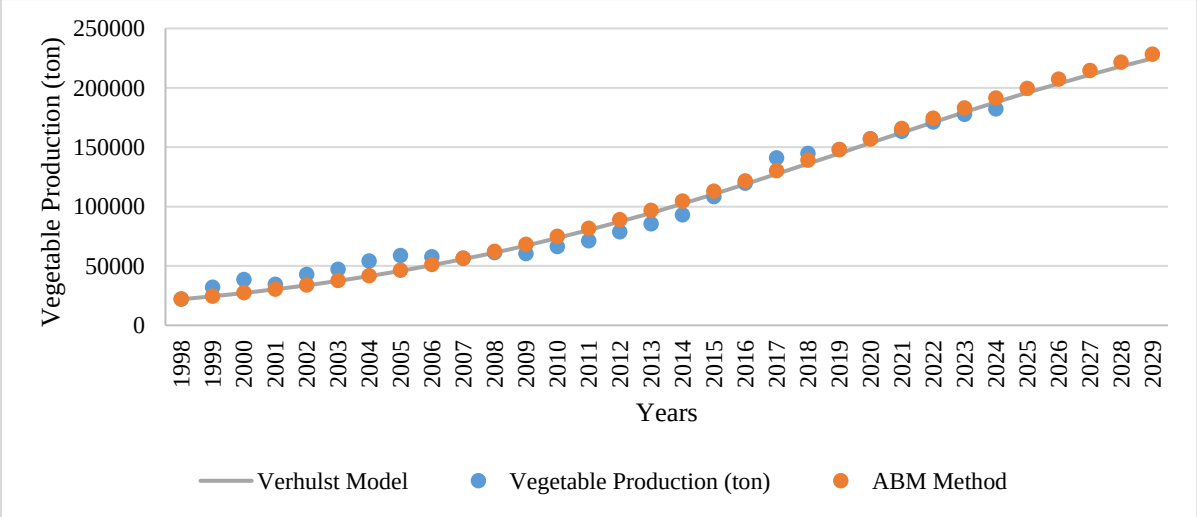

Figure 3. Comparison of the real data of vegetable production with ABM method and analytic solution of Verhulst model

Source: Developed by the authors

It is seen that the approximation results for the vegetable production are close to the real data. In this figure are also shown the results of the prediction of vegetable production for the period 2025-2029 by ABM method. This research can serve as a foundation for more effective agricultural production planning and supply chain optimization in Albania. Accurate prediction projections of the study can help the decision makers in designing more effective strategies for the future. This study also shows how numerical methods such as ABM method can be used into applied mathematical modeling to address real-life affairs. Despite the positive results, this study has certain limitations. In the study is assumed a constant growth rate ($r = 0.12$) and carrying capacity ($\mathrm{K} = 297742$), which may not reflect the dynamic environmental factors that influence the vegetable production changes over time. In addition, the use of a fixed step size ($h = 1$) may affect the accuracy of the results in case of modeling over a longer time period or under rapidly changing conditions. These limitations highlight the need for more complex and adaptive models in future research.

CONCLUSIONS

This study discusses the application of the ABM method to the Verhults model. Based on the obtained results in the study, can be concluded that the ABM method can be used to describe and predict the vegetable production in Albania. The results showed that the vegetable production increased every year. This trend suggests that the ABM method successfully describes the positive growth pattern of the vegetable production. This makes the method a valuable tool for agricultural planning. Based on numerical simulation results, it was found that there will be an increase in vegetable production from 2025 to 2029. The estimated vegetable production in Albania in 2025 is projected to be 199412.7407 tons, in 2026 is projected to be 207148.6218 tons, in 2027 is projected to be 214529.8489 tons, in 2028 is projected to be 221530.9265 tons and in 2029 is projected to be 228134.0802 tons. The total vegetable production in the period 2025-2029 is increased with an average percentage increase of 3.58%. The practical implementation of ABM method may require more complex calculations and more computations compared to other simpler approaches. In the future research could be explored the integration of this method with other predictive and numerical techniques in order to enhance prediction accuracy.

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CYTOTOXIC EFFECTS OF OLIGOMERIC PROANTHOCYANIDINS OBTAINED FROM Pinus brutia Ten. BARKS

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ABSTARCT

Pinus brutia Ten., a widely distributed pine species in the Eastern Mediterranean and Aegean regions, is not only ecologically valuable but also a potential source of bioactive compounds such as oligomeric proanthocyanidins (OPCs) found in its bark. This study aimed to evaluate the cytotoxic effects of OPC-rich bark extracts obtained using two different solvent systems (PBET: ethanol and PBAS: ethanol:water) on human lung (A549) and colon (HT-29) cancer cell lines, along with healthy human colon fibroblast cells (CCD18-Co).

The cytotoxic activity of the extracts was assessed using the MTT assay. Cells were treated with increasing concentrations of each extract (0–200 µg/mL) and incubated for 72 hours. IC_{50} values were determined based on absorbance measured at 540 nm. According to the results, PBAS exhibited the strongest cytotoxic activity on the HT-29 colon cancer cell line with an IC_{50} of 40.87 ± 0.15 µg/mL, followed by PBET with an IC_{50} of 95.10 ± 0.07 µg/mL. In both extracts, no cytotoxic effect was observed on the healthy CCD18-Co cell line (IC_{50} > 200 µg/mL), indicating selective cytotoxicity toward cancerous cells.

These findings suggest that OPCs extracted from *P. brutia* bark have potential as natural, selective anticancer agents. The ethanol:water extract, in particular, demonstrated more potent cytotoxicity, possibly due to enhanced OPC solubility and bioavailability in a mixed solvent system. This activity can be led to consider new studies on characterization of the compounds in the extract and further isolation and detection the more active compounds by enrichment studies. Further studies including mechanistic insights and in vivo validation are needed to fully assess their therapeutic potential.

Keywords: *Pinus brutia*, OPC, HT-29, A549, Cytotoxicity, MTT assay, Selective activity

INTRODUCTION

Pinus brutia Ten. (Turkish red pine), widely distributed in the Eastern Mediterranean and Aegean regions, is an ecologically and economically valuable tree species. In recent years, its bark has gained significant scientific interest as a rich source of phenolic compounds, particularly oligomeric proanthocyanidins (OPCs)(Cretu et al., 2013). OPCs are well documented for their strong antioxidant, anti-inflammatory, and potential anticancer properties. Previous studies on *P. brutia* bark have mainly focused on antioxidant and antimicrobial activities, while limited research has addressed its cytotoxic potential on cancer cell lines (Secim-Karakaya et al., 2021; Dönmez & Önem, 2024). A few studies demonstrated moderate cytotoxic effects of crude extracts or fractions from *Pinus* species against various tumor cells; however, detailed investigations targeting colon and lung cancer models with selective toxicity against malignant cells remain scarce (Sharifan et al., 2021).

Given the increasing interest in natural, selective anticancer agents, the cytotoxic potential of OPCs derived from *P. brutia* bark represents an important research gap. In particular, the solvent system employed during extraction may critically influence the solubility, bioavailability, and biological activity of these compounds (Kaczorová, et al., 2021). Therefore, the present study aimed to evaluate the cytotoxic effects of OPC-rich ethanol (PBET) and ethanol:water (PBAS) extracts from *P. brutia* bark against human lung carcinoma (A549), colon adenocarcinoma (HT-29), and normal human colon fibroblast (CCD18-Co) cell lines. This study not only provides new insights into the anticancer potential of *P. brutia* bark OPCs but also contributes to the growing evidence supporting their role as selective, plant-derived therapeutic agents.

MATERIAL AND METHOD

Cytotoxic Activity

The cytotoxic effects of PBET and PBAS were evaluated against two human cancer cell lines A549 (lung cancer cell line) and HT-29 (kolon cancer cell line) as well as a non-tumorigenic normal human colon fibroblast cell line (CCD18-Co). Cytotoxicity was assessed using the MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), according to the method described by Plumb (2003) and Belhouala et al. (2024). Cells were seeded in 96-well plates at a density of 1,000 cells per well in triplicate and incubated for 24 hours under standard conditions (37 °C, 5% CO_2, and 95% relative humidity). The extracts were applied at different concentrations (0-200 µg/mL), and the cells were further incubated for 72 hours. Cells treated with cisplatin or doxorubicin were used as positive controls. After each incubation period, 10 µL of MTT reagent (5 mg/mL in phosphate-buffered saline; Applichem, USA) was added to each well. Following a 4-hour incubation, the medium was carefully removed, and 100 µL of pure DMSO was added to solubilize the purple formazan crystals formed by viable cells. The absorbance was measured at 540 nm using a microplate reader. The IC_{50} values (± standard deviation) for each extract were calculated for the 72-hour treatment period to assess the cytotoxic potential against cancer cell lines and normal cell lines.

Statistical Analysis

For cytotoxic activity, statistical analysis was performed using GraphPad Prism version 8, including one-way ANOVA followed by Tukey's multiple comparison tests. A p value < 0.05 was considered statistically significant. The IC_{50} values were determined from regression curves generated from the percentages of inhibition or absorbance at different concentrations, as previously described.

RESULTS AND DISCUSSION

The cytotoxic activity of PBET and PBAS extracts was assessed using the MTT assay after 72 hours of treatment across a concentration range of 0–200 µg/mL. As shown in Table 1, PBAS exhibited the most potent cytotoxicity against the HT-29 colon cancer cell line, with an IC_{50} value of 40.87 ± 0.15 µg/mL, nearly twice as active as PBET (IC_{50}: 95.10 ± 0.07 µg/mL). On the A549 lung cancer cell line, both extracts showed relatively weaker activity (IC_{50} > 100 µg/mL), indicating that colon cancer cells were more susceptible to OPC treatment. Importantly, neither PBET nor PBAS displayed significant cytotoxicity on healthy CCD18-Co fibroblast cells (IC_{50} > 200 µg/mL), highlighting the selective nature of these extracts toward malignant cells.

The enhanced cytotoxicity of PBAS compared to PBET may be attributed to the ethanol:water solvent system, which likely improved the solubility and extraction efficiency of OPCs, resulting in higher bioactive content. Selective cytotoxicity is a highly desirable property in anticancer drug discovery, as it minimizes damage to healthy cells. Compared with cisplatin, a standard chemotherapeutic drug that showed strong activity but also non-selective toxicity, OPC-rich extracts demonstrated a safer profile with potential therapeutic relevance.

These findings are consistent with previous reports highlighting the anticancer potential of proanthocyanidins from other plant species, which act through mechanisms such as induction of apoptosis, inhibition of cell proliferation, and modulation of oxidative stress (Wang et al., 2019; Albogami, 2020). However, further studies are necessary to elucidate the precise molecular pathways underlying the activity of *P. brutia* OPCs.

Table 1. Cytotoxic activities of extracts of various plants against lung and colon (A549 and HT-29) cancer cell lines and their cytoxicity against healthy human (CCD18Co) cell line

Extracts/ Referance of plant codes	**Cancer Cell Lines**		**Human Cell Line**
	A549	**HT-29**	**CCD18-Co**
	IC_{50}(µg/mL)	**IC_{50}(µg/mL)**	**IC_{50}(µg/mL)**
PBET	>100	95.10± 0.07*	>200
PBAS	>100	40.87± 0.15*	>200
Cisplatin	4.01± 0.05	4.05± 0.04	NT

Values are shown as mean ± SD (n = 3) (* significant at p< 0.05) (Table 1) (NT; not tested)

CONCLUSIONS

This study demonstrates, for the first time, the selective cytotoxic potential of OPC-rich extracts from *Pinus brutia* bark against human colon (HT-29) and lung (A549) cancer cell lines. The ethanol:water extract (PBAS) displayed the strongest cytotoxicity on HT-29 cells, while both PBAS and PBET were non-toxic toward normal fibroblast cells, underlining their selective activity. These results suggest that *P. brutia* bark OPCs could serve as promising candidates for the development of natural anticancer agents with reduced side effects compared to conventional chemotherapeutics.

The findings of this study are particularly important as they highlight the role of extraction solvent systems in modulating biological activity and provide a foundation for further research on compound isolation, structural characterization, and mechanistic studies. Moreover, the selective anticancer activity observed here may encourage in vivo investigations and inspire the development of OPC-enriched formulations or functional nutraceuticals for cancer prevention and therapy. Ultimately, this work contributes to filling a significant gap in the literature on *P. brutia* and positions its bark as a valuable bioresource for anticancer drug discovery.

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EXPLORATORY FACTOR ANALYSIS OF FRUIT FARMERS' ECO-FRIENDLY AGRICULTURAL TECHNIQUES APPLICATIONS IN TERMS OF SUSTAINABLE AGRICULTURE-ENVIRONMENT RELATIONS: EVIDENCE FROM EĞIRDIR LAKE IN TURKEY

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ABSTRACT

The agricultural sector today faces multidimensional threats such as climate change, depletion of natural resources, environmental pollution, and biodiversity loss. This necessitates the widespread adoption of environmentally friendly practices, particularly in input-intensive agricultural systems such as fruit growing. The Lake Eğirdir basin, located in Türkiye's Western Mediterranean Region, serves as a notable example in this regard. Lake Eğirdir, Turkey's fourth largest freshwater source, is both a drinking water reservoir and the center of the region's agricultural activities, based on apple and cherry production. However, the increased use of pesticides and chemical fertilizers leads to deterioration of water quality in the lake ecosystem, posing a significant risk to environmental sustainability. This study examined the level of adoption of environmentally friendly agricultural techniques by fruit farmers around Lake Eğirdir using factor analysis. The Cronbach's alpha value for a 24-item scale, based on data obtained from 135 farmers, was found to be 0.733. As a result of the factor analysis, eight main factors were revealed that explain 64.72% of the total variance: Factor (1) Sustainable soil and pest management; Factor (2) Controlled Input Use; Factor (3) Sustainable and traceable agricultural practices; Factor (4) Conscious approach to input and product processes; Factor (5) Conscious weed control; Factor (6) Ecological sensitivity in agricultural practices; Factor (7) Environmental awareness and access to information; Factor (8) Conscious and controlled agricultural production. The results obtained reveal that sustainability and ecological awareness are also important determinants in farmers' agricultural activities. They also emphasize the necessity of disseminating an environmentally sensitive production approach for the protection of Lake Eğirdir.

Keywords: Sustainable agriculture, environmentally friendly practices, fruit growing, Lake Eğirdir

INTRODUCTION

Agricultural production faces multifaceted and increasingly pressing threats, including climate change, depletion of soil and water resources, loss of biodiversity, and environmental pollution. These environmental pressures question the sustainability of traditional production and necessitate the adoption of ecologically based approaches to agricultural production. A sustainable agriculture approach requires the implementation of environmentally friendly practices and calls for comprehensive policies and practices that include reducing chemical

inputs, protecting natural resources, and restructuring agro-ecosystems. (Altieri, 2018; Pretty, 2008; FAO, 2017).

Türkiye, which hosts ecological diversity and different climate zones, has a significant production potential in terms of fruit growing. (Uzun et al., 2009; FAO, 2009; Wikipedia, 2025). The Lakes Region in the Western Mediterranean, one of the regions where this potential is prominent, stands out nationwide, particularly in apple and cherry production. The Lake Eğirdir basin, one of the region's most important natural resources, is central to agricultural production for the region's population with its freshwater resources and favorable climate conditions. Fruit growing, in particular, stands out as a key source of income for the local economy.

Lake Eğirdir is under threat of losing its ecosystem function due to increasing environmental pressures in recent years. Climate change, unregulated water use, and intensive agricultural activities in particular have led to significant declines in the lake's water level and quality, threatening the ecosystem. (Ministry of Environment, Urbanization and Climate Change, 2021). Compounds such as nitrogen and phosphorus, especially those carried into the lake along with pesticides and chemical fertilizers, have led to deterioration in the lake's physicochemical parameters, which in turn threaten aquatic life by causing algal blooms and reduced dissolved oxygen. (Bulut, Atay & Uysal, 2009; Bulut et al., 2018). This lack of oxygen poses a vital threat to many aquatic organisms, especially fish; this situation reduces the biodiversity in the lake and endangers the sustainability of the ecosystem. (FAO, 2017).

These environmental pressures necessitate the expansion of new agricultural policies and production techniques based on sustainability principles. Farmers shift towards environmentally friendly techniques not only reduces pressure on ecosystems but also plays a critical role in ensuring food security, adapting to climate change, and supporting rural development. (UNEP, 2011; FAO, 2017).

This study examines the adoption of environmentally friendly agricultural techniques by fruit farmers operating around Lake Eğirdir and aims to analyze the behavioral and structural factors underlying these techniques using factor analysis. In addition to contributing to the reduction of environmental risks in natural resource use at the regional level, the study also aims to provide scientific evidence for sustainable agricultural-environmental policies that consider agriculture-environmental relationships.

MATERIALS AND METHODS

Data Collection

Primary data for this study were obtained through face-to-face surveys conducted with 135 farmers in agricultural holdings in and around the Eğirdir district of Isparta province, located in Turkey's Lakes Region. Lake Eğirdir and its surrounding area was chosen as the research area because Lake Eğirdir is a significant source of drinking and agricultural irrigation water; it holds a significant share of the region's intensive fruit production, particularly apple and cherry production (Boyraz et al., 2005; Emre et al., 2011), which is a significant part of the country; it has a high level of agricultural mechanization (Pressurized irrigation system)

(Gökdoğan et al., 2011) and various studies have demonstrated farmers tendency to adapt to innovations (Öztürk et al., 2013).

During fieldwork, farmers agricultural production techniques, environmental awareness levels, and attitudes toward sustainable practices were analyzed in detail.

The sampling frame consisted of a total of 1,529 agricultural enterprises engaged in fruit cultivation around Lake Eğirdir. Farmer lists from these enterprises were obtained from the relevant district directorates and were used as the population. The sample size for the study was determined using a simple random sampling method. The sample size was calculated according to the formula below. (Çiçek et al., 1996).

The number of sample businesses was calculated using equation (1).

$$n = \frac{N * S^2 * t^2}{(N-1) * d^2 + S^2 * t^2}$$

In the formula:

n: Required sample size,
N: Population size (1,529 farmers),
S^2: Population variance (766.76),
d: Acceptable margin of error (10% of the mean),
t: t-value corresponding to a 90% confidence level (1.65)

In this study, the sample size that would statistically represent the target population was calculated using standard sampling formulas. Based on calculations based on a 90% confidence level and a ±10% margin of error, it was determined that 135 agricultural enterprises would be appropriate to include in the sample. This sample size, in light of basic statistical principles, allows for generalizable results to be obtained regarding the research population and supports the reliability of the study. (Newbold et al., 2013).

Data Analysis

Factor Analysis

To ensure the sustainable use and protection of Lake Eğirdir, one of Turkey's important freshwater resources, factor analysis was conducted to reveal the attitudes and approaches of farmers engaged in intensive fruit growing around the lake toward environmentally friendly agricultural techniques. In this context, farmers were presented with a scale consisting of 24 statements covering topics such as environmental awareness, nature conservation approaches, and sustainable agricultural preferences and practices. Agreeing with each statement was measured using a 5-point Likert-type scale (1 = Strongly disagree, 5 = Strongly agree).

Factor analysis was used during the data analysis process to uncover underlying patterns among variables and reduce the size of the data set. This method simplifies complex data structures by summarizing a large number of variables into a smaller number of meaningful constructs. (Costello et al., 2005; Williams et al., 2010). Factor analysis allows the interpretation of a large number of variables, especially those with high correlations, by grouping them under common factors. (Beavers et al., 2013; Watkins, 2018).

This analysis technique aims to uncover non-independent (latent) structures among variables. However, for factor analysis to yield reliable results, several fundamental assumptions must be met. The most important of these is the existence of significant

correlations between variables. If there is insufficient correlation between variables, factor analysis may not be an appropriate method. (Field, 2018; Tabachnick et al., 2013).

Before analysis, it is recommended to apply the KMO and Bartlett tests to determine whether the relationships between variables are suitable for factor analysis (Suhr, 2006). The KMO value measures whether there is a sufficient level of common variance among variables, and values above 0.80 are considered "excellent," between 0.70 and 0.79 are "good," between 0.60 and 0.69 are "moderate," between 0.50 and 0.59 are "poor," and values below 0.50 are "unacceptable" (Büyüköztürk, 2002; Hair et al., 2010). A significant Bartlett test of sphericity ($p < 0.05$) indicates that there is a sufficient level of relationship between the variables and that they are suitable for factor analysis (Tabachnick et al., 2013; Williams et al., 2010).

Principal Component Analysis (PCA) was applied to determine the factor structure. This method aims to maximize the total variance in the data set (Smith, 2002). The Kaiser criterion was used to determine the number of factors, and only factors with an eigenvalue >1 were included in the analysis. (Wikipedia, 2025).

The Varimax orthogonal rotation technique was used to facilitate easier and more meaningful interpretation of the resulting factor structure. This method ensures that each variable loads heavily on only one factor, allowing for easier and more meaningful interpretation of the factor structure. Factor loadings of 0.50 and above were considered significant, and cross-loading items were excluded from the analysis (Williams et al., 2010).

Additionally, Spearman rank correlation coefficients were calculated and consistency analyses were conducted to examine the relationships among variables within each factor. Internal consistency of the scale was assessed using Cronbach's alpha, and a value of $\alpha \geq 0.70$ generally indicates reliability (Nunnally, 1978; Tavakol et al., 2011). Within the scope of this approach, the Cronbach Alpha value of the developed scale was calculated as 0.77 and it was determined that the scale was reliable. (Türel et al., 2019).

All statistical analyses were performed using SPSS for Windows 16.0 package program. (SPSS Inc., 2008).

RESEARCH FINDINGS AND DISCUSSION

Table 1 presents 24 propositions developed to identify the factors that play a role in determining fruit farmers' environmentally friendly agricultural practices for the sustainable use and protection of Lake Eğirdir. Some of these propositions were derived from previous studies, while others were determined through interviews with District Directorate of Agriculture technical staff and experts in the field. These propositions cover the following topics: protecting soil and water resources, controlled chemical use, biological control, analysis-based fertilization, use of certified inputs, and environmentally friendly practices related to ecological production processes. These approaches reflect the environmentally sensitive, planned, and conscious production approach that forms the basis of sustainable agriculture.

Descriptive statistics for the 24 variables used in the study are presented in Table 2. The means obtained from the assessment of fruit farmers' perceptions of the application of environmentally friendly agricultural techniques in terms of sustainable agriculture-environment relationships are shown in descending order. Farmers' levels of agreement with recommendations regarding the application of environmentally friendly agricultural techniques varied (X19 = 4.6370; X5 = 1.800). Accordingly, the environmental practice with the highest agreement among farmers was "paying attention to the time between the last pesticide application and harvest" (X19, Mean = 4.64, SD = 0.94). This finding indicates that farmers are particularly aware of food safety and chemical residue issues. Similarly, high means were also obtained for practices such as controlled use of pesticides (X3, Mean = 4.44) and controlled use

of fertilizers (X4, Mean = 4.41). This indicates that farmers exhibit environmentally sensitive behaviors regarding plant protection and nutrition.

On the other hand, some environmental practices have low mean values. Particularly low means were observed for techniques such as controlled hormone use (X5, Mean = 1.80), prioritizing non-chemical (biological, cultural, etc.) control methods (X8, Mean = 2.10), and implementing crop rotation (X24, Mean = 2.44). These results suggest that some environmentally friendly agricultural practices are not being adopted sufficiently due to lack of knowledge, inadequate infrastructure, or cost. (Altieri, 2018; Dessart et al., 2019).

In addition, high averages in variables such as the use of organic fertilizer (X12, Average = 3.90), the use of environmentally friendly irrigation techniques (X11, Average = 4.30) and the effective use of communication tools to raise awareness (X15, Average = 4.24) indicate a positive trend towards sustainable agriculture.

Table 1. Farmers Practices of Environmentally Friendly Agricultural Production Techniques

Codes	Variables
X1	I apply techniques in soil tillage that protect the physical structure of the soil and reduce erosion.
X2	I carry out land reclamation practices while engaging in agricultural production.
X3	I use pesticides in a controlled manner during agricultural production.
X4	I use fertilizers in a controlled manner during agricultural production.
X5	I use growth hormones in a controlled manner during agricultural production.
X6	In agricultural production, I use registered and certified seeds, seedlings, and saplings.
X7	To ensure proper timing and dosage of fertilization, I have soil analyses done at least once a year and leaf analyses when needed.
X8	In combating plant diseases and pests, I first apply cultural measures, physical control, biological control, mechanical control, and biotechnical methods and only as a last resort do I turn to chemical control.
X9	In chemical control, I use selective, environmentally friendly pesticides with low residue risk.
X10	I keep records of all pesticide applications used in chemical control.
X11	In agricultural production, I use appropriate irrigation techniques that provide the water needed by the plant while preventing water waste.
X12	In my agricultural production, I use organic, green, farmyard manure, and compost.
X13	In animal production (if available), I avoid overgrazing on pastures.
X14	I take necessary measures to maintain soil fertility (crop rotation, fallow, forage crops, or legume planting, etc.).
X15	I effectively use communication tools to raise my awareness level in agricultural practices
X16	I ensure that harvesting, storage, processing, and packaging activities are carried out within ecological methods.
X17	I pay attention to preventing microbiological contamination at all stages of production.
X18	I use sprayers suitable for applying pesticides that do not cause environmental pollution.
X19	I pay attention to the interval between the last pesticide application and harvest.
X20	I avoid pesticide application during the flowering period of plants.
X21	I avoid pesticide application in ecologically sensitive areas of regional importance (such as drinking water basins).
X22	I do not apply pesticides against diseases and pests below the economic threshold.
X23	I apply herbicides consciously.
X24	I practice proper and economical crop rotation.

According to the descriptive statistics presented in Table 2, farmers' attitudes toward environmentally friendly agricultural techniques vary. The average scores for 11 of the 24 propositions are above 3.50, indicating a generally positive perception of these techniques by farmers. This group of propositions includes practices such as paying attention to the time between the last spraying and harvest (X19, Mean = 4.6370), controlled use of pesticides and fertilizers (X3, X4), choosing appropriate irrigation techniques (X11), effective use of communication tools (X15), organic fertilizer applications (X12), avoiding spraying during flowering (X20), selective use of pesticides (X9), land reclamation (X2), keeping pesticide records (X10), and pesticide sensitivity based on ecosystem sensitivity (X21). This suggests

that farmers have environmental awareness primarily focused on input management, timing, recording, and conscious chemical use.

In contrast, the remaining 13 statements, where the mean values remain below 3.50, indicate that farmers have either not sufficiently adopted some environmentally friendly practices or have low implementation levels. These negatively perceived statements include fertilization based on soil and leaf analysis (X7), conscious weed control (X23), avoiding overgrazing (X13), soil protection techniques (X1), avoiding economical subthreshold spraying (X22), microbiological contamination measures (X17), soil fertility preservation methods (X14), appropriate sprayer use (X18), certified input use (X6), ecological harvesting and packaging methods (X16), economical crop rotation (X24), integrated pest control (X8), and controlled hormone use (X5). These findings reveal a low level of awareness, particularly in areas such as field-level analysis-based planning, biological control techniques, and certified production.

These results suggest that farmers have a positive approach to some environmentally friendly practices, but are more hesitant, particularly in practices requiring technical knowledge and cost. This situation points to the areas where training programs should be targeted and provides guidance for more effective planning of environmentally sensitive agricultural policies. (Pretty, 2008).

Table 2. Descriptive Statistics on Environmentally Friendly Agricultural Production Techniques Applications

Variables	Mean.	SE ORT. SD Mean	Ss. SS	Min.	Max.	Ordered variables	Mean
X1	2,9630	,13726	1,59480	1,00	5,00	X19	4,6370
X2	3,6148	,12351	1,43509	1,00	5,00	X3	4,4370
X3	4,4370	,07406	,86047	1,00	5,00	X4	4,4074
X4	4,4074	,07959	,92475	1,00	5,00	X11	4,3037
X5	1,8000	,12117	1,40787	1,00	5,00	X15	4,2370
X6	2,5778	,15362	1,78496	1,00	5,00	X12	3,9037
X7	3,1259	,15107	1,75529	1,00	5,00	X20	3,8074
X8	2,1037	,12139	1,41038	1,00	5,00	X2	3,6148
X9	3,6000	,10473	1,21680	1,00	5,00	X9	3,6000
X10	3,5630	,13894	1,61434	1,00	5,00	X10	3,5630
X11	4,3037	,11289	1,31168	1,00	5,00	X21	3,5111
X12	3,9037	,12480	1,45003	1,00	5,00	X7	3,1259
X13	3,0741	,15794	1,83510	1,00	5,00	X23	3,0815
X14	2,7778	,14783	1,71763	1,00	5,00	X13	3,0741
X15	4,2370	,08159	,94795	1,00	5,00	X1	2,9630
X16	2,5407	,12251	1,42349	1,00	5,00	X22	2,9481
X17	2,8370	,14463	1,68047	1,00	5,00	X17	2,8370
X18	2,6000	,15244	1,77125	1,00	5,00	X14	2,7778
X19	4,6370	,08050	,93527	1,00	5,00	X18	2,6000
X20	3,8074	,12770	1,48372	1,00	5,00	X6	2,5778
X21	3,5111	,11269	1,30936	1,00	5,00	X16	2,5407
X22	2,9481	,13146	1,52745	1,00	5,00	X24	2,4370
X23	3,0815	,14304	1,66192	1,00	5,00	X8	2,1037
X24	2,4370	,13854	1,60971	1,00	5,00	X5	1,8000
Valid N (listwise)							

* Likert Scale: 1-5, 5= Strongly Agree, 4= Agree, 3 = Undecided, 2= Disagree, 1= Strongly Disagree

Factor analysis results revealed an eight-factor structure explaining 64.72% of the total variance. Factor 1 explained 11.57% of the total variance and was named "Sustainable Soil and Pest Management." This factor 1 was explained by variables X1, X8, X14, X21, X22, and X24. In this context, it was determined that farmers:

• Implemented soil tillage techniques that would preserve the physical structure of the soil and reduce erosion (X1),

• Prioritized cultural, biological, mechanical, and biotechnical methods for controlling plant diseases and pests, resorting to chemical control as a last resort (X8),

• Implemented measures such as crop rotation, fallow, and forage or legume planting to maintain soil fertility (X14),

• Avoided spraying in sensitive ecosystems such as drinking water basins (X21),

• Did not spray against diseases and pests below the economic threshold (X22),

• And implemented appropriate and economical crop rotation (X24).

These findings demonstrate that farmers are developing an environmentally conscious approach to agricultural production. The literature indicates that nitrate and phosphate pollution resulting from pesticide and chemical fertilizer use poses serious ecological risks, particularly for surface and groundwater resources (Singh et al., 2021; OECD, 2012). This highlights the importance of farmers adopting environmentally conscious practices to reduce the environmental impact of agricultural activities. These practices significantly contribute to the protection of water and soil resources.

Factor 2, which explains 10.06% of the total variance, is defined as "Controlled Input Use." This factor is strongly correlated with variables X3 (controlled pesticide use) and X4 (controlled fertilizer use). The findings indicate that farmers are adopting controlled approaches to the use of agricultural inputs (especially pesticides and fertilizers). This is a crucial behavior for both environmental sustainability and economic efficiency. Careful and prudent use of input management contributes to the conservation of natural resources and can also reduce production costs. (Pretty, 2008).

Factor 3 explains 8.57% of the total variance and is labeled "Sustainable and Traceable Agricultural Practices." This factor is represented by variables X10 (keeping records of pesticide application), X11 (using appropriate irrigation techniques), and X12 (using organic, green manure, and compost). Findings indicate that farmers are increasing their awareness of environmentally friendly production processes and are adopting conscious practices accordingly. Providing the plants with the necessary water efficiently, adopting irrigation techniques that prevent water waste, and expanding the use of organic fertilizers instead of chemical fertilizers are critical to the development of sustainable production systems. Furthermore, keeping records of pesticide application operations is a valuable practice for both traceability and food safety. (Altieri, 2018).

Factor 4, "Conscious Approach to Input and Product Activities," explains 8.14% of the total variance. This factor is highly loaded with variables X5 (controlled hormone use), X18 (environmentally friendly sprayer use), and X16 (ecological harvesting, storage, processing, and packaging). The variables included in this factor demonstrate that inputs used in the production process should be evaluated not only by their content but also by their application methods and post-harvest processes. Controlled hormone applications, in particular, are critical for both plant development and product quality. Uncontrolled use of hormone residues poses serious threats to the environment and food safety. (Türkyılmaz et al., 2022).

Endocrine-disrupting chemicals (EDCs), in particular, are known to cause adverse consequences on animal and human health, such as reproductive disorders, hormonal imbalances, and neurodevelopmental effects (Damstra et al., 2002). This poses a threat to both human health and the sustainability of ecosystems. Similarly, directing pesticides to the target plant using appropriate nozzle (sprayer) technology and correct application techniques ensures that the pesticide reaches the correct location. This minimizes spread to non-target areas, reduces environmental impact, and reduces operator exposure to chemicals (Van de Zande et al., 2021).

Furthermore, carrying out harvesting, storage, processing, and packaging processes in accordance with ecological principles ensures sustainability not only in field production but throughout the entire chain from product delivery to consumer. This holistic approach should be considered an essential component of sustainable agricultural systems (UN ESCAP, 2016; Whitfield et al., 2023).

Therefore, this factor represents a production approach that includes not only the correct selection of inputs but also environmentally friendly, technically supported, and hygienic practices.

Factor 5, explaining 6.78% of the total variance, was labeled "Conscious Weed Control." This factor is highly correlated only with the X23 (I use weed control consciously) variable (factor loading = 0.817), representing a univariate but significant structure.

This prominent factor variable demonstrates that farmers adopt a conscious, selective, and targeted approach to weed control. Applying pesticides at the right time, at the right dose, and in the right place is crucial for reducing both economic and environmental impacts in controlling weeds. Unnecessary or broad-spectrum pesticide applications can harm non-target plants and organisms, disrupt soil microbial structure, and cause pesticide accumulation in water resources. (*Drocco et al.,* 2025).

In this context, this factor reflects the applicability of environmentally sensitive agricultural pest control methods and farmers' awareness levels on this issue. In particular, conscious pest control based on economic thresholds, rather than indiscriminate and high-dose pesticide use, is a fundamental approach to ensuring environmental sustainability (Karaca et al., 2020). Furthermore, such practices demonstrate that farmers have a production approach that considers both environmental and human health.

Factor 6, explaining 6.59% of the total variance, was labeled "Ecological Sensitivity in Agricultural Practices." This factor loaded highly with variables X2 (I conduct land reclamation work during agricultural production) and X20 (I avoid pesticide application during the flowering period).

This structure demonstrates that farmers plan their agricultural practices not only with a yield focus but also with an ecological sensitivity framework. Land reclamation provides long-term sustainability benefits such as improving soil physical properties, increasing water retention capacity, and preventing erosion (Gökoğlu et al., 2021). In this respect, it is a protective strategy aimed at preventing the depletion of natural resources.

Another prominent factor within this factor, avoiding pesticide application during the flowering period, is critical for protecting pollinator populations, especially bees. The inappropriate timing and application of pesticides can harm beneficial organisms, disrupt pollination services, and reduce biodiversity. (Basu et al., 2024).

These findings demonstrate that sustainable agriculture must be supported not only by technical knowledge but also by the ability to make and implement decisions in harmony with nature. Therefore, this factor demonstrates that farmers make agricultural decisions based on sensitive, long-term environmental planning that prioritizes ecosystem services (Şahin et al., 2020).

Factor 7, which explains 6.53% of the total variance, is labeled "Environmental Awareness and Access to Information." This factor is highly correlated with variables X13 (avoiding overgrazing in pastures) and X15 (effective use of information and communication tools in agricultural practices).

This factor demonstrates farmers' sustainable use of natural resources and informed decision-making. Preventing overgrazing of pastures is particularly important for protecting biodiversity, maintaining soil structure, and preventing erosion (Milazzo et al., 2023). Overgrazing causes irreversible damage to pasture areas, which negatively affects both animal production and the environmental balance in the long term.

On the other hand, the effective use of information and communication tools facilitates farmers' access to environmentally friendly agricultural techniques and ensures that practices are implemented based on scientific knowledge. This demonstrates the development of a knowledge-based production culture, a fundamental component of agricultural sustainability (Demirtaş et al., 2022).

The findings obtained under Factor 7 reveal that farmers act not only for production purposes but also with an awareness of environmental and social responsibility. Sustaining animal production in harmony with nature is considered a fundamental necessity not only for animal welfare but also for the protection of soil, water, and vegetation (Şahin et al., 2020).

Factor 8, which explains 6.47% of the total variance, is labeled "Conscious and Controlled Agricultural Production." This factor is represented by variables X7 (I have soil and leaf analysis performed for appropriate fertilization) and X19 (I pay attention to the time between the last spraying and harvest).

This structure reveals that farmers plan their agricultural practices based not only on traditional knowledge but also on measurements and analysis results. Soil and leaf analyses ensure correct fertilization in agriculture, increasing productivity and reducing environmental damage (Kaya et al., 2021).

Furthermore, allowing sufficient time between the last spraying and harvest plays a critical role in protecting food safety by preventing pesticide residues from remaining on the crop. This practice also serves as a preventative measure to prevent residues that could harm human health from reaching the market (Çetin et al., 2020).

Therefore, this factor is closely related to scientifically based decision-making, precision in timing, and a producer's awareness of prioritizing product safety. For sustainable agriculture, both natural resource use and pesticide applications must be carefully planned (Demirtaş et al., 2019).

Overall, examining the eight factors together reveals that farmers evaluate environmentally friendly agricultural practices with a multidimensional approach. While awareness of some factors (e.g., controlled use of chemical inputs) is high, implementation levels are lower for others (e.g., crop rotation or alternative pest management methods). These findings highlight the importance of supporting farmers in the region with training, technical support, and financial incentives for sustainable agricultural practices.

Table 3. Factor Analysis Results of Environmentally Friendly Agricultural Production Techniques Applications

VARIABLE CODES	FACTORS								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**h^2**
X22	**,668**	,040	,136	-,107	,312	,053	-,075	-,177	,615
X1	**,628**	,046	,255	,176	,144	,314	,067	,072	,621
X24	**,661**	-,132	-,106	,239	,400	-,075	,235	-,039	,745
X21	**,556**	,117	-,224	,017	-,231	,090	,376	,074	,582
X8	**,564**	,135	-,102	,163	-,020	-,363	-,031	,247	,568
X14	**,510**	-,170	,088	,368	-,232	-,007	,030	-,239	,544
X3	-,032	**,943**	,059	,060	,005	,090	,014	-,031	,907
X4	,027	**,923**	,048	,031	,051	,135	-,006	-,041	,878
X10	,071	,044	**,666**	,075	,242	-,149	,209	,041	,582
X12	,047	,026	**,644**	,098	-,315	,113	-,046	-,226	,592
X11	-,063	,172	**,634**	,001	-,128	,101	,126	,208	,521
X5	-,025	-,065	,213	**,785**	,058	,207	-,155	-,003	,737
X18	,386	,102	-,381	**,629**	-,021	-,041	-,046	,119	,718
X16	,206	,284	,024	**,592**	,157	-,253	,380	-,156	,732
X23	,142	,009	-,104	,082	**,817**	,060	,018	-,031	,710
X2	,025	,275	,144	,059	,038	**,693**	,064	,069	,591
X20	,505	-,048	-,181	-,005	-,047	**,586**	,070	,254	,705
X13	,172	-,028	,052	-,020	,125	-,024	**,712**	,091	,564
X15	-,076	-,038	,227	-,075	-,150	,202	**,658**	-,147	,582
X7	-,116	,053	,269	,100	,104	,092	,174	**,701**	,641
X19	,099	-,094	-,154	-,100	-,119	,054	-,185	**,729**	,635
X6	,247	,360	,241	,406	,375	-,032	-,080	,138	,581
X9	,057	,491	,366	-,101	-,172	-,430	-,131	,067	,625
X17	,095	-,046	,173	,314	-,365	-,299	,299	,325	,558
Eigenvalues	2,777	2,415	2,056	1,954	1,628	1,582	1,568	1,552	-
Total varianceexplained	11,571	10,064	8,565	8,140	6,784	6,593	6,532	6,466	-
Cumulativevarianceexplained	11,571	21,635	30,200	38,340	45,125	51,718	58,250	64,717	-

Values in italics = factor loads ≥0,5

(–) A factor loading with a negative sign indicates the inverse relationship

These findings suggest that overall, farmers in the region have a moderate to high level of awareness of environmentally friendly agricultural techniques, but some techniques need to be expanded and supported. Providing training, technical support, and financial incentives, particularly for areas with low averages, could contribute to sustainability goals. (Pretty, 2008; Tekeli et al., 2022).

Table 4. Application of Environmentally Friendly Production Techniques - Spearman's Rho Correlation Matrix

Faktör 1		**Faktör 2**									
	1	22	24	21	8	14			3	4	
1	1,000	,362**	,468**	,320**	,212*	,314**		3	1,000	,968**	
22	,314**	1,000	,450**	,142	,226**	,141		4	,968**	1,000	
24	,468**	,450**	1,000	,365**	,371**	,321**					
21	,320**	,142	,365**	1,000	,289**	,243**					
8	,212*	,226**	,371**	,289**	1,000	,237**					
14	,314**	,141	,321**	,243**	,237**	1,000					
Faktör 3		**Faktör 4**		**Faktör 5**							
	10	11	12			5	16	18			23
10	1,000	,266**	,221*		5	1,000	,211*	,237**		23	1,000
11	,266**	1,000	,309**		16	,211*	1,000	,382**			
12	,221*	,309**	1,000		18	,237**	,382**	1,000			
Faktör 6		**Faktör 7**		**Faktör 8**							
	2	20			13	15			7	19	
2	1,000	,332**		13	1,000	,304**		7	1,000	,203*	
20	,332**	1,000		15	,304**	1,000		19	,2039	1,000	
Faktör 1		**Faktör 2**									
	1	22	24	21	8	14			3	4	
1	1,000	,362**	,468**	,320**	,212*	,314**		3	1,000	,968**	
22	,314**	1,000	,450**	,142	,226**	,141		4	,968**	1,000	
24	,468**	,450**	1,000	,365**	,371**	,321**					
21	,320**	,142	,365**	1,000	,289**	,243**					
8	,212*	,226**	,371**	,289**	1,000	,237**					
14	,314**	,141	,321**	,243**	,237**	1,000					
Faktör 3		**Faktör 4**		**Faktör 5**							
	10	11	12			5	16	18			23
10	1,000	,266**	,221*		5	1,000	,211*	,237**		23	1,000
11	,266**	1,000	,309**		16	,211*	1,000	,382**			
12	,221*	,309**	1,000		18	,237**	,382**	1,000			
Faktör 6		**Faktör 7**		**Faktör 8**							
	2	20			13	15			7	19	
2	1,000	,332**		13	1,000	,304**		7	1,000	,203*	
20	,332**	1,000		15	,304**	1,000		19	,2039	1,000	

** 0.01 important at the level (2-tailed

* 0.05 important at the level (2-tailed)

Spearman rho correlation analysis was applied to assess the relationship between the factors. The correlation coefficients presented in Table 4 reveal statistically significant relationships ($p < 0.01$) between all pairs of variables. However, considering the magnitudes of the correlation values obtained, these relationships appear to be weak in practice. This suggests that the factors possess independent structures but may represent different dimensions of environmental sensitivity.

CONCLUSION AND RECOMMENDATIONS

As part of this study, factor analysis conducted to determine the attitudes and practices of fruit farmers around Lake Eğirdir toward environmentally friendly agricultural production techniques yielded an eight-factor structure. These factors reflect a wide range of behaviors, from practices that cause agricultural pollution to controlled chemical use, from ecological harvesting methods to analysis-based decision-making.

Each factor revealed how farmers define and implement environmental sustainability across different dimensions. Factors such as "Practices Causing Agricultural Pollution" and "Practices Sensitive to Time and Analysis," in particular, demonstrate the multidimensional nature of environmental sustainability at both the structural and administrative levels.

Spearman rho correlation analysis, conducted to determine the relationships between factors, revealed statistically significant relationships ($p < 0.01$) between all factor pairs. However, the generally low correlation coefficients suggest that these relationships have a weak impact on implementation. This suggests that each factor influences farmers' sensitivity to environmentally friendly agriculture in different ways, indicating that there is no strong structural dependence between them.

Therefore, farmers' environmental sensitivity, rather than being a homogeneous attitude, exhibits a multidimensional, area-based structure that varies by behavior type. This result indicates the need to develop holistic and multi-level policy tools that address different dimensions rather than a single, discrete approach to disseminating environmentally sensitive agricultural practices. In this context, it is crucial to increase and strengthen farmer training activities on sustainable agricultural techniques in the study region. Agricultural environmental policies and legal regulations should be implemented with a proactive approach to ensure the sustainability of Lake Eğirdir, an ecologically and economically important natural resource, and to implement them for sustainable water use.

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IMAGES OF EĞIRDIR LAKE AND ITS SURROUNDINGS

SOME TECHNOLOGICAL PROPERTIES AND BIOACTIVE COMPONENTS OF LEAVENED AND UNLEAVENED FLATBREADS SUBSTITUTED WITH GERMINATED MILLET FLOUR

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ABSTRACT

In this study, millet (Panicum miliaceum L.) was germinated for three days to improve bioactive components. Flour obtained from germinated millet was used at different ratios (0-20%) in the production of leavened and unleavened flatbread with replacing wheat flour. Control leavened and unleavened flatbreads were produced from wheat flour. Color, diameter, thickness, spread ratio, antioxidant activity (DPPH, FRAP and CUPRAC) and phenolic (free, bound and total) contents of all breads were determined. The use of high ratios of germinated millet flour (GMF) increased the diameter, and surface a* and b* color values of both. The bound, free and total phenolic content of leavened flatbread increased up to 2522.22 mg GAE/kg, 5250.83 mg GAE/kg and 7773.04 mg GAE/kg, respectively with 20% GMF usage. As the GMF ratio increased, antioxidant activity values (DPPH, FRAP and CUPRAC) of leavened and unleavened flatbreads were also increased. The highest phenolic contents and antioxidant activity values were achieved especially at the 20% GMF addition ratio.

Keywords: Germination, millet, leavened bread, unleavened bread, flatbread.

INTRODUCTION

Proso millet (*Panicum miliaceum* L.) is one of the oldest cereals. The caryopsis of millet is rich in carbohydrates, protein, mineral substances, and vitamins and its nutritive parameters are comparable or better than common cereals. Millet was richer in essential amino acids (leucine, isoleucine, methionine) than wheat. Functional components found in millet such as phenolic, antioxidant, beta-glucan and dietary fibers have positive effects on health and nutrition. On the other hand, some antinutritional factors such as phytic acid, oxalate, and tannins decrease the nutritional value of millet (Kalinova and Moudry, 2006; Kalinova, 2007). Germination is an economical and effective bioprocessing technique that increases digestibility and bioavailability of nutrients by reducing antinutritional factors. Germination results in the biosynthesis and accumulation of various secondary metabolites such as vitamin C, tocopherols, flavonoids, tocotrienols, γ-aminobutyric acid and phenolic compounds. Germinated grains have beneficial effects on health and free radical scavenging abilities (Koehler et al., 2007; Azeke et al., 2011; Kaur and Gill, 2020; Dhillon et al., 2020; Ceccaroni et al., 2020). There have been conducted many studies in the literature on the use of germinated cereals, legumes and pseudocereals in the preparation of cereal products (Torres et al., 2007; Tok, 2017; Zhu et al., 2017; Demir and Bilgiçli, 2020). In these studies, significant improvement in the nutritional and functional properties of end products has been determined with the use of germinated grains.

Bread is a cereal product that has an important place in meeting daily energy requirements. In recent years, interest in leavened and unleavened traditional flatbreads has increased. Various studies have been carried out to improve the nutritional and functional properties of these breads, which are commonly produced from refined wheat flour (Başman and Köksel, 1999, 2001; Coşkuner and Karababa, 2005; Levent et al., 2012; Levent and Bilgiçli, 2012a; Levent and Bilgiçli, 2012b; Madenci

et al., 2012; Yıldız and Bilgiçli, 2012). In this study, the effect of GMF on some technological and functional properties of leavened and unleavened flatbreads was investigated.

MATERIAL AND METHOD

Materials

Millet was purchased from Taşan Ticaret, Konya, Turkey. Wheat flour, salt, baker's yeast and sugar were procured from a local market in Konya.

Germination of millet

The millet germination process was performed according to Parameswaran and Sadasivam (1994) and Li et al., (2017) with some modifications. The surfaces of the samples were sterilized by soaking in a solution of 1.0% aqueous sodium hypochlorite for 15 minutes at room temperature and then rinsed with distilled water. Before germination, the grains were soaked overnight in distilled water (at room temperature) and germinated in the dark for 4 days at room temperature. The germinated grain was dried in an oven at 45 °C for 12 h and milled (< 500 µm) on a laboratory grinder with a 100% extraction ratio.

Leavened and unleavened breadmaking

Leavened flatbread samples were prepared according to the method given by Akbaş (2000). Wheat flour (200 g), salt (3 g), sugar (2 g), fresh yeast (5 g) and water were used to control leavened flatbread production. Unleavened flatbread was prepared according to Başman and Köksel (2001). To control unleavened flatbread production, wheat flour (200 g), salt (3 g) and water were used. In other leavened and unleavened flatbread formulations, wheat flour was replaced with GMF at 5, 10, 15 and 20% ratios.

Color measurement

The color measurements of flatbreads were performed using a chromometer Minolta CR-400 (Minolta Camera, Co., Ltd., Osaka, Japan). Parameters L*, a* and b* determine a three-dimensional color space, in which L* indicates lightness (100 = white; 0 = black), a* values determine the redness (+) and greenness (–), and b* values determine yellowness (+) and blueness (–). Hue angle (arctan (b*/a*)) and SI value ($(a^{*2}+b^{*2})^{1/2}$) was calculated.

Technologic properties

The diameter and thickness of leavened flatbread and unleavened flatbread samples were determined according to Yıldız and Bilgiçli (2012). The spread ratio values of samples were found by dividing the diameter to the thickness value of flatbreads.

Antioxidant activity and phenolic content

The antioxidant activity of leavened flatbread and unleavened flatbread samples was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay (Gyamfi et al., 1999; Beta et al., 2005), ferric reducing antioxidant power assay (FRAP) (Yılmaz, 2019) and cupric ion reducing antioxidant activity assay (CUPRAC) (Apak et al., 2008). The free and bound phenolic content was determined based on the Folin-Ciocalteu colorimetric method as described by Naczk and Shahidi (2004). Total phenolic content was calculated as the sum of free and bound phenolic content. Phenolic content was expressed as gallic acid equivalents (mg of GAE/100 kg).

Statistical analysis

Leavened and unleavened flatbread results were analyzed separately. SPSS statistical program version 22.0 (SAS Institute Inc., Cary, NC, USA) was used for statistical data analysis. Mean values were compared with Duncan's multiple range test.

RESULTS AND DISCUSSION

Color values of leavened and unleavened flatbreads

Color values of leavened and unleavened flatbread containing different ratios of GMF are shown in Table 1. Increasing usage ratios of GMF decreased the L* value of both bread types. For leavened flatbread, a* value increased in all GMF usage ratios, but for unleavened flatbreads, 10% or more GMF usage increased a* value. The reason for the decrease in lightness of the flatbread surface is probably

due to the Maillard reaction. Increasing amylase and protease enzyme activity with germination causes the Maillard reaction by increasing the amount of free sugar and amino acids (Goesaert et al., 2009).

Table 1. Color values of leavened and unleavened flatbread containing different ratios of GMF

Leavened flatbread					
GMF (%)	**L***	**a***	**b***	**SI**	**Hue**
0	79.01±0.31a	-4.95±0.05e	19.10±0.59c	104.52±0.29a	19.73±0.58c
5	74.19±0.12b	-4.22±0.13d	21.91±0.69b	100.92±0.65b	22.31±0.65b
10	72.99±0.99bc	-3.35±0.00c	22.45±0.38b	98.48±0.13c	22.70±0.37b
15	71.82±0.13c	-3.06±0.10b	24.57±0.80a	97.15±1.41cd	24.76±0.17a
20	69.37±0.43d	-2.77±0.17a	24.97±0.86a	96.31±0.24d	25.13±1.02a
Unleavened flatbread					
GMF (%)	**L***	**a***	**b***	**SI**	**Hue**
0	77.89±0.24a	-4.41±0.20d	21.05±0.12c	101.88±0.51a	21.51±0.17c
5	75.76±0.05b	-3.84±0.28cd	23.84±0.12b	99.14±0.63b	24.15±0.16b
10	70.44±0.75c	-3.25±0.57bc	24.07±0.01b	97.70±1.31bc	24.30±0.07b
15	67.29±0.55d	-2.40±0.63b	24.78±1.37ab	95.51±1.11c	24.90±1.42ab
20	65.86±0.16e	-1.20±0.24a	26.41±1.17a	92.57±0.44d	26.46±1.16a

Means followed by the different letters within a column are significantly ($p < 0.05$) different. GMF: Germinated millet flour.

Marti et al. (2017) reported that the use of germinated wheat flour increased the crumb a*, crust a* and b* values but decreased the bread crumb and crust L* values. The use of GMF in both bread formulations increased the yellowness value and the highest values were reached with the use of 15-20% GMF. The high carotenoid pigment content of millet affected the b* value of flatbreads. While the SI values of the bread decreased with the use of GMF, the Hue values increased.

Technologic properties of leavened and unleavened flatbreads

The diameter, thickness and spread ratio values of leavened and unleavened flatbread containing different ratios of GMF are given in Table 2. The diameter of the leavened flatbreads ranged from 17.32 to 18.28 cm and there was a slight increase in the diameter value with the use of GMF. The diameter value of bread using only 20% GMF was significantly ($p<0.05$) higher than the control bread.

Table 2. Technologic properties of leavened and unleavened flatbread containing different ratios of GMF

Leavened flatbread			
GMF (%)	**Diameter (cm)**	**Thickness (cm)**	**Spread ratio**
0	17.32±0.16b	1.63±0.65a	10.63±0.05c
5	17.64±0.34ab	1.47±0.71ab	12.00±0.08bc
10	17.82±0.11ab	1.46±1.24ab	12.21±0.09bc
15	18.00±0.57ab	1.27±1.24bc	14.17±0.10ab
20	18.28±.11a	1.18±1.33c	15.49±0.18a
Unleavened flatbread			
GMF (%)	**Diameter (cm)**	**Thickness (cm)**	**Spread ratio**
0	24.48±0.45c	0.16±0.17a	154.94±2.12e
5	26.34±0.48c	0.12±0.20b	227.07±1.52d
10	30.28±0.68b	0.10±0.03bc	296.86±0.11c
15	31.62±0.31b	0.08±0.03c	405.38±1.94b
20	34.92±2.15a	0.07±0.06c	471.89±0.39a

Means followed by the different letters within a column are significantly ($p < 0.05$) different. GMF: Germinated millet flour.

In unleavened flatbreads, the diameter value increased with the use of 10% or more GMF. The use of 15-20% GMF in leavened flatbreads decreased the thickness value; on the other hand, all usage ratios of GMF decreased the thickness of unleavened flatbreads compared to control. High GMF usage ratios increased the spread ratio in leavened flatbreads and all GMF usage ratios in unleavened breads. Diluting gluten content with GMF addition may cause a decrease in thickness. There are numerous studies in the literature about decreasing the volume or thickness of breads with the substitution of non-gluten flours or bran (Sidhu et al., 2001; Gómez et al., 2012; Levent and Bilgiçli 2012a; Levent and Bilgiçli 2012b).

Antioxidant activities of leavened and unleavened flatbreads

Antioxidant activities leavened and unleavened bread containing different ratios of GMF are presented in Table 5. DPPH, FRAP and CUPRAC antioxidant activity values for leavened bread ranged between 181.77-344.93 mg TE/kg, 0.54-1.39 µmol TE/g and 3.61-8.34 µmol TE/g, and for unleavened bread changed between 152.89-300.91 mg TE/kg, 0.33-1.35 µmol TE/g and 2.91-6.73 µmol TE/g, respectively. With the increasing use of GMF in leavened flatbreads, DPPH, FRAP and CUPRAC antioxidant activity values increased. In unleavened flatbreads, DPPH and CURAC values increased with increasing GMF ratio, while FRAP value increased with 10% or more GMF use. The highest antioxidant activity values were achieved with the use of 20% GMF in both bread types.

Table 5. Antioxidant activities leavened and unleavened flatbread containing different ratios of GMF

Leavened flatbread			
GMF (%)	**DPHH (mg TE/kg)**	**FRAP (µmol TE/g)**	**CUPRAC (µmol TE/g)**
0	181.77±1.39e	0.54±0.05e	3.61±0.05e
5	204.21±1.29d	0.67±0.02d	4.10±0.04d
10	232.13±0.00c	0.88±0.01c	5.20±0.14c
15	292.33±2.97b	1.02±0.02b	6.56±0.002b
20	344.93±1.40a	1.39±0.04a	8.34±0.27a
Unleavened flatbread			
GMF (%)	**DPHH (mg TE/kg)**	**FRAP (µmol TE/g)**	**CUPRAC (µmol TE/g)**
0	152.89±0.87e	0.33±0.08d	2.91±0.05e
5	187.20±1.45d	0.42±0.01d	3.74±0.05d
10	224.87±1.52c	0.84±0.05c	4.15±±0.06c
15	240.18±1.38b	0.96±0.02b	5.03±0.02b
20	300.91±0.48a	1.35±0.01a	6.73±0.12a

Means followed by the different letters within a column are significantly ($p < 0.05$) different. DPPH: 2,2-diphenyl-1picrylhydrazyl radical scavenging (TE: Trolox equivalent). FRAP: Ferric reducing antioxidant power CUPRAC: Cupric ion reducing antioxidant capacity.

Phenolic contents of leavened and unleavened flatbreads

Phenolic contents of leavened and unleavened bread containing different ratios of GMF are given in Table 4. Bound, free and total phenolic content of leavened flatbread increased up to 2522.22 mg GAE/kg, 5250.83 mg GAE/kg and 7773.04 mg GAE/kg with GMF usage. All addition levels of GMF increased the bound, free and total phenolic content of leavened flatbread. Bound, free and total phenolic content of unleavened flatbreads increased with the use of GMF as in leavened flatbreads, and the highest values were reached with the use of 20% GMF.

There are many studies reporting an increase in antioxidant activity and phenolic content in cereals and legumes with germination (Demir and Bilgiçli, 2021; Alvarez-Jubete et al., 2010; Žilić et al., 2014; Swieca and Dziki, 2015; Cankurtaran-Kömürcü, 2021). Sharma et al. (2015) determined that the total phenolic content (free/bound) increased significantly with the germination of foxtail millet. Cankurtaran-Kömürcü, (2021) used germinated modern and primitive wheat in different ratios to produce bread (0, 5, 10, 15 and 20%) and noodles (0, 15, 30, 45 and 60%). It has been reported that germinated wheat significantly increased the antioxidant activity and phenolic content of bread and

noodles. Demir and Bilgiçli (2021) used germinated quinoa flour at the rates of 0, 10, 20 and 30% in gluten-free pasta production and found that the antioxidant activity and phenolic content increased with increasing germinated quinoa flour.

Table 5. Phenolic contents of leavened and unleavened flatbread containing different ratios of GMF

Leavened flatbread			
GMF (%)	**BPC (mg GAE/kg)**	**FPC (mg GAE/kg)**	**TPC (mg GAE/kg)**
0	2095.60±20.86d	4167.68±16.22d	6263.28±37.08e
5	2264.83±12.87c	4523.44±26.27c	6788.27±13.40d
10	2336.61±6.52bc	4683.43±83.63c	7020.04±77.16c
15	2404.78±31.23b	4912.21±29.15b	7316.48±2.08b
20	2522.22±13.99a	5250.83±16.33a	7773.04±30.32a
Unleavened bread			
GMF (%)	**BPC (mg GAE/kg)**	**FPC (mg GAE/kg)**	**TPC (mg GAE/kg)**
0	2042.01±6.85e	4078.20±35.99d	6120.20±42.84e
5	2194.54±10.59d	4486.62±12.35c	6681.15±1.76d
10	2280.36±7.15c	4698.82±37.56b	6979.17±30.40c
15	2357.65±13.49b	4758.87±7.87b	7116.52±5.62b
20	2425.90±10.99a	5159.43±4.27a	7585.33±15.26a

Means followed by the different letters within a column are significantly ($p < 0.05$) different. BFC: Bound phenolic content, FPC: Free phenolic content TPC: Total phenolic content (GAE, gallic acid equivalent).

CONCLUSIONS

In this study, the effects of GMF on the technological properties and bioactive components of leavened and unleavened flatbreads were examined. The findings were evaluated separately for leavened and unleavened flat breads. While the increase in the use of GMF decreased the L* and SI values of the bread, it increased the a*, b* and Hue values. While higher a* and b* color values were determined in unleavened bread samples compared to leavened breads, lower L* values were generally measured. Especially high utilization ratios of GMF increased the spread ratio values of the both breads. As predicted, unleavened flatbreads were determined as breads with higher diameter and spread ratio than leavened flatbreads. The use of GMF in flatbread production significantly ($p<0.05$) increased DPPH, FRAP and CUPRAC antioxidant activity values. The highest antioxidant activity values were obtained with the use of 20% GMF. In addition, it was determined that the amount of free, bound and total phenolic content increased in both bread types with the use of GMF, and these values reached the highest amounts at the highest use of GMF. Utilization of 20% GMF resulted in the greatest redness, yellowness, diameter, spread ratio value and bioactive components of the breads.

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OPTIMISATION OF THE CHEMICAL COMPOSTING OF CATTLE MANURE BY MEANS OF NITRIC ACID

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ABSTRACT

The composting of cattle manure is a crucial process for transforming this organic waste into a soil amendment that is rich in nutrients. This practice plays an instrumental role in reducing the amount of waste sent to landfills and in mitigating greenhouse gas emissions. This environmentally friendly method sustainably enriches soils, which improves crop quality and plant health. There are two main types of composting: biological and chemical. While beneficial, biological composting is slow and requires rigorous management of environmental conditions to optimize decomposition by microorganisms. In contrast, chemical composting is faster and more economical, where chemical additives are used to accelerate decomposition. In this study we investigated a chemical method using nitric acid to oxidize cattle manure. The excess acid was neutralized with potassium hydroxide, leaving harmless residues such as potassium and nitrates, which are also powerful fertilizers. Scanning electron microscopy and energy dispersive X-ray spectroscopy (SEM-EDX) analyses showed that the concentration of nitric acid had a significant effect on compost quality. The results show a decrease in carbon concentration and an increase in nitrogen and potassium concentrations with higher nitric acid concentrations used in the composting process, illustrating the oxidation of organic matter and the incorporation of nitrogen and potassium into the compost. The C/N ratio, which is crucial for decomposition and plant nutrition, is optimal within a range of nitric acid concentrations from 0.05 Nto 1.00 N, facilitating the production of high-quality compost. The texture of the samples analyzed by SEM also varies with nitric acid concentration, with higher and more uniform degradation of organic matter and a finer texture with improved porosity.

Keywords: Cattle Manure Composting, Chemical Composting, SEM-EDX, Soil Amendments, Fertilizer Production.

INTRODUCTION

Cattle manure composting is a vital process for efficiently converting it into a highly beneficial, nutrient-rich soil amendment (Larney et al., 2006). By reducing the amount of manure sent to landfills and greenhouse gas emissions, this practice promotes the recycling of organic materials and contributes to environmental preservation. The compost produced can be used to enrich soils in an ecological and economical manner, thereby improving crop quality and plant health.

Cattle manure composting can be achieved either biologically or chemically. Biological composting relies on the action of natural microorganisms, such as bacteria and fungi, which decompose the manure in the presence of oxygen. This aerobic process produces nutrient-rich compost that benefits soil structure and fertility. However, biological composting has some drawbacks. It is much slower than chemical composting, requiring careful management of temperature, humidity, and aeration conditions to optimize the process. Additionally, due to handling and exposure to organic materials, there is a potential risk of pathogen spread, including viruses such as COVID-19, though this risk can be mitigated by proper composting practices and health safety measures (Zeqiri &Biçoku, 2021).

In contrast, chemical oxidation uses chemical additives to accelerate manure decomposition (Jensen et al., 2013). In this case, care must be taken to ensure that the chemicals used are not hazardous and that chemical residues in the compost do not affect its quality and environmental impact. Chemical composting is significantly more economical than biological composting.
In our study, aiming to replace the traditional composting process, we conducted the chemical oxidation of cattle manure using nitric acid. The excess nitric acid at the end of the process is neutralized using potassium hydroxide. The remaining residues of this process are potassium and nitrates, which are not only harmless but also constitute potent fertilizers, thereby enriching our compost.

MATERIALS AND METHODS

Scanning electron microscopy and elemental analyses

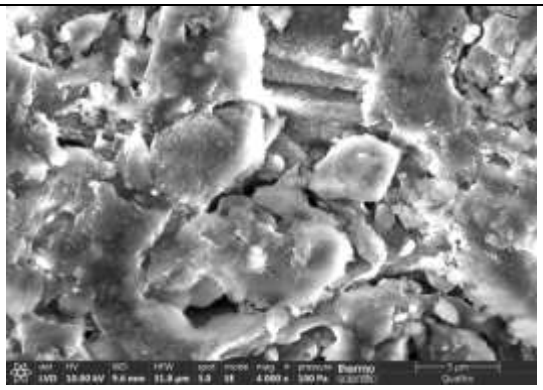
Scanning electron microscopy (SEM) images and elemental analyses were performed using a Thermo Fisher Scientific Helios G4 UC system, equipped with Energy Dispersive X-ray Spectroscopy (EDX). The EDX analysis was conducted at an accelerating voltage of 20 kV and a beam current of 1.6 nA, with a spectrum acquisition time of 60 seconds.

Compost Preparation

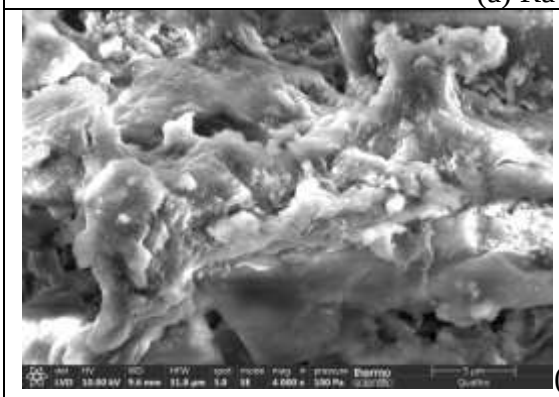
A fixed amount of collected manure is weighed before and after drying to determine the moisture content. Once this step is completed, the manure is ground using a ball mill to obtain a homogeneous texture. Particles smaller than 250 micrometers are recovered by sieving and then stored dry in bags for later use. Three grams of this dried and ground manure are mixed with 15 ml of nitric acid (at concentrations of 1 M, 0.5 M, 0.1 M, 0.05 M, and 14 M), until a paste is formed. Finally, the paste's pH is adjusted using a potassium hydroxide solution to achieve a neutral pH. The resulting product is dried for characterization.

RESULTS

The pH of the compost obtained for samples treated with nitric acid showed an acidic pH, ranging from 3 to 5 depending on the acid concentration used before neutralization with KOH. After neutralization, the pH was adjusted between 6.5 and 7.5. The texture of the obtained samples appears to depend on the concentration of nitric acid used during their preparation. Higher concentrations of nitric acid result in more uniform degradation of organic matter, leading to a finer texture with improved porosity.
The texture of the prepared samples is shown in Figure 1. Image (a) presents raw cattle manure at a magnification of x4000. Images (b), (c), (d), and (e), also at x4000 magnification, show cattle manure samples treated with nitric acid at concentrations of 0.05 M, 0.5 M, 1 M, and 14 M, respectively.

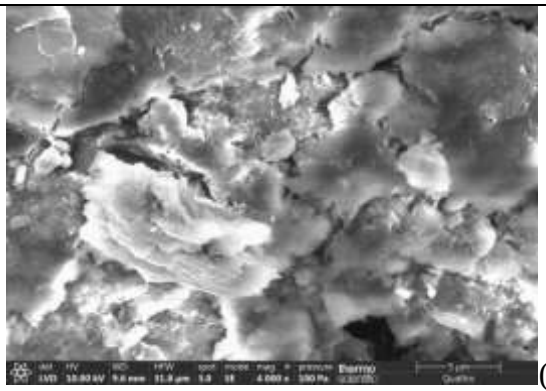


(a) Raw cattle manure



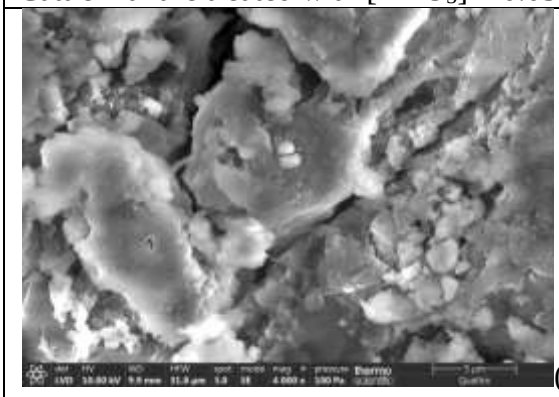
(b)

Cattle manure treated with [HNO_3] = 0.05 M



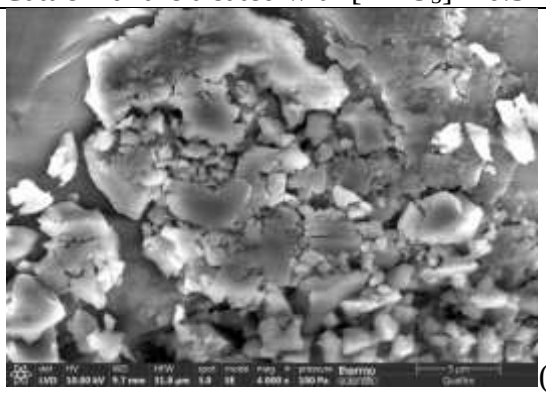
(c)

Cattle manure treated with [HNO_3] = 0.5 M



(d)

Cattle manure treated with [HNO_3] = 1 M



(e)

Cattle manure treated with [HNO_3] = 14 M

Figure 1 SEM Images of (a) Raw cattle manure, (b) Cattle manure treated with [HNO3] = 0.05 M, (d) Cattle manure treated with [HNO3] = 1 M and (e) Cattle manure treated with [HNO3] = 14 M

SEM-EDX is often used for the characterization analysis of composts (Manohara et al., 2017, Diquattro et al., 2018,Khare et al., 2023). The images show the presence of two types of structures in each sample. The first consists of particles of varying sizes and shapes, which may be dispersed or clustered. The images also demonstrate the formation of particle aggregates, which are sets of interconnected particles, indicating the presence of organic matter.

The quality of the obtained product was characterized by scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy (SEM-EDX). This technique was used to study the shape and structure of the compost samples as well as their chemical composition. The effect of nitric acid concentration on the quality of the obtained compost was the first parameter we studied. For this purpose, we used the following concentrations: 0.05 M, 0.5 M, 1 M, and 14 M.

EDX analysis allowed us to identify the elements present in the compost, and the results are presented in Table 1. We focused particularly on the following elements: C, N, O, Al, Si, P, K, and Ca, which compose the compost. Special attention was given to C and N as they are key indicators of compost quality.

Table 1: EDX Analysis Results of raw cattle manure, cattle manure treated with [HNO_3] = 0.05 M, cattle manure treated with [HNO_3] = 0.5 M, cattle manure treated with [HNO_3] = 1 Mand, cattle manure treated with [HNO_3] = 14 M

Table 1: EDX analysis results of raw cattle manure and cattle manure treated with [HNO_3] at concentrations of 0.05 M, 0.5 M, 1 M, and 14 M.

Element (%)	Raw cattle manure	[HNO_3] = 0.05 M	[HNO_3] = 0.5 M	[HNO_3] = 1 M	[HNO_3] = 14 M
C	40.21	43.87	36.68	38.30	8.51
N	0.08	4.97	6.47	5.63	10.07
O	42.67	41.89	39.92	45.94	39.16
Al	2.28	1.09	1.60	1.32	1.24
Si	5.42	1.05	1.65	0.96	0.18
P	0.77	1.37	2.24	0.78	0.12
K	1.01	1.22	7.62	2.04	39.67
Ca	7.57	3.67	2.31	3.04	0.39
C/N	502,63	8,83	5,67	6,80	0,85

The results show a decrease in carbon concentration with increasing nitric acid concentration during treatment, a consequence of the oxidation of organic matter. Nitrogen concentration in the compost increases in parallel with the nitric acid concentration used, which is expected as nitrogen comes from the acid. Additionally, potassium concentration in the compost increases with higher nitric acid concentrations because more potassium hydroxide is needed to neutralize the compost when the acid concentration is high. These observations highlight the significant impact of nitric acid concentration on the final chemical composition of the compost.

The C/N ratio decreases with the increase in nitric acid concentration used during oxidation. It remains favorable within a broad range of nitric acid concentrations from 0.05 N to 1.00 N.

Maintaining an optimal C/N ratio is essential for supporting decomposition and providing the necessary nutrients to plants after compost application (Azim et al., 2018). Achieving an optimal result over a wide range of nitric acid concentrations reduces manufacturing constraints.

CONCLUSION

This study highlights the effectiveness of chemical oxidation with nitric acid for cattle manure composting, demonstrating its potential to produce high-quality compost quickly and economically. Using nitric acid to oxidize manure, followed by neutralization with potassium hydroxide, not only accelerates the decomposition of organic matter but also enriches the compost with essential nutrients such as nitrogen and potassium. SEM and EDX analyses revealed that nitric acid concentration plays a crucial role in the final compost quality, influencing both its chemical composition and texture. In particular, the C/N ratio, a key indicator of compost quality, was found to be optimal within a range of nitric acid concentrations from 0.05 N to 1.00 N. This flexibility in nitric acid concentrations facilitates the production of high-quality compost without requiring extremely precise control of composting conditions. Chemical oxidation with nitric acid represents a promising method for valorizing cattle manure, providing a practical and eco-friendly solution for agricultural waste management and soil improvement.

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NEW DATA ON DISTRIBUTION OF WULFENIA BALDACCII DEGEN

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ABSTRACT

Wulfenia baldaccii is an Albanian endemic and a Tertiary relict species, currently listed as Vulnerable (VU) on the Albanian Red List (2013). Its distribution is limited to a few sites in the western Albanian Alps, mainly on north-facing limestone grasslands at 900–2200 m a.s.l. We report a newly discovered population at Syri i Kaltër, Theth (600 m a.s.l.), representing the lowest known occurrence of the species. The plants were found on humid limestone walls, predominantly covered by mosses and frequently associated with *Ramonda serbica*, *Pinguicula hirtiflora*, *Parnassia palustris* and the liverwort *Marchantia polymorpha*. Its presence in permanently humid microhabitats of a major karstic spring highlights the site's exceptional ecological value, particularly for conserving Tertiary relict flora that are highly vulnerable to climate change and drought. Yet Syri i Kaltër is a popular tourist site, where increasing human activity threatens this vulnerable species. This finding underscores the need for targeted conservation measures and careful management of natural monuments hosting rare plants.
Keywords: *Wulfenia baldaccii*; Tertiary relict flora; Albanian Alps; karstic spring; conservation.

INTRODUCTION

The genus *Wulfenia* (Plantaginaceae) exhibits a remarkable disjunct distribution, occurring in the southeastern Alps (Carnic Alps), the southeastern Dinaric Alps, and the Amanos Mountains of southern Turkey. It is widely regarded as a Tertiary relict in southeastern Europe, with origins dating back to at least the Miocene. Except for the nominate variety of *W. orientalis*, all *Wulfenia* species are confined to montane or subalpine habitats. Growth in the genus is nearly continuous, interrupted only briefly by winter frost. All species require high light levels and depend on moist soils and humid air (Surina et al., 2014).

In Albania, two species occur: *W. carinthiaca*, native to the southeastern Alps and extending into northeastern Albania and northwestern North Macedonia, and *W. baldaccii*, a narrow endemic restricted to the Albanian Alps (Degen, 1897). According to Barina et al. (2017), *W. baldaccii* is restricted to the western Albanian Alps, where it occurs in a few localities on limestone substrates, usually in north-facing mountain grasslands at 1400–2200 m a.s.l. Herbarium records from the National Herbarium of Albania document the species at 14 sites (Tab. 1), mostly in the Shkodër and Malësi e Madhe districts, at 900–2100 m a.s.l. An early report by Lemperg from Ura e Djallit (Kukës) has not been confirmed in later surveys.

Table 1. Localities of *Wulfenia baldaccii* recorded in the National Herbarium of Albania.

No.	Date	Locality	District	Phytoclimatic zone/ Elevation	Collector
1.	22.07.2000	Gropa e Shtrelit(Plan-Shkodër)	Shkodër	I	Kashta
2.	-	Gura e Strashës	Malësi e Madhe	III (1000 m)	Baldacci
3.	-	Parun	Shkodër	I	Baldacci
4.	-	Plan (Shkodër)	Shkodër	II	Baldacci
5.	-	Qafa e Shtogut (Shkrel)	Malësi e Madhe	III-IV (1600-2100 m)	Hayek
6.	22.07.2000	Qafa e Shtogut (Shkrel)	Malësi e Madhe	III-IV (1600-2100 m)	Kashta
7.	-	Qafa e Shtogut (Shkrel)	Malësi e Madhe	III-IV (1600-2100 m)	Markgraf
8.	-	Raba e Bogës	Malësi e Madhe	III	Lemperg
9.	-	Rrjoll	Shkodër	II	Baldacci
10.	-	Shtegu i Dhënve (Theth)	Malësi e Madhe	III (1500-1780 m)	Baldacci
11.	19.06.2003	Shtegu i Dhënve (Theth)	Malësi e Madhe	III (1800 m)	Kashta
12.	01.07.1978	Shtegu i Dhënve (Theth)	Malësi e Madhe	III (1600 m)	Vangjeli, Tartari
13.	-	Tarabosh i Bzhetës (Parun)	Shkodër	III (900 m)	Schütt
14.	-	Ura e Djallit	Kukës	II	Lemperg

Tertiary relict species such as *W. baldaccii* are among the most vulnerable plant groups under climate change, particularly in Mediterranean and montane ecosystems where increasing aridity and warming are projected. Due to their narrow ecological tolerance, these species are highly sensitive to changes in temperature and moisture. Documenting the distribution and ecological conditions of *W. baldaccii* populations is therefore essential to provide baseline data for effective management and conservation strategies.

METHODOLOGY

Fieldwork at Syri i Kaltër was carried out within the project *Achieving Biodiversity Conservation through Effective Management and Enhanced Resilience to Climate Change in the Existing Protected Area of the North Albanian Mountainous Region*, implemented by the Urban Research Institute (URI) and funded by UNEP.

New localities of *Wulfenia baldaccii* were georeferenced with a Garmin Dakota 10 GPS. Ecological parameters were recorded, including substrate type, slope exposure, associated plant species, and population size. Both actual and potential threats to the species were also assessed.

Taxonomic identification followed Illustrated Flora of Albania (Pils, 2016) and Flora europaea (Tutin et al., 1964–1980). Distribution data were compiled from the Atlas of Vascular Plants of Albania (Barina et al., 2017) and from the register of the National Herbarium of Albania at the Research Center for Flora and Fauna, University of Tirana.

RESULTS AND DISCUSSIONS

We report a new locality of *W. baldaccii* at Syri i Kaltër, Theth (Albanian Alps; Fig. 1) at ~600 m a.s.l., the lowest altitude recorded for the species. Although this site lies relatively near previously known populations, the species' overall distribution remains highly restricted. Ecological conditions at this site may be especially favorable: the permanently humid environment supplies the soil and atmospheric moisture required by *W. baldaccii* as other Tertiary relicts.

Figure 1. Distribution of *Wulfenia baldaccii* in Albania (after Barina et al., 2017). The red dot marks the newly recorded locality at Syri i Kaltër, Theth.

The surveyed population occurs on shaded, humid limestone walls (Fig. 2), predominantly covered by the moss *Brachythecium* sp. Associated species include the relict *Ramonda serbica* and hygrophilous taxa such as *Pinguicula hirtiflora*, *Parnassia palustris*, *Cystopteris fragilis*, *Marchantia polymorpha*, and the orchid *Dactylorhiza saccifera*. Valley slopes support broad-leaved forests dominated by *Ostrya carpinifolia*, *Carpinus orientalis*, *Acer pseudoplatanus*, and *Acer obtusatum*, whereas upper slopes are characterized by beech (*Fagus sylvatica*) forests. The population size is estimated at 50–70 mature individuals.

Phytosociologically, the assemblage belongs to the alliance **Ramondion serbicae** (Blečić & Lakšić, 1970), comprising paleoendemic, hygrophilous chasmophytic communities on shaded calcareous cliffs of the Balkan Peninsula. The habitat's ecological specificity and the presence of glacial relicts underscore its conservation value.

The occurrence of *W. baldaccii* in permanently humid microhabitats of a major karstic spring highlights the site's exceptional ecological value, particularly for conserving Tertiary relict flora that depend on consistently high humidity and are highly vulnerable to climate change and increasing drought.

However, Syri i Kaltër is a popular tourist destination, and increasing human activity poses significant threats to this vulnerable species. The creation of informal paths, use of rock slopes as diving platforms, and similar disturbances may directly impact this rare endemic species. Furthermore, promoting the species as a tourist attraction could present an additional risk, as visitors may be tempted to collect flowers or even attempt to transplant individuals into private gardens. These findings underscore the urgent need for targeted conservation measures and careful management of natural monuments that harbor rare plant populations.

Figure 2. (1) Syri i Kaltër; (2) humid limestone walls with hygrophilous vegetation; (3) *W. baldaccii* with plant species and mosses; (4) W. baldaccii in fructification. *Photos by. M. Meço, Jul. 2025*

CONCLUSIONS

The discovery of *W. baldaccii* at Syri i Kaltër, Theth, extends the known distribution of this Albanian endemic to its lowest recorded altitude and identifies a particularly favorable habitat, characterized by the high moisture levels essential for the species' survival. The coexistence of *W. baldaccii* with other Tertiary relict such as *Ramonda serbica* and other hygrophilous taxa emphasizes the exceptional ecological and phytogeographical significance of this site. Despite its favorable ecological conditions, the population remains very small and highly vulnerable to anthropogenic pressures, particularly tourism-related disturbance. These findings underscore the urgent need for targeted conservation actions, including habitat protection, monitoring, and visitor management, to ensure the long-term survival of *W. baldaccii* and the unique relict flora of the Albanian Alps.

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SOCIO-ECONOMIC IMPACTS OF REDUCING AIR PARTICULATE MATTER (PM10) POLLUTION IN KOCAELI USING BENMAP-CE MODEL

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ABSTRACT

Air pollution has long been a significant factor affecting both human and environmental health. With increasing human population and industrialization, various pollutants have been released into the atmosphere. Among these, particulate matter is one of the most critical pollutants and poses a serious threat to living organisms. In particular, it contributes to various health problems, such as respiratory and cardiovascular diseases. Long-term exposure can lead to workforce loss and, in severe cases, even death. Each year, deaths related to air pollution also have a negative impact on national economies. To address these challenges and minimize potential harm, researchers have developed various approaches and models. One such tool is the Benefits Mapping and Analysis Program – Community Edition (BenMAP-CE), developed by the U.S. Environmental Protection Agency (EPA). BenMAP-CE enables health and economic risk assessments based on regional data. It utilizes air quality data, along with mortality, hospital admission, and economic metrics, to estimate improvements in quality of life and potential economic benefits resulting from better air quality. In this study, air quality monitoring data and WRF-Chem model simulations of particulate matter concentrations were used for the Kocaeli region. While the Kocaeli region is one of Türkiye's most industrialized and densely populated areas, it is also exposed to severe air pollution. Therefore, poor air quality can lead to serious health problems and even death. Cause-specific mortality data related to cardiovascular, respiratory, and other diseases were incorporated, and the BenMAP-CE model was applied to assess potential improvements in quality of life and associated economic gains under a scenario of a 50% reduction in particulate matter concentrations. The results estimate that more than one hundred deaths from cardiovascular and respiratory causes could be prevented, and the economic benefits could exceed one billion dollars. As a result of the Kocaeli districts, Izmit, Gebze and Darica within the Kocaeli province demonstrated the highest potential for benefit, whereas Dilovasi yielded relatively lower benefit, a more limited impact compared to the other districts.

Keywords: Air pollution, PM10, BENMAP-CE, Health benefit, Economic benefit, Kocaeli, Türkiye.

INTRODUCTION

Air pollution remains one of the most important problems of the 21st century (Bridgman, 1991; Fang et al., 2013; Likhvar et al., 2015). As urbanization, industrialization, and population increase accelerate worldwide, the degradation of air quality has become an unavoidable serious health and environmental problem, especially in heavily populated and industrial areas. The World Health Organization (WHO) recognizes air pollution as a major public health risk, contributing significantly to the global burden of disease (McDuffie et al., 2021). Air pollution is caused by different pollutants, especially particulate matter (PM) is the most well-known pollutant due to its environmental and health impacts (Mukherjee and Agrawal, 2017; Zhao et al., 2013). Exposure to these airborne particles is

strongly linked to respiratory and cardiovascular diseases, premature mortality, and other health issues (Brook, 2008; Hoek et al., 2013; Lee et al., 2014; Shahriyari et al., 2022). Short-term exposure to particulate matter can cause acute health effects, including asthma attacks and hospital admissions for respiratory and cardiovascular complications, while long-term exposure may lead to premature death (Franchini and Mannucci, 2009). In addition, air pollution has substantial economic effects on countries. These include direct costs such as increased healthcare fees, as well as indirect costs related to productivity losses, reduced labor output, and premature deaths (Pandey et al., 2021). According to the Organisation for Economic Cooperation and Development (OECD), the global economic cost of air pollution caused by premature mortality alone might reach trillions of dollars per year if adequate control measures are not implemented (Lanzi, 2016). To analyze the health and economic impacts of air pollution, researchers and agencies have developed several methodologies and models. One of the most popular models is the Benefits Mapping and Analysis Program - Community Edition (BenMAP-CE). This model is designed to estimate the health benefits and related economic gains of air quality improvements, using data such as pollutant concentrations, population demographics, health incidence rates, and economic valuation metrics. BenMAP-CE gives comparative risk assessment by predicting avoided cases of disease and mortality under various pollution management scenarios.

The causes of air pollution can be examined as anthropogenic emissions and natural. The local air pollution is mainly affected by industrial areas, traffic, mining and residential heating. Kocaeli well know intense industrialization and high population density area. Studies conducted on health issues and air pollution have shown that pollutant concentrations in Kocaeli consistently exceed both national and international standards (Hamzaoglu et al., 2011; Unal, 2021). Therefore, Kocaeli region is important for the health risk. In the literature review of Kocaeli health risk from air pollution, Palanbek Yavaş et al. (2021) found that high levels of PM10 and NO2 in the Marmara and Kocaeli region, were linked to an increased risk of chronic bronchitis and mortality, highlighting air pollution as a serious public health issue. Öztürk et al. (2021) found that PM2.5 concentrations in Kocaeli's Izmit district reached significantly high levels in winter and often exceeding WHO and EPA limits, especially in traffic and urban areas, indicating a need for winter seasonal air quality controls and fossil fuel reduction to control health risks. Pekey et al. (2010) emphasize that indoor and outdoor PM2.5 and PM10 concentrations measured in 15 homes across Kocaeli were significantly higher in winter, with outdoor PM10 averaging 102.3 µg/m³, and that industrial emissions, vehicular traffic, and fossil fuel combustion were identified as major sources of pollution, while long-term exposure to these pollutants is believed to increase health risks. In this study, health and economic benefits of a 50% reduction in PM concentrations across the Koceli region were specifically investigated and analyzed.

MATERIAL AND METHOD

Kocaeli is one of the most crowded cities in Türkiye, hosting approximately 2.1 million residents as of 2023, and Izmit, the capital district of Kocaeli, covers an area of about 480 km² and is home to around 378,000 inhabitants (TURKSTAT, 2023a). The province is geographically connected to both the Marmara and Black Sea regions. Kocaeli consists of 12 districts: Izmit, Derince, Gebze, Golcuk, Kandira, Karamursel, Korfez, Basiskele, Cayirova, Darica, Dilovasi, and Kartepe. The city is mostly known for its industrial activities, including automotive, petrochemical, machinery, mining, metal, food, paper, cement, and tire production sectors (Sarper Erdogan et al., 2005). These industries have a significant effect on air pollution, releasing various pollutants into the atmosphere. Kocaeli is also a major transportation hub, intersected by key highways such as the D-100 and the Trans-European Motorway (TEM), which connect industrial areas with residential zones. Due to the traffic, industrial, and residential emissions, Kocaeli serves as a critical region for assessing the impacts of air pollution. All these factors negatively affected the air quality in the Kocaeli region, thereby posing a significant risk to public health (Hamzaoglu et al., 2011; Unal, 2021). The locations on the map of Kocaeli districts are given in Figure 1.

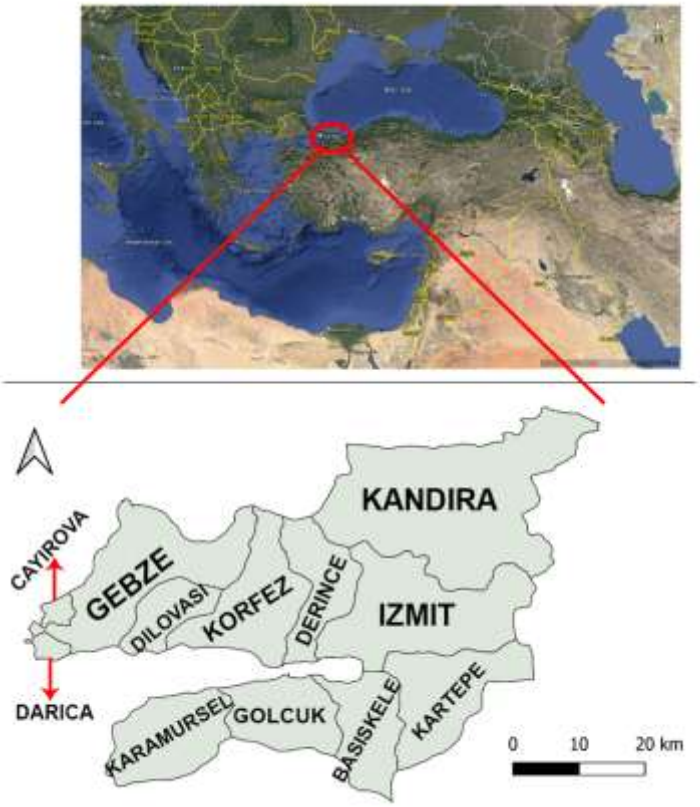
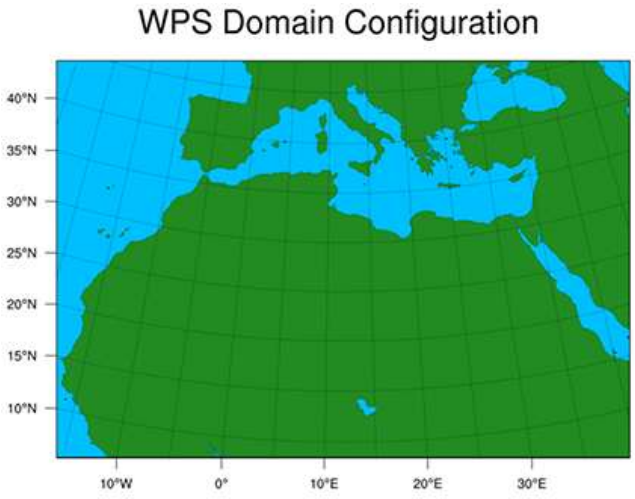


Figure 3. Study area and Kocaeli district locations

In this study, we used air quality monitoring station (AQMS) and WRF-Chem model data for the year 2023. This year was selected as the reference period because the most recent mortality dataset was available for this year. AQMS measurements were the main source of air quality data for the BenMAP-CE model. However, the model data is only used to identify missing values and to fill in missing stations in three districts: Yenikoy, Cayirova, and Kartepe. There are nine AQMS in the Kocaeli region, and each station measures different pollutants. PM10 data were obtained from all of these nine stations. In the lack of AQMS in Kocaeli districts, the closest station data were used, and missing values were predicted with Weather Research and Forecasting model coupled with Chemistry (WRF-Chem) simulations (Figure 2). WRF-Chem is a mesoscale model developed to simulate the distribution of air pollutants and is particularly known for its capability to predict atmospheric chemical reactions (Peckham et al., 2018; Sicard et al., 2021). Air pollution is known to cause various adverse effects on human health, particularly in relation to respiratory and cardiovascular diseases. Therefore, in order to predict health risk assessments, both mortality and hospital admissions data can be used with air quality data. In this case study, health impacts were estimated based on mortality data, which were obtained for each district within the Kocaeli region. Additionally, Kocaeli University Research and Application Hospital data were used to improve health incidence estimates for cardiovascular and respiratory diseases. In Türkiye, mortality data were obtained from the Turkish Statistical Institute (TURKSTAT), while hospital admission data were only provided by Kocaeli University Hospital (TURKSTAT, 2023b). Therefore, hospital administration data were used only to support the mortality data. The last dataset, includes the economic valuation of the country, specifically for Türkiye. The economic calculation method followed the user guide example from Santiago, as provided in the BenMAP-CE training materials. Various economic data sources are available globally, such as calculation methods, data sets and standards. Therefore, in this study, the World Bank's World Development Indicators were primarily used, and the necessary data were supported by the The Organisation for Economic Co-operation and Development (OECD) and The International Monetary Fund (IMF) (IMF, 2025; OECD, 2023; World Bank, 2025).



Physics and Chemistry options	Schemes
Microphysics	Thompson
Longwave radiation	RRTMG
Shortwave radiation	RRTMG
Surface layer	ETA
Planetary boundary layer	MYJ
Cumulus parametrization	Tiedke
Chem_opt	300
Dust_opt	4
Dust_schemes	1

Figure 4. The domain and namelist used in WRF-Chem model

BenMAP-CE is an open-source software tool developed by the United States Environmental Protection Agency (US EPA). The model was developed to estimate the health and economic impacts under scenarios of improving air quality or reducing air pollution. In this study, BenMAP-CE version 1.5.8 was selected as the health impact assessment tool. This version has been modified to enable the use of various pollutants and health impact functions across different scenarios to calculate health risks (Coffman et al., 2024). The model needs mortality data, population statistics, economic indicators, air quality data, hospital admissions in order to perform its calculations (US EPA, 2023). The BenMAP-CE model flow diagram can be shown in Figure 3.

The input datasets used in the model are listed below to provide a clearer understanding of the methodology. The model uses different parameters and methods that are designed for socio-economic calculations. As the calculation algorithm of BenMAP-CE, the model utilizes a specific health impact function, which is illustrated in Figure 4. In this study, the BenMAP-CE model was prepared by adapting the Santiago scenario provided in the official user guide training materials. Reference values required for the formulas, such as relative risk, odds ratio, and hazard ratio, were obtained from the example data in the training materials. We used cardiovascular, respiratory, and all-cause mortality data specific to the Kocaeli region for this case scenario. Additionally, prevalence data for cardiovascular and respiratory cases were collected from Kocaeli University Research Hospital. After that, air pollution reducing scenario needs to be chose for model. The final dataset required for the model is the economic data. Therefore, BenMAP-CE allows users to estimate the health and economic impacts based on the reduction in air pollution.

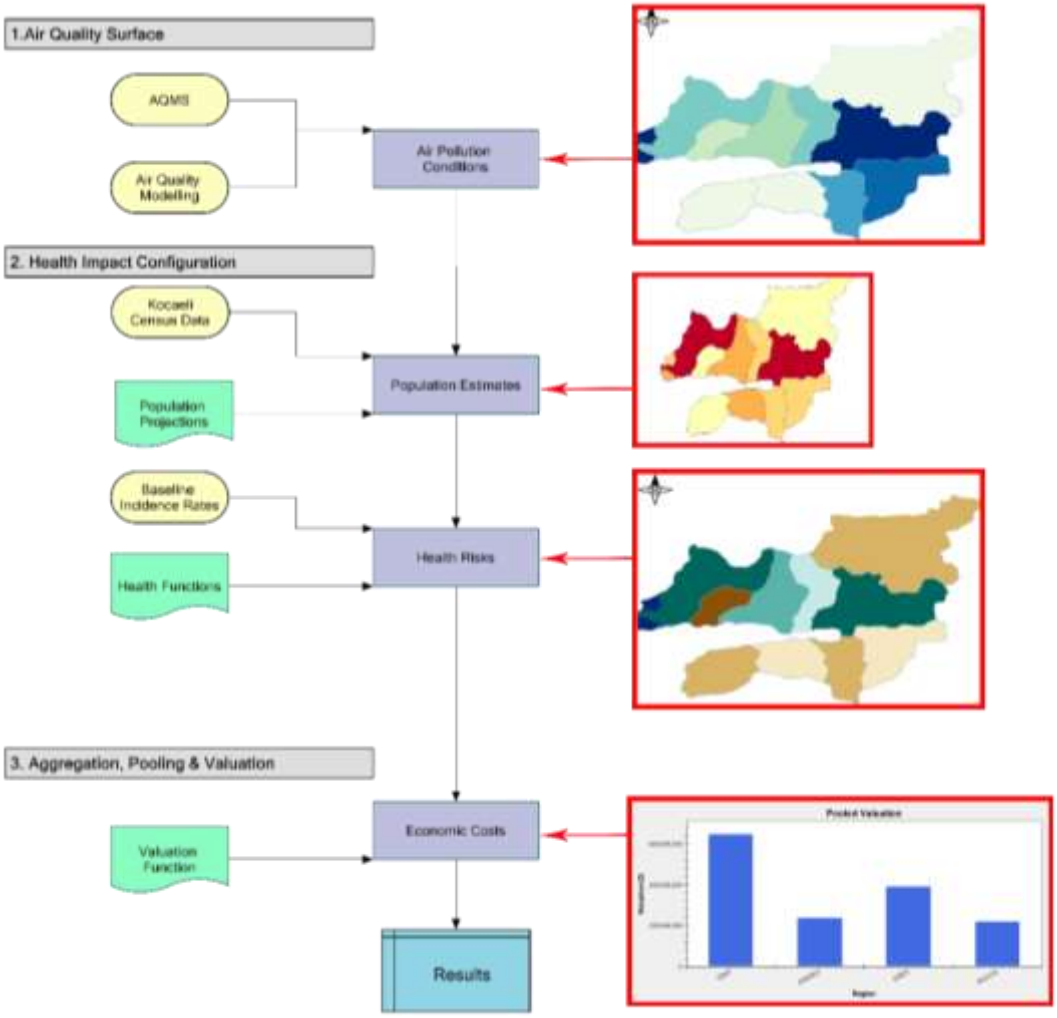


Figure 5. Inputs and outputs of BenMAP-CE model analysis

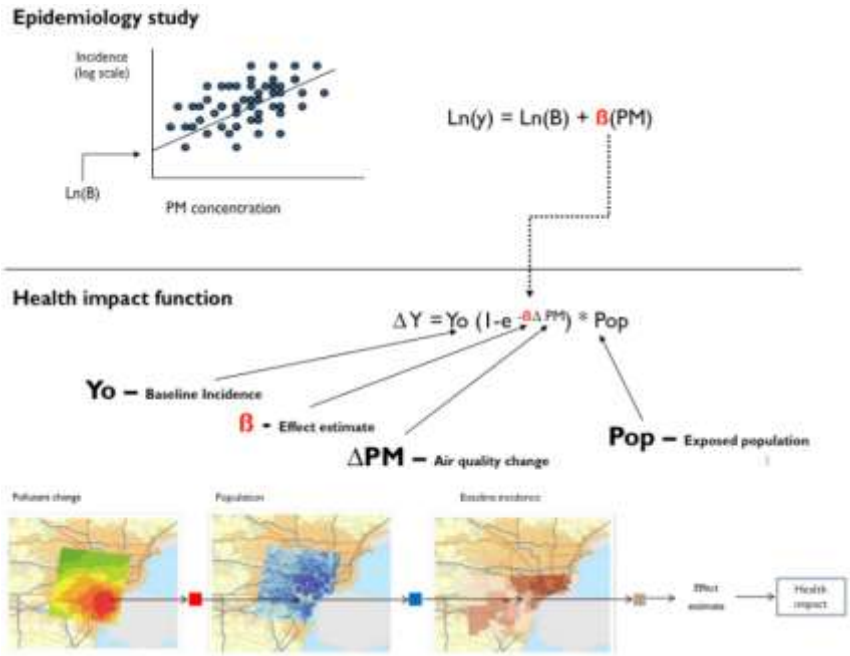


Figure 6. Health impact functions of BenMAP-CE (US EPA, 2023)

The BenMAP model allows for economic calculations to be performed using the preferred formulation method. In the case study, the valuation function employed follows the same methodological approach as in the Santiago case scenario. Because of this, 2010 was selected as the base year for data adaptation. The primary reason for choosing this year is the availability of a wide range of required data from the World Bank database. In summary, the U.S. dollar index for 2010 was used as the basis for calculating the economic outputs and economic valuations employed in this study for the year 2023. Assuming that ε, the income elasticity of the Value of a Statistical Life (VSL), the sensitivity of VSL to changes in income is constant both across countries (ε_1) and over time (ε_2), the equation simplifies to Equation (1), with Equation (2) representing the corresponding adjusted form.

$$VSL_{Türkiye,2023} = VSL_{US,1990} * \left(\frac{Y_{Türkiye,1990}}{Y_{US,1990}}\right)^{\varepsilon_1} * \left(\frac{Y_{Türkiye,2023}}{Y_{Türkiye,1990}}\right)^{\varepsilon_2} * PPP_{1990} * \frac{CPI_{Türkiye\ 2023}}{CPI_{Türkiye\ 1990}}$$

(1)

$$VSL_{Türkiye,2023} = VSL_{US,1990} * \left(\frac{Y_{Türkiye,2023}}{Y_{US,1990}}\right)^{\varepsilon} * PPP_{1990} * \frac{CPI_{Türkiye\ 2023}}{CPI_{Türkiye\ 1990}} \quad (2)$$

Most of the economic data required for the analysis were obtained from the World Bank database. However, the most recent data for GDP per capita (constant 2010 US$) were only available up to the year 2019. Therefore, the GDP per capita value for 2023 was estimated by projecting the missing years based on Türkiye's annual GDP growth rates published by the IMF. The economic parameters used in the model are presented in Table 1. In this study, Kocaeli districts were examined to highlight the potential health and economic benefits under a case scenario where PM10 concentrations are reduced by 50%.

Table 1. Economic data of Türkiye used in BenMAP-CE

Country	Year	VSL (Mean)	GDP PER Capita (Constant 2010 US $)	PPP (Turkish liras per ınternational $)	Consumer Price Index (2010 = 100)
United States	1990	$4,800,000	$36,312	-	-
Türkiye	1990	-	$6,774	0.0012	0.0716
	2023	Estimated by equation	$18,782	7.3503	834.5931

RESULTS AND DISCUSSION

The Kocaeli region shows variation in land area and population distribution. In addition, air pollution in Kocaeli can be caused by both local sources and the transport of pollutants from the other territories. In this study, air pollutant data were mostly obtained based on measurements from air quality monitoring stations. Air pollution levels reduction in Kocaeli show that PM10 varied the most in Izmit and Cayirova, ranging from 18.85 to 25.64 µg/m³ (Figure 5). Apart from Kandira, the other districts showed relatively similar average PM10 concentration levels. Gebze and Dilovasi, which are important districts known for their large populations and heavy industry, had PM10 levels ranging from 13.06 - 16.11 µg/m³.

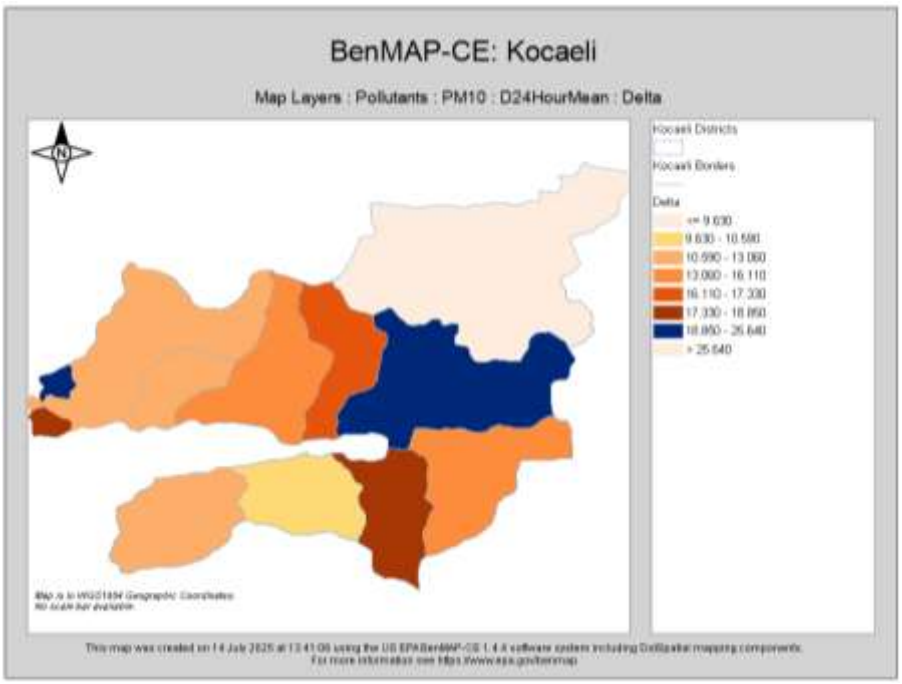


Figure 7. Air quality provincies map of Kocaeli

As a result of the model predictions, avoidable deaths due to cardiovascular and respiratory causes are showed in Figures 6 and 7. According to the data obtained from TURKSTAT, the number of deaths attributed to cardiovascular diseases is higher than those related to respiratory diseases. Therefore, BenMAP-CE predicted a higher reduction in deaths from cardiovascular disease compared to respiratory. When the overall mortality estimates (Figure 8) are examined, the most significant reduction in deaths observed in Izmit, Gebze, and Darica. In this study, based on the 2023 model data, the results show that the highest estimated avoidable mortality predicted Izmit, Gebze, and Darica. The main reason these three regions stand out is primarily due to PM pollutant. As shown in Figure 5, the case scenario of PM pollutant is reduced by 50%, the most significant decrease also occurs in these regions. Additionally, mortality and population rates further support these outcomes. Exposure to cardiovascular and respiratory risks is closely linked to air pollution. Over the past five years, the year 2023 recorded the highest annual average concentration of particulate matter in the Kocaeli region, with a value of 56.61 µg/m³, according to data from AQMS (Kocaeli 2023 Environmental Status Report, 2024). However, it is difficult to understand past data due to the inclusion of the COVID-19 time, and the most recent "Kocaeli 2024 Environmental Status Report" has yet to be released. Based on the available data, 2023 appears to be the year with the highest recorded PM pollution levels. AQMS in the Kocaeli region conduct measurements based on prevailing environmental conditions. In particular, variables such as wind speed and rainfall considerably impact the observed pollutant concentrations. The Kocaeli region is predominantly affected by northerly winds, and this prevailing wind direction has remained consistent over long-term wind patterns. Rainy days also positively affect the removal rate of airborne pollutants. In this study, to help make sense of the findings, number of rainy days and average rainfall over the past five years were taken from Station 17066. In the Kocaeli region, 146 rainy days were recorded, with an average daily rainfall of 6.62 mm. In previous studies, Ouyang et al. (2015) examined the impact of rainfall on PM2.5 levels and found that even 5 mm of rainfall could reduce the pollutant by 10–30 µg/m³. Similarly, Bodor et al. (2025), in a study conducted in Romania, observed a decrease in PM10 concentrations on rainy days. Since both the number of rainy days and total rainfall amounts have remained relatively stable over the last five years, it can be concluded that the effect of rainfall on pollutant levels has shown similar behavior each year (Table 2).

Table 2. Kocaeli 17066 meteorological station of rainy days

Year	Rainy Days	Rainfall (mm)
2019	124	6.59
2020	130	5.33
2021	150	5.69
2022	133	5.05
2023	146	6.61

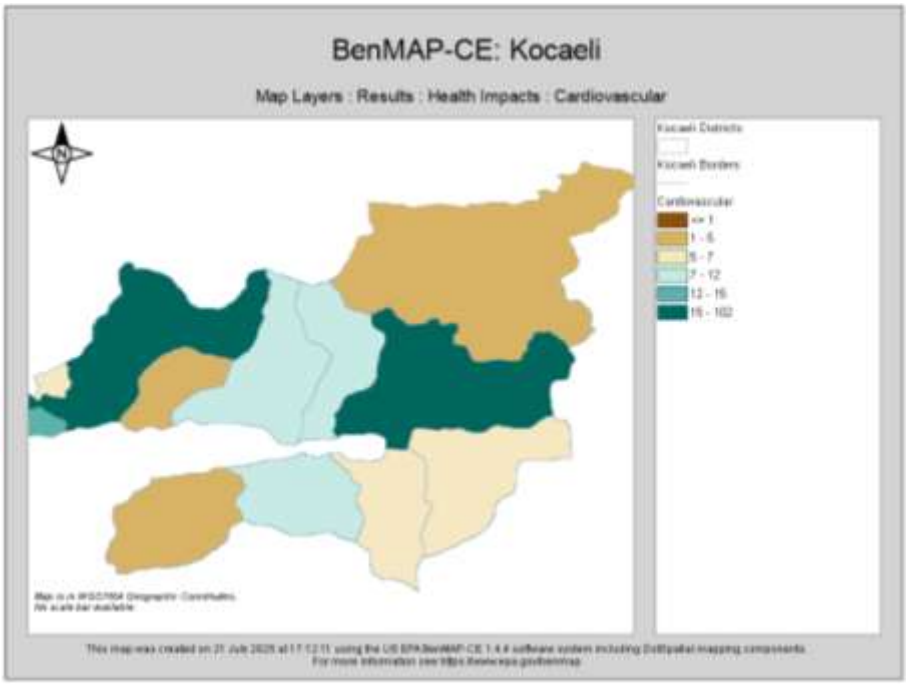


Figure 8. Cardiovascular model predicted map of Kocaeli districts

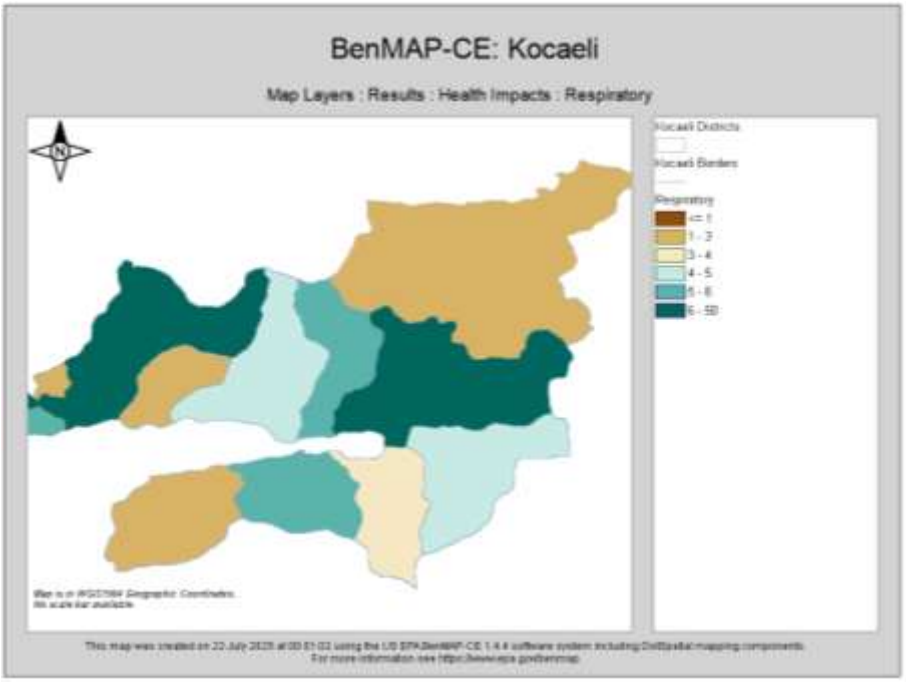


Figure 9. Respiratory model predicted map of Kocaeli districts

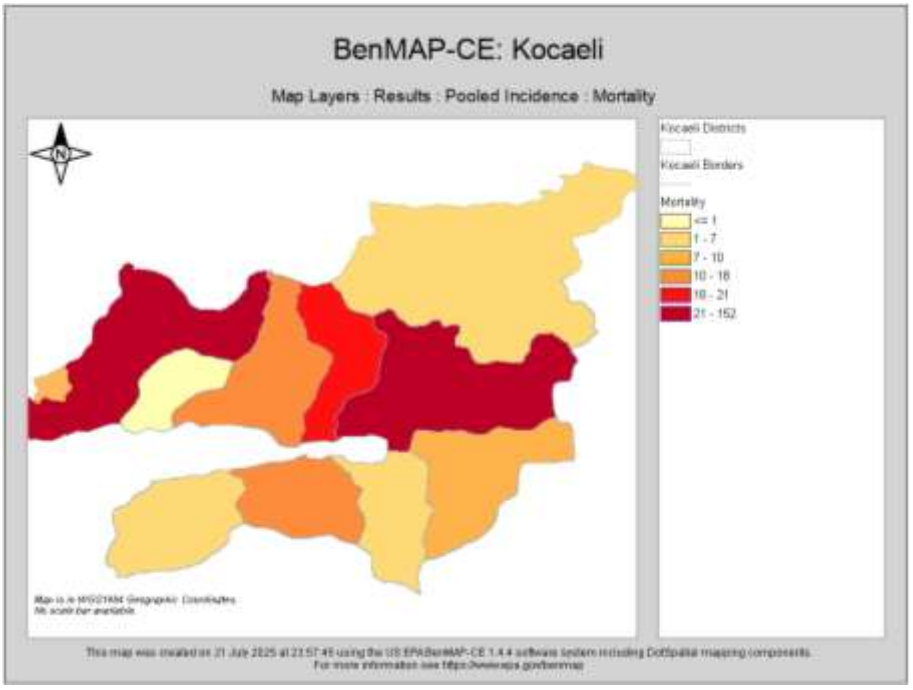


Figure 10. Predicted of reducing mortality map

The results of the economic impacts showed that in the Figure 9. Health effect costs are estimated based on air quality, mortality or incidence rates, and population data. Model results indicated that the highest economic impact occurred in Izmit, followed by the Gebze region. Another notable area is Dilovasi, which exhibited a low economic benefit due to its relatively lower population density compared to other districts. All of the BenMAP-CE model results were given in Table 3 Limited research has been conducted on the socio-economic benefits of improved air quality in the Kocaeli region. Thus, the model results are validated using data from the Santiago scenario. Accordingly, the data obtained for Kocaeli were directly adapted to the model's formulation without modification. In this study, the improvements in model predictions were based on an assumed 50% reduction in pollutant levels to explore possible outcomes under a representative scenario. Consequently, if pollutant levels in the region stay the same or increase, the model is expected to give higher estimates. According to the "Kocaeli 2023 Environmental Status Report", it was stated that climate change action plans would be developed within the scope of the 2019–2023 strategic plan. To reduce air pollution, the focus was on stopping leaks, using continuous monitoring systems, and controlling emissions during loading and unloading. Based on this report, current control measures and European Union (EU) air quality alignment efforts are expected to lower pollutant levels in the Kocaeli region; however, no specific or numerical data has been published yet. In the current situation, industrial activities in Kocaeli are continuous, and new areas continue to appear. If no further mitigation steps are taken, the economic losses caused by air pollution in the region are likely to grow even further.

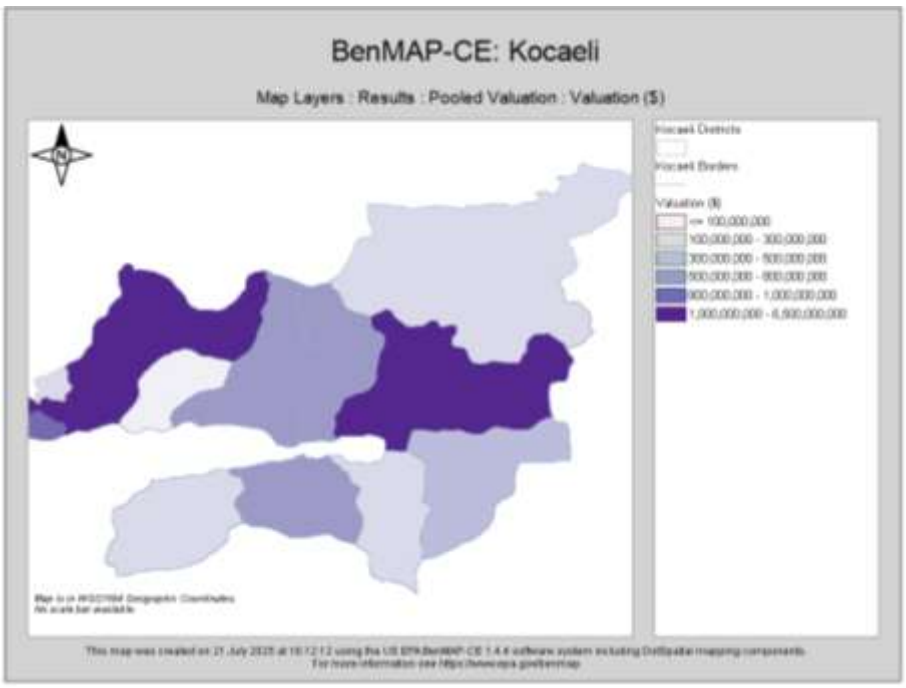


Figure 11. Predicted valuation of health costs

Table 3. BenMAP-CE predicted results of Kocaeli districs

Number	Kocaeli Districts	Avoided of Cardiovascular Deaths	Avoided of Respiratory Deaths	Total Avoided Death Cases	Valuation of Prevented Expenses
1	Izmit	102	50	152	6.5 billion $
2	Derince	12	6	18	718 million $
3	Gebze	34	14	48	2 billion $
4	Golcuk	11	6	17	681 million $
5	Kandira	3	2	5	178 million $
6	Karamursel	3	2	5	174 million $
7	Korfez	12	5	17	708 million $
8	Basiskele	5	3	8	268 million $
9	Cayirova	5	2	7	289 million $
10	Darica	15	6	21	860 million $
11	Dilovasi	1	1	2	25 million $
12	Kartepe	7	4	11	418 million $

CONCLUSIONS

In this study, the BenMAP-CE model was employed to evaluate the potential health and economic impacts of the Kocaeli case scenario, in which air pollution specifically PM10 is reduced by 50%. The required input data for the model were obtained from AQMS, and the WRF-Chem model results were used for missing data and stations. Due to the inadequacy of local data availability, missing parameters were obtained from the Santiago scenario provided in the BenMAP-CE training materials. Kocaeli is among the most significant provinces in Türkiye in terms of both economic and population density. The results of this study show that, improvement of air quality scenario, a considerable number of premature deaths could be prevented, which would consequently reduce associated economic losses. Izmit and Gebze areas were particularly prominent in the model predictions and should be considered as

priority areas compared to other districts. Although the model estimated lower health and economic impacts for some districts such as Dilovasi due to relatively less mortality. This situation is not to be underestimated. Because the Dilovasi district hosts a large industrial zone and many people work there during the day, it is considered a critical area for air pollution risks. The model predictions were mostly based on official mortality data and also included records from hospital administrations. However, illness cases were obtained from only one hospital, so the evaluation was primarily based on mortality data. Therefore, the number of cases is an important parameter, given that particulate matter pollutants are capable of causing both acute and chronic health effects. It is possible to reduce air pollution in the local region. However, the main problem is the source of the pollution, as pollutants can originate from either local sources or long-range transport. Local sources of pollution can be controlled, but long-range pollution transport cannot be prevented. Therefore, both anthropogenic and natural events, especially large-scale dust events such as Saharan dust can significantly affect air quality through atmospheric transport, and this makes it difficult to control air quality. Kocaeli region is an important area that requires special attention due to environmental and health issues. Therefore, air pollution in Kocaeli requires further research, particularly studies that examine both local sources and long-range pollution transport.

ACKNOWLEDFEMENTS

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INFLUENCE OF SYNTHESIS METHODS ON STRUCTURAL AND MORPHOLOGICAL PROPERTIES OF MAGNETITE NANOPARTICLES: A COMPARATIVE STUDY OF PRECIPITATION AND SOLVOTHERMAL TECHNIQUES

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ABSTRACT

With the rapid expansion of applications across various fields, particularly in biology for the development of antibacterial agents, new high-performance strategies are attracting growing attention, especially when based on materials synthesized at the nanoscale. At this scale, the emergence of novel phenomena such as superparamagnetic and an increased specific surface area endows these materials with unique functional properties. These properties are strongly influenced by the morphology, size, and structural organization of the nanoparticles. In the present study, particular emphasis was placed on evaluating the morphology, crystallinity, and purity of magnetite synthesized under varying conditions of temperature, pH, and reaction time, using two distinct synthesis methods: chemical precipitation and solvothermal synthesis. Scanning electron microscopy (SEM) analyses revealed that the synthesis route plays a crucial role in determining both the shape and size of the resulting nanoparticles. Specifically, chemical precipitation tends to produce cubic and hexagonal morphologies, while solvothermal synthesis predominantly yields quasi-spherical particles. X-ray diffraction (XRD) and Raman spectroscopy analyses confirmed that the obtained powders were primarily composed of pure magnetite. However, slight oxidation of the precipitated magnetite into maghemite (γ-Fe_2O_3) was detected in certain samples.

Keywords: Magnetite, Spinel, Nanoparticles, Magnetic Properties.

INTRODUCTION

Magnetite (Fe_3O_4) is among the most extensively studied materials owing to its remarkable electrical and magnetic properties [1, 2]. Recognized as the first known magnetic substance, it serves as a model compound for the investigation of magnetic phenomena. At the nanoscale, magnetite exhibits superparamagnetic behavior when particle size is sufficiently reduced. It is now well established that the size reduction of nanoparticles induces quantum effects, which in turn lead to significant alterations in their magnetic properties [3–5].

Although magnetite remains highly relevant in catalytic applications, it has been attracting increasing interest in various fields, particularly in magnetic storage and biomedical applications. These include its use as an antibacterial agent, in bone tissue engineering, in magnetic resonance imaging (MRI), and as a therapeutic agent in cancer treatment, owing to its excellent biocompatibility and low toxicity. The broadening of its application scope calls for comprehensive research based on innovative approaches and diverse experimental techniques to gain deeper insight into its physical, thermal, magnetic, and optical properties. Recent studies have primarily focused on the control of synthesis conditions and the investigation of novel properties tailored to the demands of emerging technologies [10, 11].

In recent years, extensive efforts have been devoted to optimizing synthesis methods to achieve precise control over the size, shape, crystallinity, and stability of magnetic nanoparticles. Consequently, a variety of synthesis routes have been developed to produce magnetite nanoparticles ranging from 25 nm to several hundred nanometers in size. These include coprecipitation, micro- and nanoemulsion techniques, green (biosynthetic) methods, and solvothermal approaches [12, 13].

Among the various methods for synthesizing magnetite (Fe_3O_4) nanoparticles, thermal decomposition in high-boiling organic solvents has proved particularly effective for producing very small nanospheres, typically between 4 and 30 nm in diameter. However, this technique has certain limitations, not least the difficulty of precisely controlling the reaction temperature, which is a major obstacle to large-scale production [14]. In addition, Fe_3O_4 nanoparticles are highly prone to oxidation, which compromises their long-term stability. For this reason, many studies emphasize the importance of coating these nanoparticles with protective layers, particularly in biomedical applications, in order to improve their chemical stability and prevent their oxidative degradation [15].

Currently, many studies have been conducted and are ongoing on Fe_3O_4 nanoparticles. These investigations have demonstrated that solvothermal and micro/nano-emulsion methods are preferred routes for obtaining crystalline spherical nanoparticles with controlled sizes and shapes, excellent magnetic properties, and low susceptibility to oxidation. Researchers have focused on solvothermal methods to maintain competitiveness in biotechnological applications. These approaches are recommended for various biomedical applications, such as targeted drug delivery (TDD) and magnetic hyperthermia therapy (MHT) [16-18].

Despite their potentially higher cost, solvothermal methods provide unmatched versatility in the fine control of reaction conditions. Derived from hydrothermal synthesis, they involve organic solvents under vacuum or inert gas to form precursors under controlled temperatures and pressures. These techniques enable not only the formation of spherical nanoparticles but also a variety of complex structures, such as cubes, sheets, rods, and single-crystal nanoplates of triangular or hexagonal geometry [3].

In this study, two simple methods were used to synthesize Fe_3O_4 nanoparticles: the co-precipitation method and the solvothermal method. A detailed structural analysis, based on commonly used characterization techniques such as X-ray diffraction (XRD), differential scanning calorimetry coupled with thermogravimetric analysis (DSC–TG), field emission scanning electron microscopy (FESEM), and Raman spectroscopy, provided essential information about their microstructural and physical properties, particularly their magnetic and optical characteristics.

MATERIAL AND METHOD

2.1 Materials

Ferric chloride hexahydrate (FeCl3.6H2O, 97%), cobalt acetate tetrahydrate, sodium hydroxide (NaOH) were acquired from Alfa Aesar, Sigma-Aldrich Aragonesas, respectively. While ethylene glycol (EG) and ammonium acetate (NH4Ac) were purchased from Biochem Chemopharma.

All the chemical reagents and solvents are of analytical grade and utilised without further purification.

2.2 2.2 Synthesis

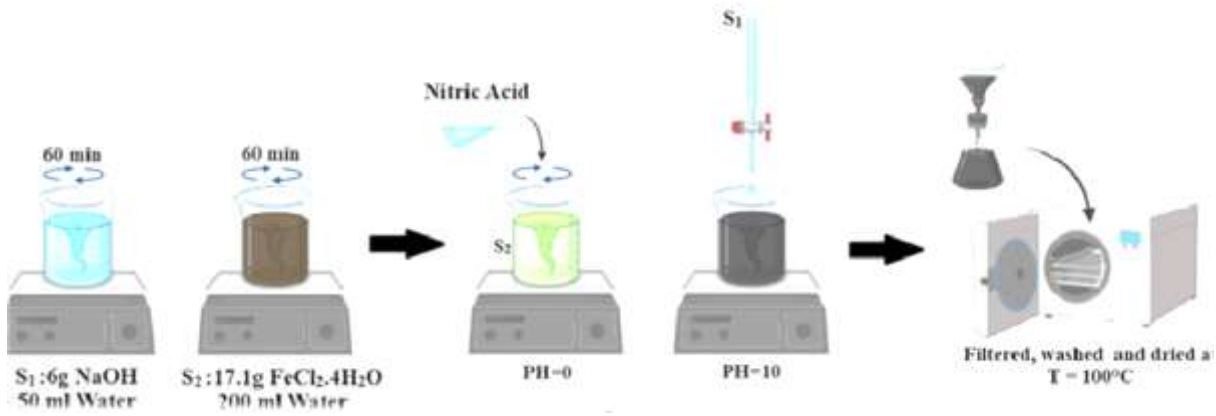


Figure 1: Precipitation Synthésis

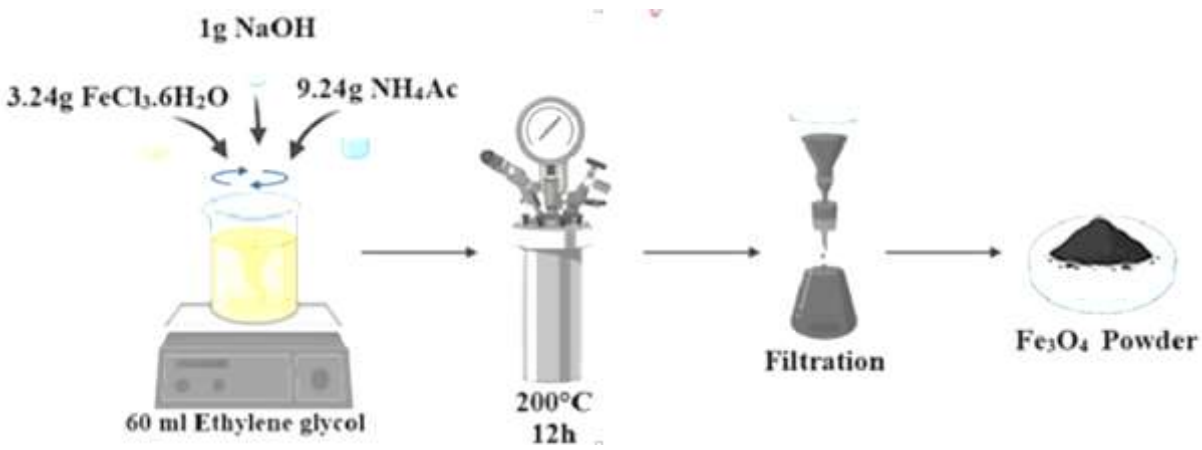


Figure 2: Solvothermal Synthésis

RESULTS AND DISCUSSION

3.1 Structural Properties

The X-ray diffraction patterns of Fe_3O_4 powders synthesized via solvothermal and precipitation methods are presented in Figure 3. The diffraction peaks were indexed with reference to the standard magnetite phase Fe_3O_4 (PDF card No. 01-075-0033), with a lattice parameter (see Table 1) closely matching the reported value for magnetite (8.39 Å) [19]. The combination of the main peak position, the refined lattice parameter, and the characteristic black color of the nanoparticles confirms the successful formation of magnetite, which crystallizes in a cubic spinel structure corresponding to the **Fd-3m** space group. Table 1 summarizes the average lattice parameters and crystallite sizes of both powders, calculated from the five most intense reflections using the Scherrer equation:

$$D = B\lambda/\beta\cos\square$$

Where, D is the average size of the crystallites responsible for the peak, B is Scherrer's constant (0.89), λ is the wavelength of the X rays beam (1.54056 Å), β is the full width at half height of the diffraction peak, and θ is the Bragg angle.

The magnetite particles synthesized by the solvothermal and precipitation methods exhibit average crystallite sizes of approximately 11 nm and 27 nm, respectively, confirming the nanometric nature of the obtained crystallites.

Table. 1 Lattice parameters and average crystallite size of Fe_3O_4 powders synthesized via solvothermal and precipitation methods

Sample	Average grain size (nm)	Lattice parameter (Å)
Fe_3O_4 (Solvothermal)	10.4	**8.388**
Fe_3O_4 (Precipitation)	27.1	**8.39**

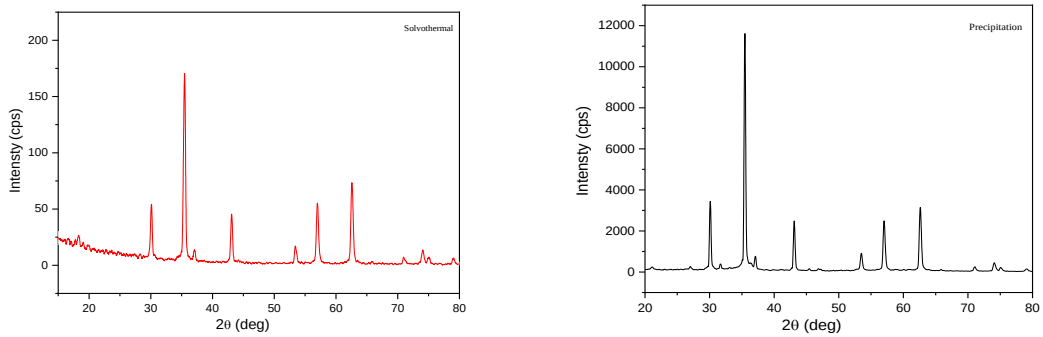


Figure 3: XRD diffractogram of magnetite synthesized by precipitation and solvothermal methods.

3. 1 Analyse Thermique

Coupled DSC–TG thermal analysis, carried out in the temperature range of 0 to 550 °C on magnetite powders synthesized by solvothermal and precipitation methods, was performed to characterize the thermal transformations of iron oxides and to assess their thermal stability. For both samples, the resulting thermograms exhibit three distinct thermal events, each corresponding to characteristic transitions associated with specific oxidized phases of iron (Figures 4).

The first stage, observed at temperatures below 200 °C, exhibits contrasting behaviors depending on the sample composition.

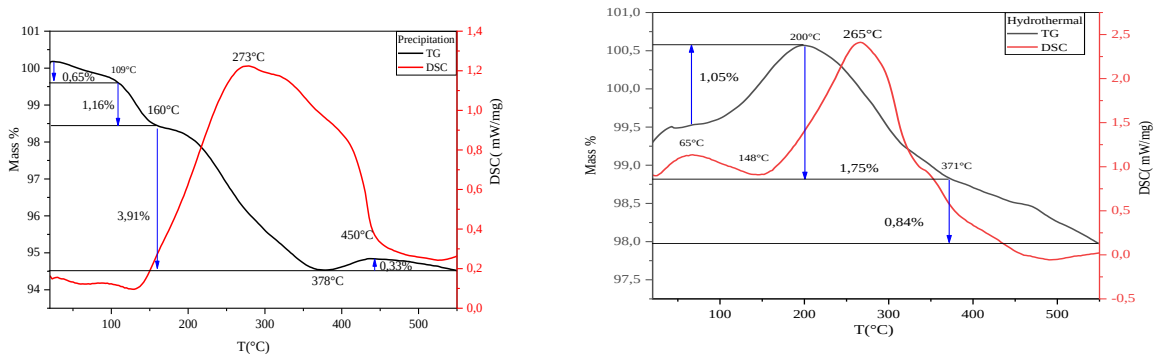


Figure 4: DSC/TG curves of magnetite synthesized by precipitation and solvothermal methods.

In the case of magnetite synthesized by the solvothermal method, the DSC curve reveals a distinct exothermic event centered around 64 °C, which coincides with a slight mass gain (1.05%) observed on the TGA curve (Figure 4). This event is most likely associated with the initial surface oxidation of Fe^{2+} ions to Fe^{3+}, marking the onset of the transformation of magnetite into maghemite (γ-Fe_2O_3), an intermediate phase frequently identified prior to the formation of hematite (α-Fe_2O_3). The observed mass increase is attributed to oxygen adsorption on surface sites deficient in Fe^{3+}, a process favored by the strong affinity of magnetite for oxygen at low temperatures [20].

Pour la magnétite synthétisée par la méthode de précipitation, la première étape se traduit par une perte de masse de 1,81 % sur la courbe TG, attribuée à un processus de déshydratation ou à un dégagement gazeux. Parallèlement, la courbe DSC présente un pic exothermique modéré centré à 90 °C, indiquant une réaction d'oxydation interne (Eq. 1). Ces résultats sont cohérents avec la transformation progressive de la magnétite (Fe_3O_4) en magnémite (γ-Fe_2O_3), un processus exothermique pouvant se produire sans variation de masse détectable à cette échelle de mesure.

$Fe_3O_4 \rightarrow \gamma$-$Fe_2O_3$ (oxidation) Eq. 1

The second thermal stage, occurring between 200 and 450 °C, is characterized by a mass loss of 3.91% for magnetite obtained by precipitation and 1.79% for that synthesized via the solvothermal route. This stage is associated with a pronounced exothermic signal, indicative of the internal oxidation of magnetite. In the solvothermal sample, the signal appears as a sharp peak centered at 265 °C, whereas in

the precipitation sample it takes the form of a broad band with a maximum at 273 °C. This behavior corresponds to the structural transformation of maghemite (γ-Fe_2O_3) into hematite (α-Fe_2O_3) [21].

γ-$Fe_2O_3 \rightarrow \alpha$-$Fe_2O_3$ (réorganisation) Éq. 2

Despite their distinct thermal behaviors, both samples undergo the onset of structural transformation to hematite at an unusually low temperature of about 150 °C.

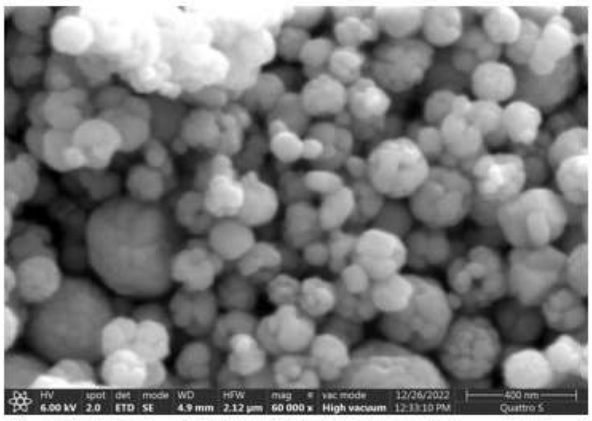
During the third thermal stage, spanning 450 °C to 550 °C, the two samples display distinct behaviors. For magnetite synthesized by the precipitation method, the TG curve reveals a relatively stable mass. In contrast, the sample produced via the solvothermal method exhibits a continuous mass loss, attributed to the evaporation of residual ethylene glycol used as a solvent during nanoparticle synthesis. These results indicate the completion of the oxidation process, culminating in the formation of a thermally stable iron oxide, identifiable by the characteristic reddish-brown color of hematite (Figure 5).

Figure 5: Visual appearance of the powder after thermal analysis (DSC/TG)

3.3 Morphological properties

The size and morphology of Fe_3O_4 nanoparticles synthesized via coprecipitation and solvothermal methods were investigated using field emission scanning electron microscopy (FE-SEM). The representative SEM image of the nanoparticles produced by coprecipitation (Figure 6) reveals faceted, cube-like aggregates, whereas those obtained via the solvothermal route exhibit quasi-spherical aggregates. In both cases, the aggregate sizes can reach approximately 100 nanometers [22–25].

The particle size distribution was determined using ImageJ software based on measurements of 200 individual particles. For the coprecipitation method, the average particle size was estimated at approximately 62.5 nm, which is slightly larger than the value obtained by X-ray diffraction (XRD). In contrast, the nanoparticles synthesized via the solvothermal method exhibit an average size of 11.2 nm, showing excellent agreement with the XRD results.



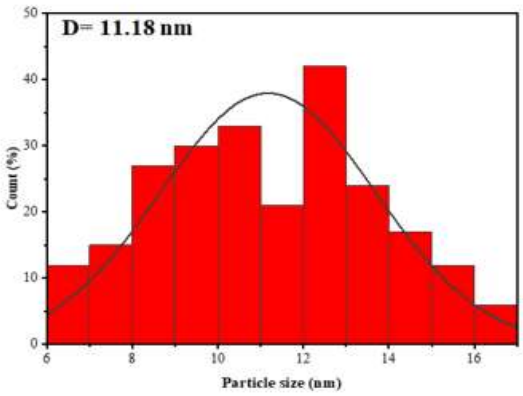


Figure 6a: Hydrothermal

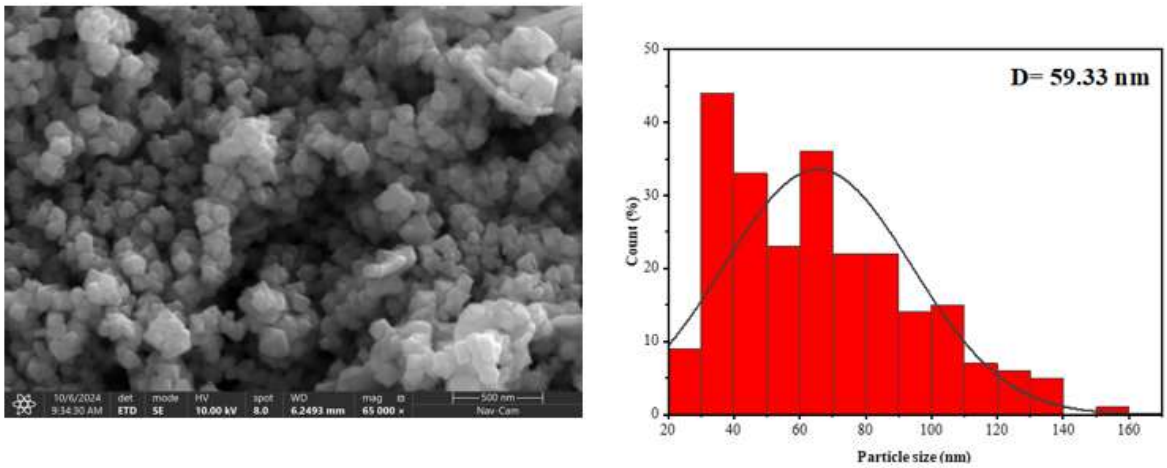


Figure 6 a: Precipitation

3.4. Raman spectroscopy analysis

Due to the wide variety of magnetite crystal structures reported in the literature [26–32], the spinel structure was selected as a reference for conducting symmetry analyses and assigning phonon modes. A comprehensive examination of the symmetry properties of the inverse spinel structure, described by the cubic space group *Fd3m* (O^7_Fh_), enables the prediction of five Raman-active modes, summarized by the following representation:

$\Gamma = A_1g + E_g + 3T_2g$.

Figure 7 presents the spectral deconvolution of the Raman spectra of nanoparticles synthesized by both methods, represented as Gaussian bands within the 150–800 cm^{-1} range. Four distinct peaks are observed at approximately 180, 330, 500, and 666 cm^{-1} for both samples. In agreement with the literature, the bands at 180 and 500 cm^{-1}, assigned to T_2g symmetry, correspond to the asymmetric vibrations of Fe^{3+} and O^{2-} in tetrahedral sites, and are designated as the $T_2g(1)$ and $T_2g(2)$ modes, respectively. The bands at 330 and 666 cm^{-1} are attributed to the Eg and A_1g modes: the former arises from symmetric and asymmetric bending of oxygen atoms around iron, while the latter corresponds to the symmetric stretching of Fe–O bonds within the same sites [23, 24].

In addition to the main bands, a shoulder at 718 cm^{-1}, characteristic of maghemite, is observed in both spectra. Furthermore, additional bands at 258, 287, and 600 cm^{-1} are present in the spectrum of the powder obtained by co-precipitation. These bands, attributed to hematite, indicate oxidation of magnetite.

As hematite, the most stable phase of iron oxides, was not detected by XRD, it is likely that its formation occurred during the micro-Raman analysis. This is plausible given that the laser beam was focused on an area only a few micrometers in diameter, which can cause a local temperature rise of several hundred degrees [33].

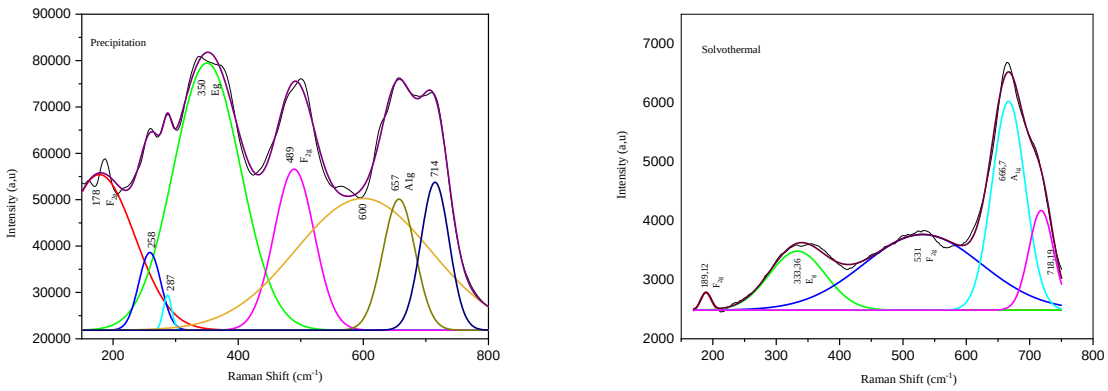


Figure 7: Raman spectra of magnetite synthesized using precipitation and solvothermal methods.

CONCLUSION

This work focuses on the controlled synthesis of nanomaterials using two distinct approaches: precipitation and solvothermal synthesis. Structural (XRD, SEM, DSC-TG) and physical (Raman) characterization confirmed the successful production of pure magnetite nanoparticles, with the smallest particle sizes achieved via the solvothermal route. Results from XRD, SEM, and Raman analyses underscore the decisive role of the synthesis method in determining the morphology, size, purity, and stability of the magnetic nanoparticles.

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PHENOTYPIC ANALYSIS OF LEAF SENESCENCE IN ANTIRRHINUM MAJUS L.

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ABSTRACT

Leaf senescence represents the final phase of development, culminating in cell death. While leaf senescence poses challenges in the post-harvest lifespan of horticultural plants and the yield of agricultural plants, it plays a crucial role in facilitating the re-mobilization of nutrients within plants. Various factors can influence the regulation of leaf senescence. The natural senescence of the leaf starts and progresses according to a regular schedule. During senescence, morphological and physiological events occur along with intracellular changes. Despite this, these processes have not been thoroughly explored across many species. The snapdragon (Antirrhinum majus L) flower serves as a valuable model system that has been extensively studied in terms of genetic traits, flower development, and cellular death. However, the senescence of its leaves has not been investigated. This study delved into the morphological attributes of senescing snapdragon leaves and revealed significant alterations in various indicators as senescence progressed. The results indicated that many indicators changed significantly with the onset and progression of senescence. Notably, there were significant decreases in fresh and dry weight, photosynthetic pigment levels, total soluble protein content, and chloroplast numbers per cell. Conversely, ion leakage and the incidence of dead cells increased with the progression of senescence. Trypan blue staining elucidated that cell death initiation occurred at the leaf tip, progressing to encompass all cells within the leaf blade during the later stages of senescence.

Keywords: Chlorophyll, Chloroplast, Ion leakage, Soluble protein, Trypan blue

INTRODUCTION

Leaf senescence, the final stage of leaf development, is associated with a wide range of morphological, physiological, biochemical, cellular, and molecular changes. Leaf senescence includes changes in leaf color, chlorophyll degradation, breakdown of macromolecules, changes in the intracellular organelles, recycling of nutrients, and the activation of senescence-associated genes (SAGs), and it mainly starts with changes in developmental age (Guo, 2019).

The initial indications of leaf senescence become apparent through changes in leaf color (Zhao et al., 2018). Following a period of rapid expansion and achieving full maturity, a leaf initiates the senescence process, influenced by either the leaf's age or developmental stage or by environmental factors. Typically, the first signs of senescence involve the yellowing of the leaf tip, which gradually spreads inward towards the leaf blade. Eventually, the entire leaf blade takes on hues of yellow, orange, or purple, and the leaf dries up and shrinks, ultimately leading to cell death (Lim et al., 2007; Woo et al., 2018; Zhao et al., 2018).

Senescence is inevitable in all organisms. However, it can be delayed. The delay in senescence can be important in agricultural and horticultural crops in terms of yield and shelf life of crops. It can also impact carbon and nitrogen consumption in plants (Liang et al., 2014). Studies have indicated that senescence syndrome can be evaluated with morphological and physiological changes such as pigment content, nutrient content, ion leakage, and organelle structure. Age-related senescence is different in plant species. At different levels, leaf senescence has been investigated in a few model plants such as Arabidopsis thaliana (Buchanan-Wollaston et al., 2005). Therefore, an accurate description of leaf senescence using quantitative methods is very important in other plant species.

For many years, snapdragon (Antirrhinum majus L.) has been used as a model system for genetic studies and flower development (Lian et al., 2020; Rabiza-Świder et al., 2020). Recently, its petals have been considered in the evaluation of the programmed cell death (Roghayeh Nabipour Sanjbod et al., 2023a, 2023b). However, there is still no information about the senescence of its leaves. In this study, age-related senescence in snapdragon leaves will be investigated through some basic methods.

MATERIAL AND METHOD

This experiment was conducted at the University of Mohaghegh Ardabili, Ardabil, Iran in 2021 to investigate the leaf senescence of snapdragon. The F_1 seeds of snapdragon (*Antirrhinum majus* cv. Legend White) were purchased from the Takii seed company. The seeds were germinated in Petri dishes (20 °C, LED light, 3000 lux intensity) and sown in plastic pots (length = 18 cm and height = 15 cm) after the selection of coeval germinated seeds. The mixture of pot soil was vermicompost and complete organic fertilizers (3:1). The snapdragon plants were transferred to a greenhouse with standard conditions (15-18 °C, natural daylight) and were grown in December. The irrigation of pots was once every 2 days. Leaf longevity was divided into ten time points and the sampling of leaves was performed at these stages (days 18, 32, 46, 60, 74, 88, 102, 116, 130, 144). The first leaf was analyzed at each time point.

The measurement of fresh and dry weight was performed with a digital Micro Balance (Semi-Micro Analytical Balances GR-200, A&D Company, JAPAN). The samples were dried in an oven (60 °C, 48 h).

For investigation of electrolyte leakage, the leaf segments (squares of 1 × 1 cm) were immersed in deionized water (10 mL) and then placed in a water bath (25 °C, 30 min). After the measurement of initial conductivity (using conductivity meter, WTW InoLab Cond 720 Conductivity Meter, Carl Stuart Limited Company, UK), the samples were incubated in a water bath of 96 °C for 15 min. Then, the total conductivity was calculated and expressed as the percentage of the initial conductivity versus the total conductivity.

The content of chlorophyll and carotenoid was evaluated with the Lichtenthaler method (Lichtenthaler and Buschmann, 2005; Lichtenthaler and Wellburn, 1983). The pigment absorption was determined at three wavelengths (470, 645, and 662 nm) using a spectrophotometer (SP-UV 200, Spectrum Instruments Limited, Australia). The concentration of pigments was calculated using the following equation.

$$C_a\ (\mu g/ml) = 11.24 \times A_{661.6} - 2.04 \times A_{644.8}$$

$$C_b\ (\mu g/ml) = 20.13 \times A_{644.8} - 4.19 \times A_{661.2}$$

$$C_{(x+c)}\ (\mu g/ml) = \frac{(1000 \times A_{470} - 1.90C_a - 63.14C_b)}{214}$$

The leaf samples were fixed in 3.5% (v/v) glutaraldehyde for 1 h in the dark for chloroplast number per cell area. The samples were washed with Na2EDTA buffer (0.1 M, pH=9) and kept in this buffer. The samples were then placed in a shaker water bath (60 °) for 2.5 h and stored at 4 °C. The pictures of leaf samples were taken by light microscopy. Image J software was applied for quantification of chloroplast count (Evans et al., 2010).

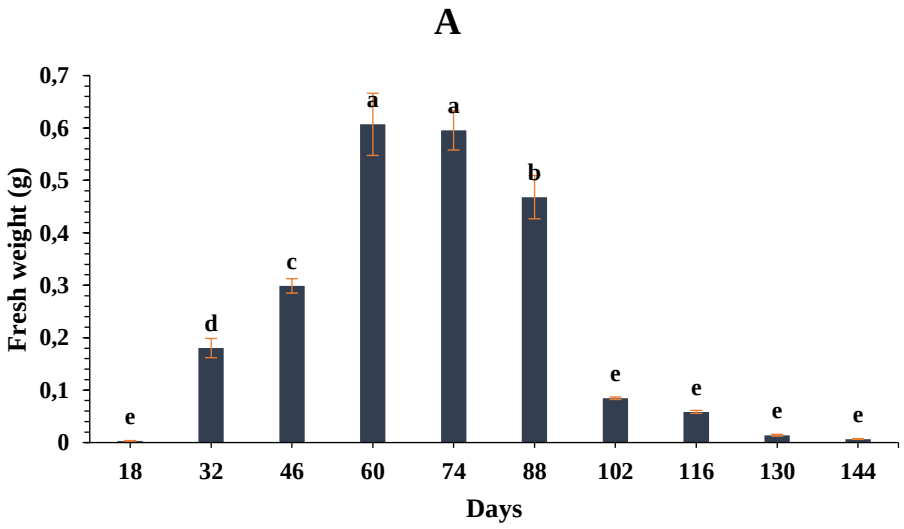
For soluble protein, the leaf tissue (100 mg) was homogenized in phosphate buffer (pH=7) and centrifuged at 10000 rpm (10 min) at 4 °C. The supernatant (100 µL) was separated and mixed with Coomassie solution (5 mL). After vortexing, absorbance was read at 595 nm. BSA standard curve was applied for the calculation of total soluble protein (Roghayeh. Nabipour Sanjbod et al., 2023).

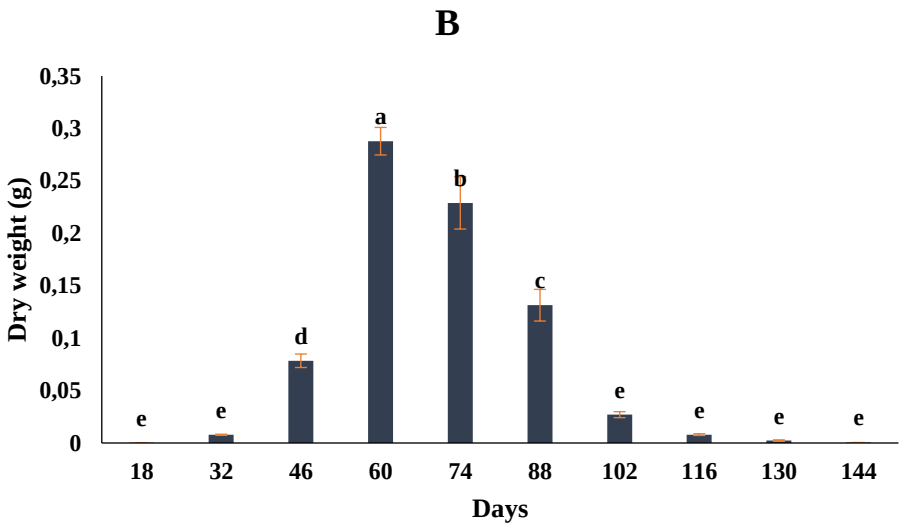
Dead cells of snapdragon leaf were detected with trypan blue staining. For this purpose, the excised leaves were placed in trypan blue staining solution at 96 °C for 1 min. The samples were left at room temperature for 1-24 h. The staining solution was removed and the samples were immersed in chloral hydrate solution for 2–4 h. After that, the chloral hydrate solution was removed and the samples were placed in 70% glycerol. The staining of leaves was recorded by taking pictures (Guo, 2019).

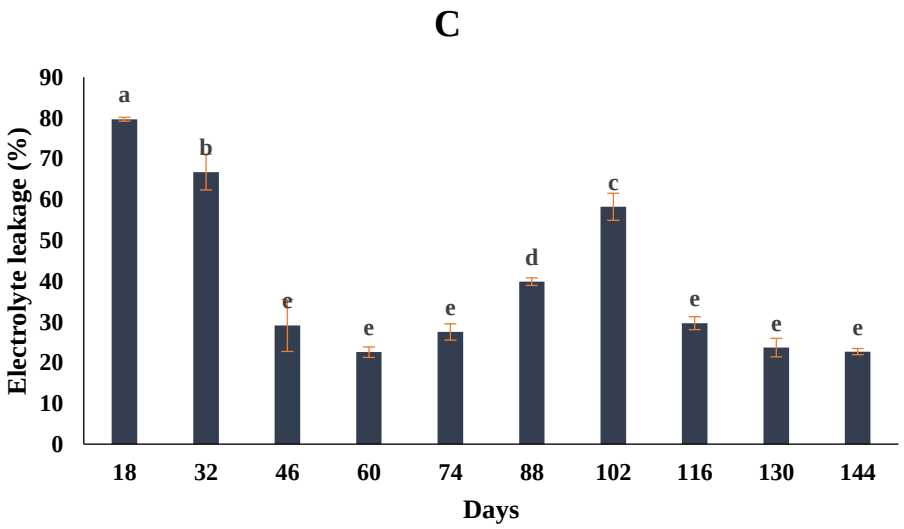
This experiment was conducted as a completely randomized design (CRD) with five replications and analyzed using SPSS software (version 16; SPSS Inc, USA). The means were evaluated with Duncan's multiple-range tests at 5% probability.

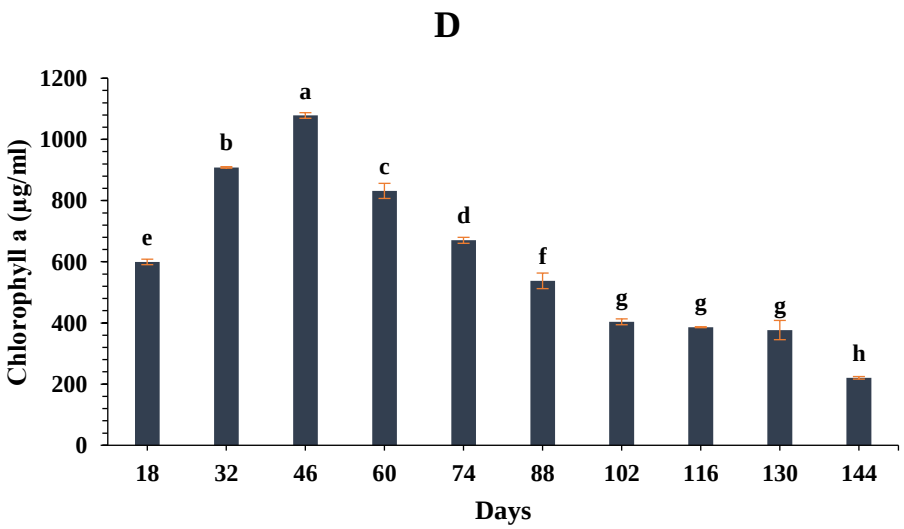
RESULTS AND DISCUSSION

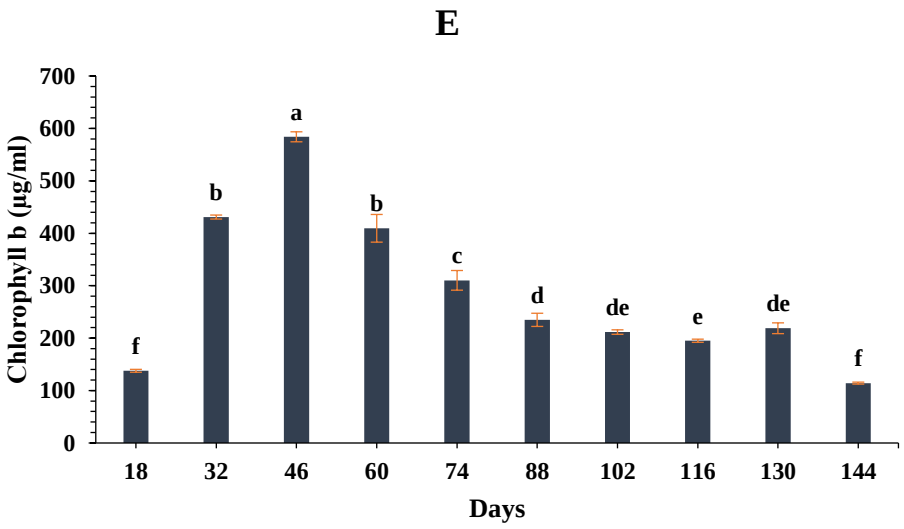
The results of this study showed that with increasing age of the plant, fresh and dry weight increased and reached the maximum value on day 60, then with the onset and progression of senescence, a severe decrease in these parameters was observed, which was visually associated with leaf desiccation (Figure 1A and 1B). Interestingly, the leaves had the highest ion leakage on day 18 in electrolyte leakage. Gradually, with increasing age, ion leakage decreased. On the 88th day, an increase in electrolyte leakage was observed, which coincided with the progress of senescence and the beginning of cell death. After that, ion leakage decreased in the stage of leaf shriveling and desiccation (days 116, 130, and 144) (Figure 1C). The highest content of photosynthetic pigments was observed on the 46th day. After that, the content of chlorophyll a, chlorophyll b, and total carotenoids gradually decreased. (Figure 1D, 1E and 1F) The examination of the total soluble protein content showed that with increasing age and reaching day 88, this parameter increased with a gentle slope, and then with the onset of cell death, cell proteins were broken down and their content significantly decreased (Figure 1G). The highest number of chloroplasts per cell was recorded on the 46th day and their number decreased in the following days (Figure 1H). Trypan blue staining showed that the number of dead cells increased with increasing age. Furthermore, the death of the cells started from the tips and margins of the leaves and gradually the whole leaf cells experienced death (Figure 2).

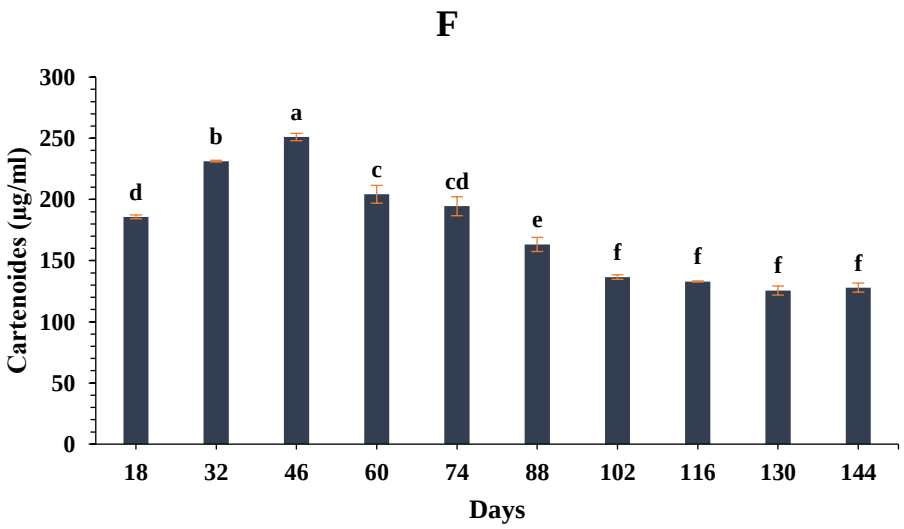


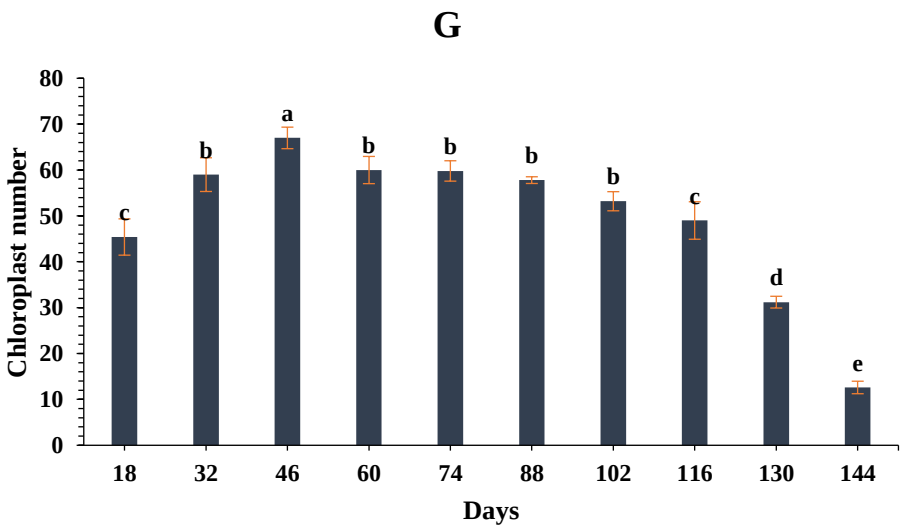

B
Dry weight (g)
0,35
0,3
0,25
0,2
0,15
0,1
0,05
0
e
e
d
a
b
c
e
e
e
e
18
32
46
60
74
88
102
116
130
144
Days


C
Electrolyte leakage (%)
90
80
70
60
50
40
30
20
10
0
a
b
e
e
e
d
c
e
e
e
18
32
46
60
74
88
102
116
130
144
Days


D
Chlorophyll a (µg/ml)
1200
1000
800
600
400
200
0
e
b
a
c
d
f
g
g
g
h
18
32
46
60
74
88
102
116
130
144
Days


E
Chlorophyll b (µg/ml)
700
600
500
400
300
200
100
0
f
b
a
b
c
d
de
e
de
f
18
32
46
60
74
88
102
116
130
144
Days


F
Cartenoides (µg/ml)
300
250
200
150
100
50
0
d
b
a
c
cd
e
f
f
f
f
18
32
46
60
74
88
102
116
130
144
Days


G
Chloroplast number
80
70
60
50
40
30
20
10
0
c
b
a
b
b
b
b
c
d
e
18
32
46
60
74
88
102
116
130
144
Days

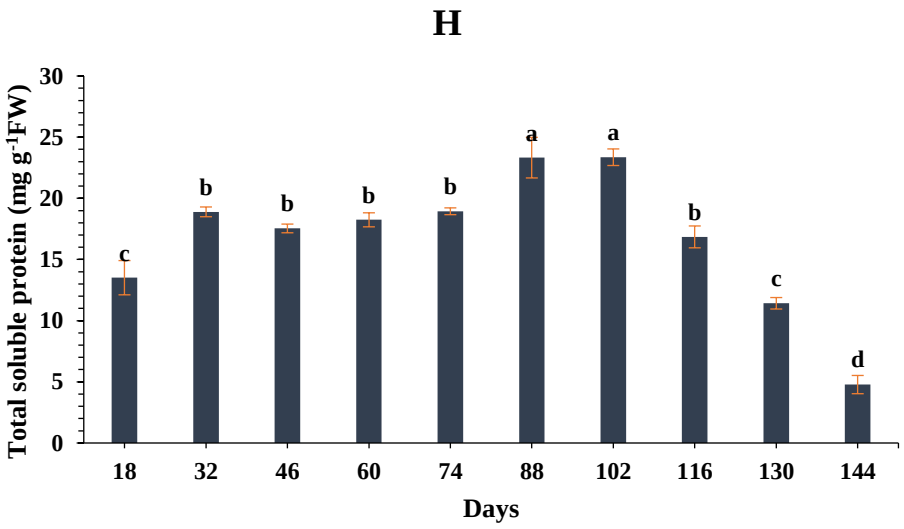


Figure 1. Phenotypic characteristics of snapdragon leaves during senescence. (A): fresh weight, (B): dry weight, (C): electrolyte leakage, (D): chlorophyll a content, (E): chlorophyll b content, (F): carotenoids content, (G): chloroplast number, (H): total soluble protein. Different letters indicate significant differences at $p \leq 0.05$ (Duncan's test). Error bars represent ±SD, n=5.

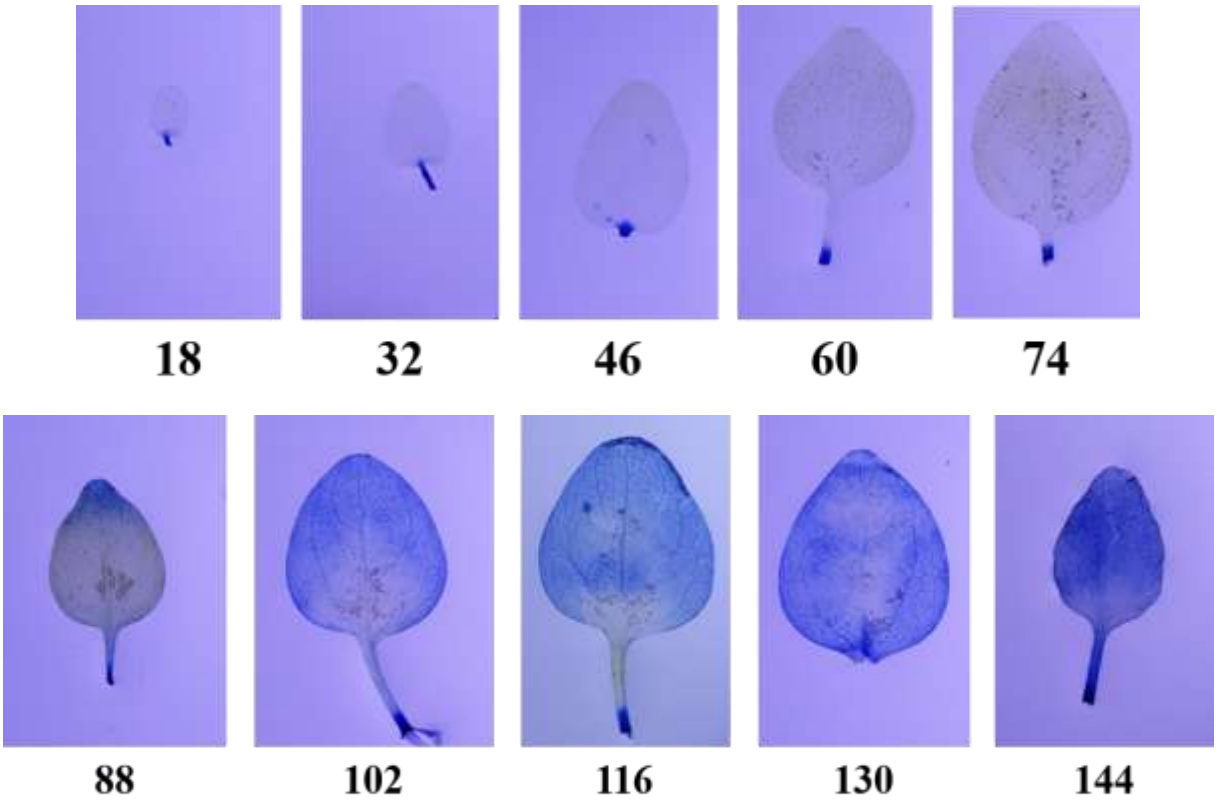


Figure 1. Progression of cell death in snapdragon leaves.

Leaves, as specialized organs for photosynthesis, require considerable energy and nutrients for production; following a productive period, leaves enter senescence where nutrients are redistributed to younger leaves, seeds, or stored for future growth, resembling a recycling program at the organismal level (Quirino et al., 2000).

The senescence of leaves can be discerned through alterations in their appearance. The initial visible indications of leaf senescence involve changes in leaf color. Following growth and maturation, a leaf enters the senescence phase. In typical circumstances, the initial signs of senescence with the yellowing of the leaf tip, progressing gradually towards the leaf blade, eventually resulting in the entire blade, followed by leaf desiccation and shrinkage, culminating in cell death (Guo, 2019) as observed with trypan blue staining.

Chloroplasts are among the primary organelles targeted for degradation at the onset of senescence. Similar to our findings, the loss of chlorophyll is a noticeable occurrence during senescence and is frequently used as a biomarker for the start of senescence. On the other hand, numerous other breakdown processes such as protein, lipid, and nucleic acid degradation also happen (Zhao et al., 2018).

Protein degradation represents a crucial process during leaf senescence, playing a pivotal role in nitrogen recycling. Studies at the transcriptomic level have revealed a significant upregulation of genes related to proteolytic activities (Breeze et al., 2011). Predominantly, serine proteases and CysProt enzymes are prominently associated with leaf senescence, although aspartic, threonine, and metalloproteases have also been identified (Diaz-Mendoza et al., 2016). Plant protease activities have been identified in various cellular compartments such as nuclei, chloroplasts, cytosol, ER, vacuoles, mitochondria, apoplast, cell wall, and specialized vesicles (Diaz-Mendoza et al., 2016). The chloroplast stands as the primary reservoir of mobilizable proteins within the cell, with Rubisco and the chlorophyll-binding light-harvesting proteins of PSII (LHCIIs) being the major proteins (Hörtensteiner and Feller, 2002). Chloroplast proteins account for up to 80% of the total leaf nitrogen and serve as a key nitrogen source during mobilization from senescing leaves. Throughout senescence, chloroplast proteins are believed to undergo degradation through coordinated actions of chloroplast proteases, senescence-associated vacuoles (SAVs), and the ubiquitin/26S proteasome pathway (Diaz-Mendoza et al., 2016).

CONCLUSIONS

The findings revealed that several markers experienced significant alterations as senescence began and developed. Noteworthy was the considerable reduction in both fresh and dry weight, levels of photosynthetic pigments, total soluble protein content, and the number of chloroplasts per cell. In contrast, there was an increase in ion leakage and the prevalence of dead cells as senescence progressed. Analysis with trypan blue staining showed that the initiation of cell death occurred at the tip of the leaf, extending to encompass all cells in the leaf blade in the advanced stages of senescence.

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REFLECTION OF NATURE ON LIFE: BIOMIMICRY AND SUSTAINABILITY

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ABSTRACT

While human beings struggle to survive in a globalizing world, nature has managed to exist on its own and has solved its problems within itself. Animals, plants, decomposers and even microbes have found their place in this order and become part of the solution. Thanks to this harmony of nature, studies conducted over the last twenty years have yielded information that can guide ecosystems, cities, architects and interior designers. Although the operation of biomimicry design may sound complicated, it is actually quite simple. Biomimetic design includes inventions and designs that are inspired by solutions developed by nature and provide sustainable solutions. Especially as we move into an ecological age that embraces nature rather than overcoming it, looking at it from a biomimicry perspective becomes meaningful. This perspective suggests acting like nature and approaching problems like nature, beyond simply coexisting with nature. In this study, the importance and examples of biomimicry, which encourages innovation in design and brings new perspectives to existing problems, are discussed.

Keywords: Biomimicry, Inspiration from nature, Sustainability

INTRODUCTION

The reason why the "eco-centric" approach came to the fore in the 20th century is today's "unsustainable" patterns. Sustainability is a widely accepted moral principle that emerged within the environmental movement and whose content is constantly being redefined within the political process (Tekeli, 2001). Although the concept developed in connection with the discussions around the concept of "eco-development" included in the Report of the World Environment Conference held in Stockholm in 1972, it is known that the questioning of the human-living environment goes back much further.

Since the day human beings came into being, they have tried various ways to find solutions to the problems they have encountered. Issues such as the ever-increasing world population, rapid industrialization, excessive fossil fuel use, and environmental destruction have become major global problems. In addition to being able to quickly find solutions to all these problems, the most important goal should be that these solutions are sustainable and support the ecological structure. Taking into account and researching thousands of years of experience of nature to find solutions to human problems and needs may be the answer to many of the problems we experience. The concept of biomimicry or biomimetics, a new innovation discipline, is defined as a ‘multidisciplinary design approach’ or ‘a new relationship with nature’ (Caferoğlu, 2021). Biologist Benyus (2002) first used the concept of biomimicry in her/his book “Innovation Inspired by Nature”. Biomimetic design includes inventions and designs that are inspired by solutions developed by nature and provide sustainable solutions.

1. What is Biomimicry?

The environmental problems we have faced in recent years are actually the result of the methods we have developed to make human life easierThe use of resources, vehicles and industry, which exceed the capacity of the planet, requires new solutions to be found today. This is where biomimicry comes in. Innovative projects focused on the environment and efficiency are rapidly gaining ground in human life (URL 1).

Biomimicry is derived from the Latin words bios (life) and mimikos (imitation) (Volstad and Boks, 2012). Biomimicry is actually defined as design inspired by nature to imitate life and nature (Kennedy, 2004) and is an important source of innovation. In the light of science and technology, it is an important contribution to the question of how we can do better rather than the question of how we can do it better in producing solutions to problems (Eryılmaz, 2015; Primlani, 2013).

Biomimicry, a concept related to innovative ideas inspired by nature, basically refers to the design of products and materials that produce solutions from living things in nature. Biomimicry forms the basis of many products used in fields such as architecture, artificial intelligence, robotics, industry, transportation, in short, today's technology (URL 2).

Biomimicry enables sustainable designs by taking advantage of the power of nature. It helps people produce environmentally friendly projects. Biomimicry studies, which have become especially popular recently, aim to use the world's dwindling natural resources effectively. This process, which prioritizes naturalness and has many examples in the history of science and art, can be applied to many different fields such as technology, art, transportation and energy (URL 3). Benyus divides biomimicry into three levels, and each level offers different lessons from nature.

Mimicking Form and Shape: Consider the sleek, streamlined body of a fish; engineers use this principle to shape wind turbine blades for maximum efficiency.

Mimicking Natural Processes: What matters is not how nature looks, but how it works. Take photosynthesis for example; scientists are studying how plants convert sunlight into energy, creating sustainable and renewable energy sources.

Learn from Ecosystems: Nature wastes nothing. Forests, coral reefs, and other ecosystems operate in perfect balance, with each byproduct feeding another process. Inspired by this, industries are now designing closed-loop systems where one company's waste becomes another company's raw material, attempting to eliminate pollution (URL 4).

2. What is the Aim of Biomimicry (Biomimetism)?

It can be said that the main reason why biomimicry, which we encounter in the history of science and art, has gained popularity in recent times is related to the effort to use the world's natural resources, which are decreasing day by day, efficiently. Nature is the basis of biomimicry, and sustainable designs can be created by imitating the perfect functioning of nature. The process, which draws its strength from nature, enables people to produce environmentally sensitive projects (URL 5). Biomimicry aims to create products, processes or systems that are in harmony with life and serve sustainability.

In nature, biological organisms such as animals, plants and microbes constantly improve themselves to survive and adapt to the harsh conditions of nature. Those who fail are wiped out, those who succeed survive. American biologist Janine Benyus, the first scientist to suggest that the ways in which living things adapt to life should be consciously imitated and who pioneered the idea of biomimicry in the late 1990s, says: "Nature has already solved all the challenges we face. Failures have become fossils, and what we are in now is the key to survival." (URL 6). Biomimicry, which has been growing in popularity since its inception and utilizes natural organisms to support innovation, aims to be sustainable (URL 3).

3. What are the Areas of Use of Biomimicry?

Biomimicry usage areas form the basis of many products used in fields such as industry, artificial intelligence, transportation, architecture, and robotics. Biomimicry, first proposed by American engineer Otto H. Schmitt in 1969, is credited with establishing the field of biomedical engineering.

4. Sustainability with Nature-Inspired Designs

Sustainability involves the beneficial use of design to meet current and future human needs without depleting a resource or causing permanent damage to the environment. Considering environmental awareness in product design and production processes has led to the emergence of the concept of sustainable design. Sustainable design is an approach that minimizes environmental impacts that may occur through product design while increasing innovation. Sustainability connects the present to the future and makes all humanity responsible for the environment (Caferoğlu, 2021).

Kanısanlı and Durmuş (1995) emphasize that various plants and insects avoid becoming a food chain for living things by resembling themselves to various living things and even to the environment they live in, and state that the imitation of a plant or an animal by living things in terms of both color and pattern is called mimicry. The green color of the tree frog and its resemblance to a leaf, and the bush crickets' resemblance to a bush and a tree stem are examples of biomimicry in nature. The examples of biomimicry here are imitations of living things in nature. Based on this, people's inspiration from or imitation of nature was also shown as an example of biomimicry, and there was no need for a different word for definition. This new approach aims to bring sustainable solutions to current problems that cause less harm to the planet, using the teachings of nature, by looking at the models, systems and processes in nature (Caferoğlu, 2021).

Nature is a system that has both the best design and the most efficient system. With the development of technology, man's ability to imitate nature has also increased. Sustainability is one of the fundamental goals of biomimicry. Biomimicry-inspired designs are designed to be both aesthetically pleasing and energy-efficient. The philosophy of biomimicry, like the philosophy of nature, aims to optimize resource consumption in its natural process. For sustainability, nature redesigns its own processes over time. The strongest evidence of this is that this process, which has been imitated over time, has been seen to continue effectively. Thus, the sustainability of biomimicry may vary depending on the development of designs derived from nature (URL 3).

5. Nature-Inspired Designs

Many of the design examples inspired by nature are innovative projects that reveal details that had not been considered before. Designs based on biomimicry imitate nature's efforts to create the best system with the least amount of resources. Some examples of biomimicry are presented below.

Concorde and Dolphin: The British-French joint-production Concorde aircraft, which managed to exceed the speed of sound, made its first flight attempt in 1969. The nose part of the dolphins was effective in its design, because it reduced the friction created by the air on the outer surface. Inspired by dolphins, whose tail fins act as engines on the surface of the water, engineers placed Concorde's engines at the rear (Figure 1). Production of these aircraft ceased in 2003 (Kılıç, 2022).

Figure 1. Dolphin-inspired Concord aircraft (Kılıç, 2022)

Velcro Tape and Burdock: One day, Swiss engineer Georges de Mestral got these plants stuck on his clothes. When Mestral saw that it was not easy to separate his clothes from this plant, he immediately came up with an idea and decided to use it in the clothing industry (Figure 2). He created the same docking system and Velcro straps are now used in astronaut suits (URL 7).

Figure 2. Velcro tapes produced using burdock (URL 7)

Radar and Bat: The vibrations emitted by bats, which have poor vision, hit obstacles and bounce back, thus determining their movement style. The operation of the radar is also based on the same system (Figure 3) (URL 7).

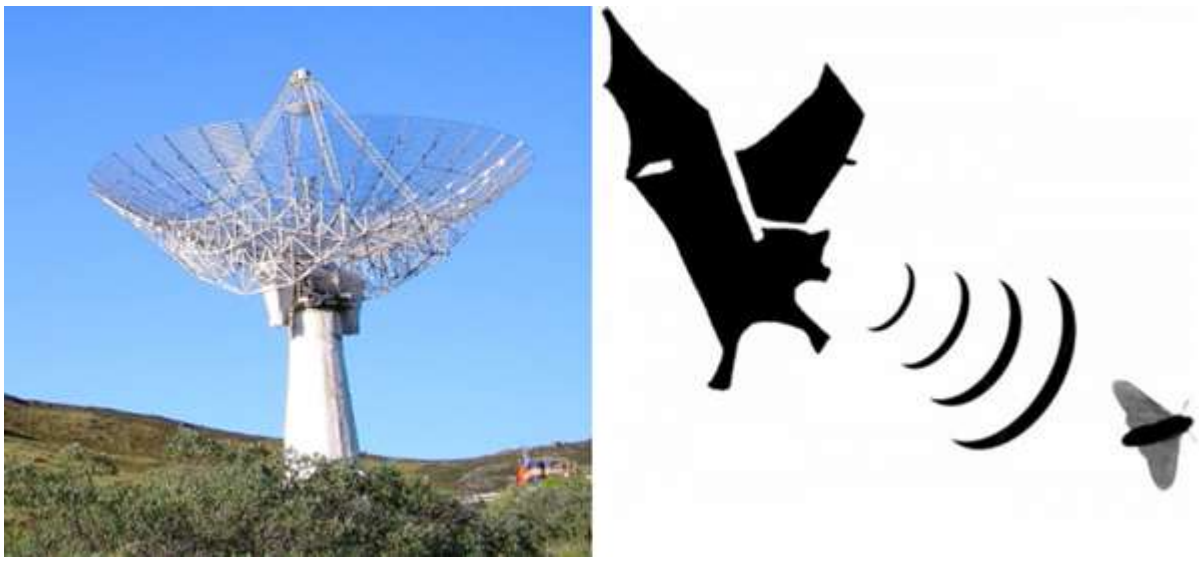

Figure 3. Radars inspired by the vibrations emitted by bats hitting obstacles and bouncing back (URL 7)

Munich Olympic Stadium and Dragonfly's Wings: Despite being so thin, the dragonfly's wings, which are only 1/3000th of a millimeter thick, are remarkable for their durability. Their durability stems from the fact that their wings are composed of nearly a thousand segments, preventing them from tearing during flight. The roof of the Munich Olympic stadium was constructed using the same method (Figure 4) (URL 7).

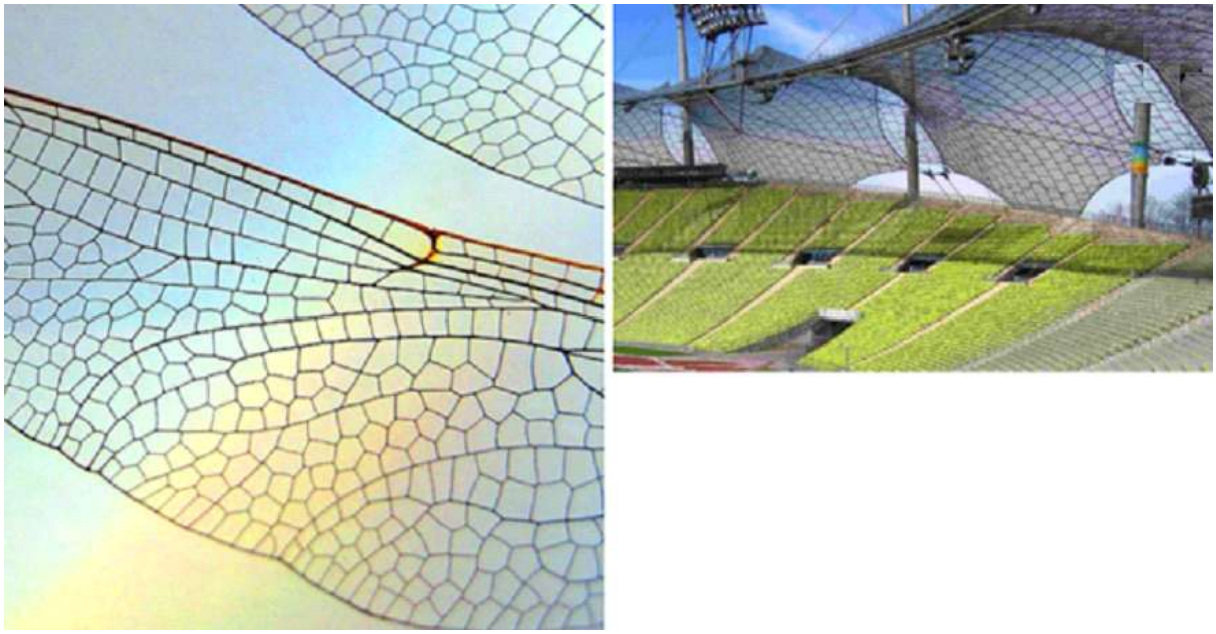

Figure 4. **The Munich Olympic Stadium was inspired by dragonfly wings** (URL 7)

Swimsuits and Sharks: Sharks have a type of tooth structure in their skin. This structure creates a low-pressure zone as the shark moves underwater, creating vortices in the water. Thanks to the vortices that form, the shark is pushed forward powerfully and gains speed in the water. This method is also applied in professional swimming trunks (Figure 5). It allows swimmers to move faster using much less

muscle force (URL 4).

Figure 5. Professional swimming trunks inspired by shark skin (URL 4)

Eastgate Centre in Harare, Zimbabwe and Termite Mounds: Designed by Mick Pearce and opened in 1996, the Eastgate Centre has a ventilation system based on the natural cooling structure of termite nests (Figure 6). Fans draw fresh air from outside and cool it as it passes through underground channels that mimic the airflow in termite nests. Cold air circulates throughout the building, creating a comfortable indoor environment without air conditioning.

Inspired by nature, this ventilation system is more environmentally friendly, consuming only 10% of the energy used by a traditional air conditioning system. The building also features a rainwater harvesting system, solar panels for hot water, and a green roof (URL 4).

Figure 6. Zimbabwe Eastgate Centre was inspired by termite nests (URL 4)

Lotusan Self-Cleaning Paint and Lotus Flower: The Lotus Effect is essentially the self-cleaning feature found in the Lotus flower leaf. This type of effect is also found in the wings and bodies of some insects (Gruber, 2011). This knowledge has led scientists to find new materials. Lotusan is a self-cleaning paint that can be used on both interior and exterior surfaces. One of a building's biggest nightmares is moisture. This design keeps moisture away from the surfaces, allowing the building's surfaces to breathe and appear clean. Lotus leaf is known as the best waterproof material in nature. The lotus, whose surface is covered with wax secretion, is hydrophobic (water repellent) thanks to this secretion (Figure 7). The University of Bonn conducted research together with several companies and designed a paint called Lotusan, which is an adhesive film containing nanoparticles such as hydrophobic silica (Shimomura, 2010). This extremely ingenious design reveals the necessity of taking inspiration from nature (Toruç, 2021).

Figure 7. Lotus effect (Toruç, 2021)

Wind Turbines and the Humpback Whale: Another prominent example of biomimicry from real life is modern wind turbines. Inspired by the fins of humpback whales, which can reach up to five meters, wind turbine designs have various humps and irregularities called pectorals, just like these creatures (Figure 8). In this way, the efficiency of the turbines increases and the noise generated by the rotation of the turbines is greatly reduced (Kılıç, 2022).

Figure 8. Wind turbine designs inspired by humpback whale fins (Kılıç, 2022)

DISCUSSION AND CONCLUSION

Nature has a flawlessly functioning system that has solved every problem that has arisen over centuries within its own system. When nature is seen as an educator rather than a resource to be constantly consumed, its importance in the continuity of life is better realized. For the survival of the human species, existing systems that destroy nature must be changed. Biomimicry can guide us in this regard. Biomimicry is a design tool that develops sustainable solutions inspired by nature to solve a human problem or need that nature has already solved. We can get answers to many problems from nature by looking in the right places to find what we need or answers to questions (Caferoğlu, 2021).

The creative and innovative potential of biomimicry is promising for providing sustainable solutions to future environmental problems in engineering, architecture, industry and other fields. It is thought that observing and applying nature's problem-solving methods with the biomimicry approach will provide the opportunity to realize innovative materials and methods that have perhaps never been produced before, in harmony with nature (Eryılmaz, 2015).

By approaching design from a holistic perspective through biomimicry, the effects of designed products on our planet's cycles, ecosystems and economy can be positively changed during the production, consumption and waste stages. Thanks to products designed with the biomimicry approach, we can help reduce the negative impact we have on the environment.

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EXPERIMENTS ON REVEALING EFFECTS OF DIFFERENT MEDIA STRENGTH, AND SUCROSE-DEPENDENT IN ADVENTITIOUS ROOT CULTURES OF RADISH (*Raphanus sativus* L.)

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ABSTRACT

Radish (*Raphanus sativus* L.), one of the important vegetables of the *Cruciferae* family, has attracted attention due to its nutritional content and health-improving properties. The adventitious root culture technique not only supports the propagation of medicinally valuable plants but also offers an alternative method to harvest valuable bioactive components from plants. In the current study, hypocotyls obtained after germination of red and black radish seeds were used as explant material, the effect of MS basic medium at different strengths (¼, ½, ¾, and 1) and the effect of different amounts of sucrose (20, 25, 40 and 50 mg L^{-1}) on adventitious root formation were evaluated in terms of biomass formation. Within the framework of the results obtained, it is thought that adventitious root cultures of radish can be used as a complementary method in the large-scale production of valuable bio-compounds to be used in the pharmaceutical industry.

Keywords: *in vitro*, root cultures, medium strength, biomass

INTRODUCTION

Plants are used as a model system and a taxonomic marker for the discovery of new compounds. Increasing awareness of the health hazards and toxicity associated with the indiscriminate use of synthetic drugs has increased interest in the use of plants and plant-based medicines. The complex structure of the compounds and the high cost of chemicals make the synthesis of bioactive compounds a difficult process. However, the production of these compounds is also affected by some factors, such as the growing period of the plant and the season in which it is collected, species and variety differences, the plant being in a certain growth and development period, and the lack of adequate methods for the production and standardization of the plant (Ahmad et al., 2015).

As an alternative solution to such problems faced by the phytopharmaceutical industry, biotechnological approaches, especially plant tissue culture methods, attract a lot of attention. Since plant tissue culture methods offer a controlled supply of biochemicals independent of plant availability with consistent product quality, it is thought that they could provide a great potential to boost the conventional agriculture for the industrial-scale production of bioactive plant metabolites (Ramachandra Rao and Ravishankar, 2002; Ahmad et al., 2015). Plant cell, tissue, and organ culture techniques are promising techniques that have the potential to be used in obtaining valuable plant metabolites, including pharmaceuticals (Murthy et al., 2008; Ahmad et al., 2015). These techniques both provide a reliable source for plant-based pharmaceutical products and can be used for large-scale cultures of plant metabolites. The increasing commercial importance of secondary metabolites in recent years has led to great interest in the diversification of the production of bioactive plant metabolites through tissue culture technology. At this point, the adventitious root culture system provides an alternative approach for the improvement and development of plant-based pharmaceutical compounds (Ahmad et al., 2015).

Plant roots serve as a source of bioactive molecules, including a surprising variety of metabolites, agrochemicals, flavors, dyes, and fragrances (Fulzele et al., 2002). Recently, organ culture, especially adventitious root culture, has been applied to many medicinal plants due to their rapid growth as well as stable production of secondary metabolites of pharmaceutical and nutraceutical interest (Murthy et al., 2008). Adventitious roots induced under aseptic conditions in a suitable nutrient medium supplemented

with plant growth regulators have a good growth rate and have a potential for accumulation and sustained production of secondary metabolites (Hahn et al., 2003).

Adventitious root formation is a complex process involving various endogenous and exogenous factors (Sorin et al., 2005). The adventitious root formation process is divided into four stages: (a) the pre-emergence stage of the root, which includes the molecular and biochemical process changes that occur prior to any cytological formation up to the emergence of primordial roots, (b) the early stage of root development, (c) the root growth stage (weight and volume increase) and (d) the final stage of root configuration (emergence of the first root) (Zhang et al., 2017). The initiation and differentiation process during the physiological stages of rooting can be triggered by changes in endogenous auxin concentrations and external addition of specific auxins (Praveen et al., 2009).

Adventitious roots show high stability, high growth rate, and continuous secondary metabolite production when stimulated in an aseptic artificial nutrient medium supplemented with optimal phytohormone (Hahn et al., 2003). These roots produce high amounts of alkaloids, terpenoids, and phenols in cell and tissue spaces and show high stability and growth rates; they can be easily produced in a suitable hormone-supplemented environment with a low amount of inoculum (Sivakumar et al., 2006). Adventitious root cultures also provide an experimental system to study the link between primary and secondary metabolism (Ahmad et al., 2015).

Radish (*Raphanus sativus* L.), one of the important vegetables of the *Cruciferae* family, is one of the functional foods that have an important place in meeting people's fresh vegetable needs and attracts attention with its nutritional content and health-improving properties (Akan et al., 2013) such as antibacterial, antioxidant, antimutagenic, hepatoprotective, nephroprotective, antidiabetic, anti-inflammatory activities (Rosés et al., 2023).

In the current study, hypocotyls obtained from red and black radish seeds germinated *in vitro* were used as starting material, and Murashige and Skoog (MS) basic medium at different strengths (¼, ½, ¾, and 1) and various amounts of sucrose (20, 25, 40 and 50 mg L^{-1}) were used to reveal their effect on adventitious root formation regarding biomass accumulation.

MATERIALS AND METHODS

The study was carried out in the Tissue Culture Laboratory of Akdeniz University, Faculty of Agriculture, and Department of Horticulture.

Explant and Media Preparation, Explant Culturing

Seeds of red and black radish cultivars were used in the study. Seeds of the cultivars were subjected to surface sterilization before being cultured. The seeds were first kept in 70% ethanol solution for 1 minute and then sterilized in 50% sodium hypochlorite solution for 12 minutes, and then, it was rinsed 3 times with sterile distilled water.

Sterilized seeds were cultured in MS (Murashige and Skoog, 1962) medium (MS0) containing 30 g L^{-1} sucrose and 6 g L^{-1} agar, without the addition of plant growth regulators, to germinate under *in vitro* conditions. Cultured seeds were incubated under 24±2 °C temperature and 16/8 hour light/dark photoperiod conditions. For seed germination under *in vitro* conditions, 15 glass jars with a volume of 660 mL were used for both cultivars, and 15 seeds were planted in each jar.

The 15-day-old hypocotyls from the germinated plantlets were used as starting material for the adventitious root culture study. Hypocotyls were cut into 0.5 - 1 cm pieces in a laminar flow and cultured in the combinations of media presented in Table 1 for the initiation of adventitious root cultures. For each cultivar, 90 mm 5 petri dishes/medium were used while 15 hypocotyl explants were cultured in each petri dish and the study was carried out in 3 replications.

Table 1. Media combinations used in the study

Media Codes	Media Combinations			
	MS	IBA (mg L^{-1})	Sucrose (g L^{-1})	Agar (g L^{-1})
1	¼	2	30	6
2	½	2	30	6
3	¾	2	30	6
4	1	2	30	6
5	¾	2	20	6
6	¾	2	25	6
7	¾	2	40	6
8	¾	2	50	6
Control	1	-	30	6

Establishing and Propagating of Adventitious Root Cultures

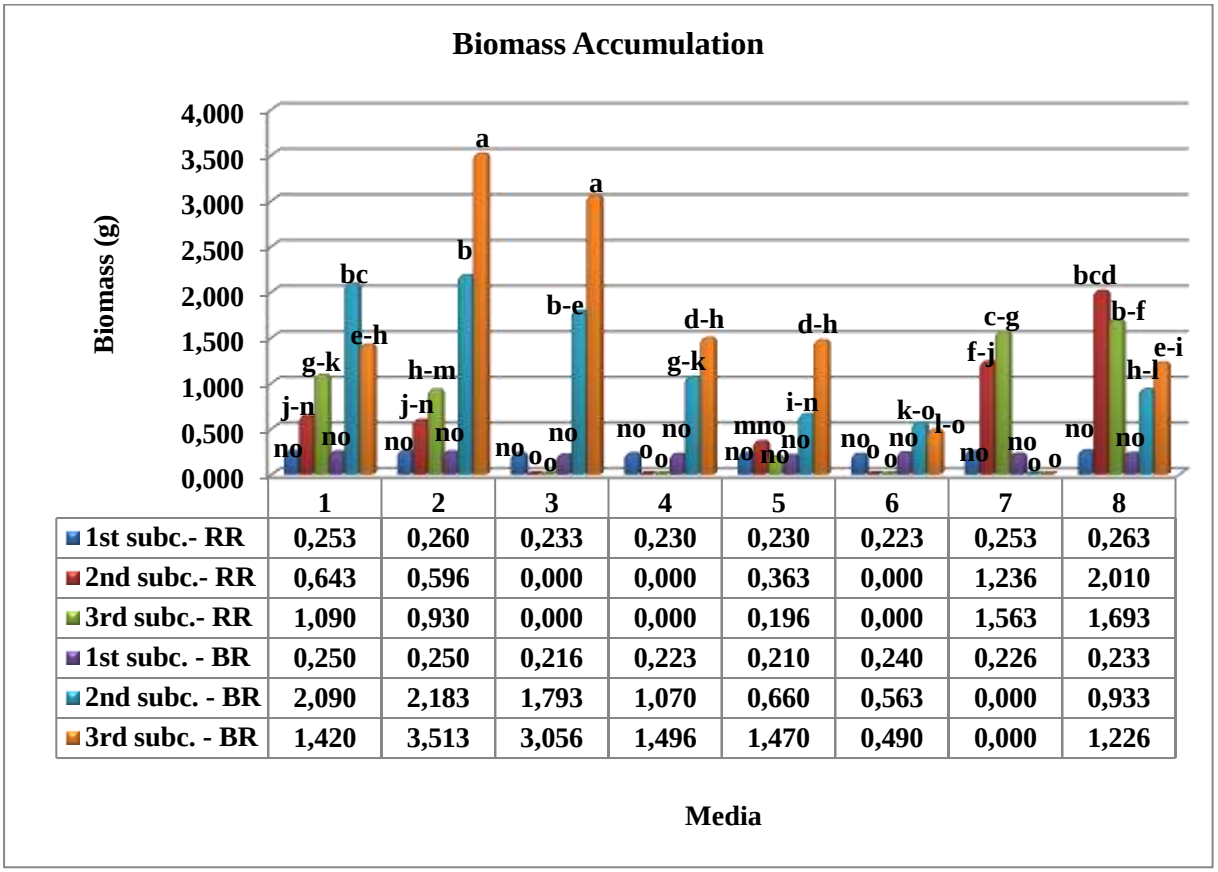
Adventitious root cultures were initiated with 2-month-old roots consisting of hypocotyl explants cultured in different combinations of media. Adventitious roots (0.15 - 0.20 g/petri) obtained from hypocotyls of black and red radish cultivars were inoculated into 100 mL conical flasks containing 30 mL of nutrient medium. The medium combinations used in the initiation of adventitious root cultures were used as liquid medium without adding agar for adventitious root propagation. Cultures were shaken at 130 rpm on an orbital shaker under dark conditions at 24±2°C for 4 weeks. Three subcultures with 4-week intervals were conducted in the study, and the weights of the proliferating adventitious roots at the end of each subculture were measured and recorded.

Statistical analysis

The experiments of the current study were conducted in three replicates with a completely random factorial design. The data obtained were made in the JMP package program and the differences between the averages were determined by the 'least significant difference' (LSD) test, and the differences were found to be statistically significant at the $p < 0.05$ level.

RESULT AND DISCUSSION

In this study, the effects of MS media of various strengths and sucrose at different concentrations on the initiation of adventitious root cultures and biomass accumulation in 2 different radish cultivars were evaluated (Figure 1 - 3).



	1	2	3	4	5	6	7	8
1st subc.- RR	0,253	0,260	0,233	0,230	0,230	0,223	0,253	0,263
2nd subc.- RR	0,643	0,596	0,000	0,000	0,363	0,000	1,236	2,010
3rd subc.- RR	1,090	0,930	0,000	0,000	0,196	0,000	1,563	1,693
1st subc. - BR	0,250	0,250	0,216	0,223	0,210	0,240	0,226	0,233
2nd subc. - BR	2,090	2,183	1,793	1,070	0,660	0,563	0,000	0,933
3rd subc. - BR	1,420	3,513	3,056	1,496	1,470	0,490	0,000	1,226

Figure 1. Biomass accumulation in radish cultivars regarding different media and subcultures
(1): Different letters among cultivars, media and subcultures denote significant differences (LSD test, $p<0.05$).
(2): LSD cultivars* = 0.115; LSD media* = 0.231; LSD subculture* = 0.142*; **(b)** LSD cult. x media x subc.* = 0.568
(3): Abbreviations; subc.= subculture; RR = Red Radish; BR = Black Radish

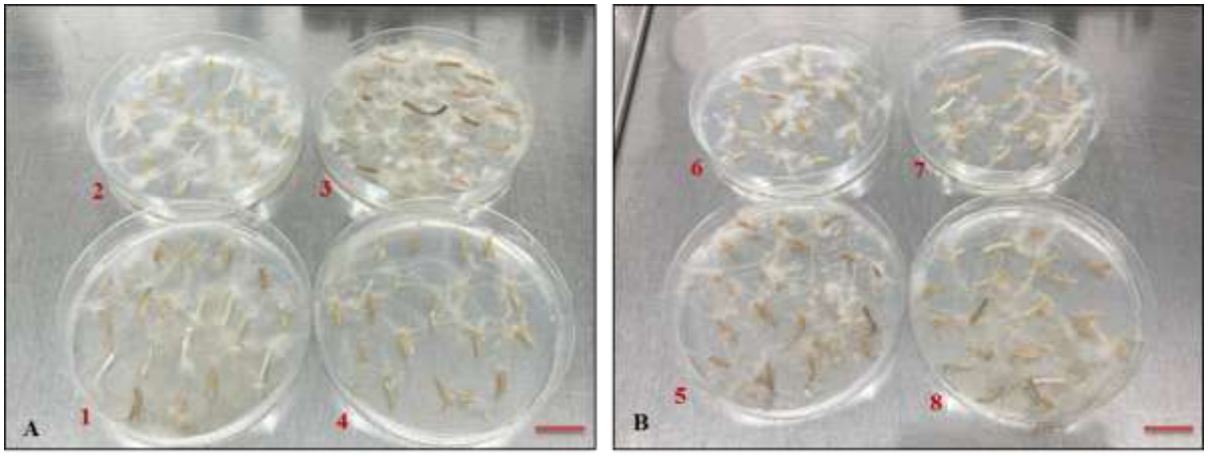


Figure 2. Adventitious root formation from hypocotyls of black (A) and red radish (B) cultivars in different media

Figure 3. Adventitious root cultures of black (A) and red radish (B) cultivars in different media

According to the findings, statistical differences were determined regarding biomass accumulation between radish cultivars. The responses of the cultivars to adventitious root cultures were found to be different in terms of biomass accumulation, and it was observed that the black radish cultivar provided higher biomass accumulation than the red radish cultivar.

Differences were detected in terms of the nutrient media combinations evaluated. The combination of media in which the ½ MS strength supplemented with 30 g L^{-1} sucrose (medium combination No. 2) gave the most positive value in terms of biomass accumulation, which was followed by the medium No. 8, contained ¾ MS strength and 50 g L^{-1} sucrose.

Among various plant cell and organ cultures, adventitious root culture is an alternative and complementary tool to whole plant cultivation to produce high-value phytochemicals (Hao and Guan, 2011; Baque et al., 2012b; Sivanandhan et al., 2013), a wide range of physiological It has a complex molecular process involving several factors (Sorin et al., 2005).

Previous studies have reported that auxins play a very important role in the adventitious root formation process, and that exogenous auxin applications as well as endogenous auxins are important in promoting the initiation and division of adventitious roots (Baque et al., 2009; Hussein et al., 2012). Indole butyric acid, IBA, is known as synthetic auxin and is among the first plant hormones used to increase root formation in plant stems (Deloso et al., 2020). For example, in a study, it was reported that IBA was more effective than IAA and NAA in stimulating adventitious roots from hypocotyl explants of *Psoralea coryfolia* (Baskaran and Jayabalan, 2009). In current study, IBA (2 mg L^{-1}) was used and had a positive effect on adventitious root development.

Success in adventitious root cultures is affected by various factors. As stated before, in addition to the effect of auxins, the salt strength of the medium, sucrose concentration, or explant type are among the determining factors for success in adventitious root cultures.

There are many studies showing that low salt strength may boost root induction and growth. Wu et al. (2006) found that *Echinacea angustifolia* adventitious roots required low levels of ambient salt strength for the production of root biomass. Baque et al. (2010) reported that a gradual decrease in fresh and dry weight was observed as the salt strength of the nutrient medium increased in the adventitious root culture study of *Morinda citrifolia*. Zhang et al. (2011) reported that a medium containing low salt strength provided a good performance in terms of induction and growth of adventitious root cultures of *Periploca sepium*. It is thought that one reason why low salt strength produces positive results on root biomass in different plant species may be that low salt strength leads to a decrease in osmotic pressure in cultures, which may be suitable for root primordium induction and growth (Zhang et al., 2011).

Another possible reason is that favorable interactions between nutrients in low salt strength cultures make it easy ions to reach to the roots (Wu et al., 2006).

In vitro plantlets require carbon sources in their artificial media for biological processes such as survival, growth, development and accumulation of bioactive compounds under aseptic and controlled conditions. Carbohydrates are an important source of carbon and energy in plant cell and organ cultures. Sugar added to the culture medium not only acts as a carbon source but also plays a role in the osmotic regulation of water stress (Hilae and Te-chato, 2005). The growth rate of biomass is directly associated with sugar consumption. It is thought that the increase in growth in adventitious roots may be due to the adventitious roots needing high sucrose for growth at the differentiation stage and structural integrity (Tremblay and Tremblay, 1995). Muthoharoh et al. (2019) investigated the effects of different sugar types and concentrations on root biomass in *in vitro* adventitious root culture of *Gynura procumbens* (Lour) Merr. Researchers obtained the highest fresh root biomass when 5% and 3% sucrose were used as carbon sources, respectively. Similarly, Manuhara et al. (2017) reported that an increase in fresh root weight was observed by increasing sucrose concentration, and the highest root weight value was obtained with the use of 5% sucrose. In another study, Ahmad et al., (2021) reported that there was an increase in biomass accumulation in stevia (*Stevia rebaudiana*) by adding a sucrose concentration as high as 50 g L^{-1} to the growing medium.

In an adventitious root culture study conducted by Adil and Abbasi (2019) in leaf cabbage (*Brassica oleracea* var. *acephala*) they found that the sucrose concentration significantly affected the adventitious root formation. Researchers, who revealed maximum adventitious root formation in MS medium supplemented with 40 g L^{-1} concentration of sucrose, reported that increasing the sucrose concentration to a level higher than 40 g L^{-1} adversely affected adventitious root formation in cotyledon explants. Similar results were reported by Wang and Weathers (2007) on *Artemisia annua* L. and by Baque et al. (2012a) on the plant *Morinda citrifolia* (L.).

Different explants, including leaves, stems, petioles, roots, and hypocotyl parts, can be used for the induction of adventitious roots *in vitro* in many industrially important plant species (Paek et al., 2009; Sharma et al., 2013; Kawakami et al., 2015; Khan et al., 2015; Khan et al., 2017; Saeed et al., 2017). For this reason, it is thought that the different results obtained in terms of biomass in adventitious root cultures of various plant species may be also due to differences in species, varieties, and explants.

CONCLUSION

In light of the results obtained from the study, differences were determined among the cultivars. Decreasing the MS strength to a certain extent or increasing the sucrose concentration added to the medium with decreasing MS strength led to an increase in the biomass obtained from adventitious root cultures in radish. Considering that adventitious root cultures are an important alternative method for producing high-value phytochemicals, it is thought that within the framework of the results obtained, adventitious root cultures of radish can be used as a complementary method in the large-scale production of valuable phytochemicals to be used in different industries such as pharmaceutical industry.

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WHAT HAPPENS WHEN PLASTICS ARE DISPOSED INTO THE ENVIRONMENT? MICROPLASTIC IN POLLUTION THE OCEANS

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ABSTRACT

Plastics are indispensable materials in daily life due to their properties such as being easy to process, durable, lightweight and cheap. Due to its widespread use, it is also one of the biggest pollutants in terms of the environment today. It is known that the increasing use of plastic creates serious pollution, especially in oceans and seas. One of the most important plastic groups that pose a problem is microplastics, which have a high probability of being ingested into living bodies due to their size. Microplastics can be directly incorporated into aquatic ecosystems, or they can emerge when large-sized plastics break down and disperse as a result of natural processes. Microplastics have become a major threat to both aquatic organisms and human health. The interdisciplinary nature of microplastics research necessitates an approach that requires integrity that transcends scientific fields. The aim here is to raise awareness of the possibility that plastic waste that is used and thrown away and whose outcome is not considered may eventually return to the human body as microplastic. To achieve this goal, the history, classification, sources, contamination with nature, disposal methods, effects on the food chain and environmental risks of microplastics were investigated.

Keywords: Plastics, Microplastics, Ecosystem pollution, Living organisms

INTRODUCTION

Plastics are a class of long-chain organic polymers with high molecular weight (Liu et al., 2016). More than 45 different types of plastics are used in commercial production, including types such as polypropylene (PP), polyethylene (PE), polyethylene terephthalate (PET), polystyrene (PS), polyurethane (PU), polyvinyl chloride (PVC) and polycarbonate (PC) (Halıcı Demir, 2024; Kannan and Vimalkumar, 2021).

In this century where we live in the plastic age, plastics are used in every field including chemistry, materials science and the food chain, and plastics are seen as the biggest pollutants of our age. Plastic materials that have entered our lives in various ways, such as simple food packaging, synthetic fibers, car tires, and clothing containing plastic, cause irreversible environmental problems in air, water, and soil quality (Fonseca et al., 2017; Aslan, 2018). Marine environments are among the places most affected by plastic pollution. It is known that plastics, which pass into the environment in different ways, create plastic litter on ocean surfaces through precipitation and flows (Akhan et al., 2022; Yurtsever, 2018; Botterell et al., 2019).

Today, plastic pollution has become a global environmental and public health problem. According to Plastics Europe, global plastic production was 390.7 million tons per year in 2021 and only 8.3% of the production was met using recycled plastic (Plastics Europe and EPRO, 2022). It has been reported that global plastic waste production was 60 to 99 million metric tons annually in 2015, and this number will triple by 2060, reaching 155-265 metric tons (Lebreton and Andrady, 2019). It is estimated that unless effective improvements are made in existing waste treatment systems, the world's environment (land, freshwater, ocean, air) will be filled with 13.2 billion plastic wastes by 2050 (Halıcı Demir et al.,

2024; Silva et al., 2020). When we add to this the fact that petroleum-derived plastics are not biodegradable and have long-term durability, it is estimated that plastic pollution resulting from human activities will continue for centuries. Therefore, plastic and especially microplastic are considered as one of the global, environmental and economic pollutants (Botterell et al., 2019).

1. What is Microplastic?

Exposure to sunlight, oxygen reaction and physical effects of plastics of various shapes and sizes entering water resources cause them to break down into small pieces as a result of their degradation. In the early 1970s, millimeter-scale plastics were first observed in the sea and in fish (Carpenter et al., 1972). These microscopic pieces of plastic are called microplastics (Fonseca et al., 2017; Aslan, 2018; Welle and Franz, 2018). The term microplastic was first defined by Thompson et al. (2004) as plastic particles smaller than 5 mm (Andrady, 2011). This definition was developed to represent synthetic solid particles or polymeric matrices with sizes ranging from 1 µm to 1 mm, of regular or irregular shape, originating from primary or secondary production and insoluble in water (Verschoor, 2015; Lusher et al., 2017).

In a study conducted by Biber et al. in 2019, the degradation and microplastic formation potential of polyethylene, polystyrene, polyethylene terephthalate and biotene (Biothene) compounds were evaluated by sampling at certain periods during a 600-day exposure period (in the shade and under sunlight, on land and in the seawater environment). While all samples kept in the land environment under sunlight decomposed faster than other conditions, it was observed that polystyrene was the fastest decomposing compound among these compounds. In addition, it has been reported that disintegration on land is faster than in seawater and direct light accelerates disintegration (Çelik et al., 2022).

2. Sources and Forms of Microplastics

Microplastics are divided into two groups according to their sources: primary and secondary microplastics. Primary microplastics are the name given to industrial microbeads of various sizes and are spread into the environment as contaminants during stages such as production and transportation (Andrady, 2017; Yurtsever, 2015). The main source of microplastics in oceans and seas is secondary microplastics (Barnes et al., 2009). These are released into the air, soil and seas as a result of the breakdown of plastic materials and garbage used by anthropogenic effects and/or natural effects such as water, air, wind and sun (UV rays) (Andrady, 2017; Esmeray and Armutçu, 2020).

Microplastics have attracted the most attention among plastic-derived pollutants in recent years. Microplastics can be found in many morphological forms, such as amorphous, spherical and long fiber forms (Abu-Hilal and Al-Najjar, 2009; Yurtsever, 2015). Most fragments found in tidal and estuarine sediments consist of fibers (Browne et al., 2011; Thompson et al., 2004). The shapes of microplastics vary depending on the duration of their residence in the environment as well as the degradation processes they undergo (Doyle et al., 2011). Microplastics also differ in many aspects such as size, composition, weight, physical and chemical properties (Esmeray and Armutçu, 2020).

3. Mikroplastiklerin Fizikokimyasal Özellikleri

Microplastics have properties such as having hydrophobic surfaces, floating ability, potential to carry pollutants, ability to absorb pollutants such as polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT), UV photo-oxidative degradation, thermo-oxidative degradation, bio- and/or thermal degradation, and binding on biomass, especially in biofilms (Hidalgo-Ruz et al., 2012; Wright et al., 2013). Because they are essentially lipophilic, persistent organic pollutants accumulate in the fatty tissues of marine organisms. Due to these features, they potentially cause many negative effects (e.g. cancer, malformation, deterioration in the immune system and reproductive ability) for humans and other living things (Esmeray and Armutçu, 2020).

4. The Effects of Microplastic on Marine Creatures and the Food Chain

The gradual breakdown of plastic waste over time and the release of microplastics from various sources have the potential to have negative impacts on aquatic life and ecosystems. These infinitesimal plastic particles can be ingested by aquatic organisms, pass into the food chain, and cause harm to various

species, including humans. The prevalence of microplastic pollution stems from inadequate control of plastic use and disposal and has become a critical focus of environmental sustainability concerns (Bozma et al., 2023; Steensgaard et al., 2017; Hartmann et al., 2019; Soysal, 2024; Toussaint et al., 2019).

Aquatic organisms can become contaminated with microplastics (and nanoplastics) by consuming water contaminated with microplastics or by feeding on other organisms exposed to microplastics, leading to human exposure to microplastics. Additionally, fish can become contaminated during storage and transportation in flexible polystyrene plastic containers after catching (Revel et al., 2017). Plastic particles have been detected in various organisms from the lowest food chain, such as zooplanktonic organisms, to the highest levels in invertebrates (Crustacea, mollusks, etc.) and vertebrates (fish) (Esmeray and Armutçu, 2020; Van Cauwenberghe et al., 2013).

It is reported that microplastics will accumulate in biological tissues if they are continuously transferred from one trophic level to another. It is reported that marine creatures, especially those consumed whole, such as mussels, can be a vector for the transmission of microplastics to humans (Gedik and Eryasar, 2020). In one study, trophic transfer of labeled microplastics found in mussels to crabs was observed. Accordingly, it was observed that the amount of microplastics in crab tissues and hemolymph fluid increased in the first 24 hours. After 24 hours, 0.28% of the microspheres estimated to be retained by the mussel were detected in the crab hemolymph (Çelik et al., 2022; Farrell and Nelson, 2013).

In the study conducted by Nelms et al., microplastic transfer was observed from Atlantic mackerel (*Scomber scombrus*) caught in the Celtic Sea to grey seals (*Halichoerus grypus*) (Nelms et al., 2018). In the study conducted by Batel et al., it was reported that microplastic particles between 1 µm and 20 µm were transferred from artemia larvae (*Artemia nauplii*) to zebrafish (*Danio rerio*) (Batel et al., 2016; Çelik et al., 2022).

The presence of microplastics in seafood is a known fact and is the most important factor endangering food safety. Since microplastics are plankton-sized, they can be ingested by marine organisms (Farrell and Nelson, 2013; Naik et al., 2020). Marine creatures cannot break down microplastics with enzymatic activity after swallowing them, and these plastics continue to exist in the gastrointestinal system of the creature. Additionally, microplastic particles remaining in the intestinal tract block the tract and lead to decreased food digestion. It is predicted that this situation may reduce nutrition and energy assimilation in aquatic organisms (Browne et al., 2015) and, consequently, a decrease in the development and fertility of the organism may be observed. It has been reported that microalgae living in the sea are also affected by the presence of micro- and nanoplastics, and that the presence of microplastics in these organisms leads to a decrease in the photosynthetic activity of the organism (Sjollema et al., 2016).

Many marine mammals, invertebrates, fish-eating birds and fish ingest these microplastics. These plastic particles are seen in carcass dissection and especially in the digestive system. It has also been determined that micro- and nanoplastic particles can pass into surrounding tissues and organs through the circulatory system (Gülçiçek Uysal, 2021; Akçay et al., 2020; Lusher et al., 2017; Smith et al., 2018).

Studies examining the presence of microplastics in aquatic areas mostly focus on the digestive systems of organisms exposed to microplastics. This is because microplastics in the environment are ingested by aquatic creatures and become part of the food pyramid. Ingested microplastics have been shown to negatively impact development, accumulate in the liver and cause inflammation, and serve as a vector for the accumulation of adsorbed chemical pollutants (Lu et al., 2016; Zhang et al., 2017). However, more studies are needed on creatures such as marine mammals located at the upper levels of the food pyramid (Akdogan and Guven, 2019; Çelik et al., 2022).

Microplastics have the potential to be consumed and inhaled by humans, especially through the water and food chains. These particles can raise questions on the overall health of the organism through a variety of biological and toxicological effects. Additionally, there are concerns about the possibility that the chemicals contained in microplastics could cause potential toxic effects when ingested. However, the fact that research in this area is limited and clear results have not been reached highlights the need for further studies on the effects of microplastics on human health. In this context, the lack of

knowledge about the potential health risks of exposure to microplastics increases the importance of research in this area (Bozma et al., 2023; Muniasamy et al., 2023; Chowdhury et al., 2023).

5. What Methods can We Use to Dispose of Microplastics?

While 380 million tons of plastic are produced annually worldwide, less than 20% of this amount can be recycled. The breakdown of plastic waste into monomers by biological methods appears to be an efficient and economical method in the process of incorporating plastics into the reuse cycle (Çelik et al., 2022). Although there are different ways to dispose of the non-recyclable part, they are not sufficient in today's conditions.

Waste incineration: While the majority of plastics that cannot be included in the recycling cycle are disposed of in waste incineration units, some are accumulated in landfills, and a significant portion mixes with terrestrial and aquatic areas.

Density separation: Density separation is the process of separating the lighter materials from the heavier ones by using the buoyancy force of the liquid and the desired materials and other undesirable materials by taking advantage of the density difference after the mixture materials are thoroughly shaken (sodium chloride, sodium iodide, zinc chloride, sodium poly tungstate) and the liquid are mixed homogeneously. Density separation is a widely applied method to isolate microplastics from environmental samples, especially sediment samples (Hidalgo-Ruz et al., 2012; Crawford and Quinn, 2017).

Sieving: This is another frequently used method for isolating microplastics from water and sediment matrices. Sieves are usually made of metal such as stainless steel or copper (Wang and Wang, 2018). The sieve physically captures solid materials larger than the mesh size and removes water and smaller particles from the sample. The mesh size of the sieves mainly depends on the desired size range of microplastics to be collected, and most of them range from 0.035 to 4.75 mm (Esmeray and Armutçu, 2020; Hidalgo-Ruz et al., 2012; Zobkov and Esiukova, 2018; Rose et al., 2016).

Filtration: Filtration is a widely used and effective method for separating microplastic particles from liquids using a filter medium that only allows the liquid to pass through. The media used for filtration include glass fibers as the most commonly used filter and some other filters such as nitro-cellulose, polycarbonate membranes, zooplankton filters or isopore filters (Hidalgo-Ruz et al., 2012; Wang and Wang, 2018; Collard et al., 2017). The pore size of filters is generally between 0.45 and 20 mm. Although filtration is a simple process to separate microplastics from liquids, complications often arise as liquids are full of various microscopic particles or debris that can quickly clog the filter media, thereby reducing its effectiveness (Rose et al., 2016). This disadvantage can be mitigated by various useful measures, such as reducing the solution volume, settling the liquids for a longer time to facilitate the separation of heavier solid particles from the supernatant, and performing a pre-filtration step using a filter with a larger pore size. Additionally, it can be alleviated by adding some chemicals (e.g., iron sulfate) to the liquid to make the solid fraction precipitate (Esmeray and Armutçu, 2020; Wang and Wang, 2018).

Chemical methods for microplastic removal: Many wastewater treatment plants worldwide use coagulation and agglomeration processes to create larger pollutant particles that are easier to separate from water (Shirasaki et al., 2016). These processes include the use of Fe and Al based salts and coagulants to bind small particles through complexation mechanisms and thereby create strong bonds between waste particles (Chorghe et al., 2017). Ariza-Tarazona and colleagues (Ariza-Tarazona et al, 2019) investigated the removal of polyethylene microplastics from water using iron, aluminum salt and ultrafiltration. Experiments were carried out under different concentrations of $Al3^{+}$ and $Fe3^{+}$ ions, and the results showed that $Al3^{+}$ had better performance than $Fe3^{+}$ (Gülçiçek Uysal, 2021).

Biological Removal: According to the results of studies published to date, biodegradation of natural and synthetic macroplastics is possible (Ahmed et al., 2018). The fragmentation and size change of polyethylene microplastics ingested by Antarctic Krill, a planktonic crustacean, was studied by a group of researchers in Australia (Dawson et al., 2018). Although the type of degradation mechanism between microplastics and zooplankton remains unclear, it has been confirmed that smaller microplastics can be degraded very easily under environmental conditions (Ter Halle et al., 2016).

Paço and colleagues (2017) investigated the use of *Zalerion maritimum*, a naturally occurring fungus in marine ecosystems, for the biodegradation of polyethylene microplastics based on mass and size variations of microplastics in a batch reactor. Results obtained from biological content measurements, such as the decrease in protein amount and the increase in carbohydrates with exposure time, indicated that the *Z. maritimum* community probably used microplastics as a food source.

Microplastic removal with bacteria: The biggest disadvantage of plastic polymers is that they accumulate in nature without breaking down due to their largely inert chemical structure. Plastics are highly resistant to the hydrolysis of microbial enzymes due to reasons such as the low activity of the functional groups in the carbon-carbon skeleton that constitutes the polymers, the high hydrophobic character of the polymers and the low mobility of the groups within the polymer chain (Taniguchi et al., 2019). On the other hand, it is known that the cutinase enzyme, which breaks down the cutin layer in plants, can also exhibit hydrolytic activity on aromatic polyesters such as PET. The polyesterase activity of cutinase was first reported in 2005 by a German research group in their study using cutinase enzyme from *Thermobifida fusca* (Müller et al., 2020). Studies in the following years revealed that cutinase plays an important role in the hydrolysis of polyesters (Tokiwa and Calabia 2007; Zimmermann and Billig 2011; Baker et al., 2011). However, more than 50 years have passed since plastics came into use. It can be assumed that the microorganisms found in areas where PET and similar polymer wastes are concentrated have adapted to break down these polymers over time. It was determined that the PET hydrolysis activity of the enzyme called PETase was 120 times higher than the enzyme from *Thermobifida fusca* and 88 times higher than the enzyme from Fusarium solani (Çelik et al., 2022).

Auta and colleagues (Auta et al., 2017) investigated the removal of different structures of microplastics consisting of polyethylene, polystyrene, polyethylene terephthalate and polypropylene by *Bacillus cereus* and *Bacillus gottheilii*. Morphological and structural changes were investigated using electron microscopy and FTIR analysis, and the biodegradation rate was evaluated by measuring microplastic weight loss. The fastest mass reduction and shortest degradation half-life were found for polystyrene microplastics using the *B. cereus* isolate. According to the degradation results scanned by FTIR analysis, by comparing bond separations and chemical changes, it was revealed that the *B. gottheilii* species was the best in microplastic degradation (Gülçiçek Uysal, 2021).

DISCUSSION AND CONCLUSION

Studies show that micro- and nanoplastics are persistent pollutants in the environment and are accumulating in terrestrial and aquatic ecosystems at increasing rates. Pollution of oceans, land and aquatic systems with microplastics is not only considered an ecological damage, but also a threat to food security and, consequently, human health. Studies have shown that marine organisms in particular are exposed to high levels of microplastics (Akçay et al., 2020).

The use of synthetic textile products has become almost irreversible due to their affordability and practicality in meeting the needs of the growing population. But it would be good if washing machine manufacturers would at least do research and produce washing machines that can retain plastic microfibers to prevent synthetic textile fibers washed in washing machines from entering the sewer system. Additionally, control and research may be required regarding the production of synthetic textile products such as fleece, polyester, and fiber and the reduction of fibers that will shed during washing (Yurtsever, 2015).

Tackling this complex problem must include strong environmental education initiatives to raise awareness and understanding of the impacts of microplastic pollution. Additionally, it is critical to develop and implement more effective polymer waste management practices to limit the spread of microplastics in various ecosystems. To complement these efforts, there is a compelling need for ongoing research to identify, develop, and utilize effective and economically sustainable technologies to systematically remove microplastics from our environment. Comprehensive and collaborative

monitoring of these strategies can pave the way for sustainable solutions to mitigate the impact of microplastics on ecosystems and human well-being (Soysal, 2024).

Various actions such as environmentally responsible behavior, sustainable use of natural resources, reuse of waste, recycling of waste, separation and disposal are very important to prevent microplastic pollution. For a sustainable environment, production and consumption must also be sustainable. Conscious consumption and sustainable use of resources focus on achieving a certain quality of life and providing basic needs without endangering the needs of future generations.

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EXAMINING THE EFFECT OF SUSTAINABILITY-FOCUSED CERTIFICATION IN PALM OIL PRODUCTION ON PRODUCER AND CONSUMER AWARENESS

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ABSTRACT

Palm oil is a vegetable oil used for many food and non-food applications. The global demand for palm oil will continue to rise, owing to the growing population and expanding economy. To meet increasing demand, the area of land dedicated to palm oil production in producer countries in Southeast Asia such as Malaysia and Indonesia has increased steadily. Palm oil establishment in these areas has resulted in widespread deforestation, deterioration of ecological balance, and increased greenhouse gas emissions. To avoid these problems and fulfill the growing global demand for palm oil, voluntary certification under the International Roundtable Palm Oil (RSPO) and Malaysian Sustainable Palm Oil (MSPO) emerges as an efficient solution. These certifications aim to ensure sustainability, optimization of productivity and efficiency while adhering to transparency, ethical, and legal principles, respecting communities, supporting smallholders, and protecting workers while conserving the ecosystem. A related study examines the impact of sustainability-focused certification on product quality characteristics and stakeholder awareness in palm oil production. An assessment was conducted with approximately 104 individuals involved in the palm oil supply chain, either as producers or users. The assessment covered recognition of sustainability concepts, awareness of health benefits associated with palm oil and brand perception about certification in the context of purchasing products containing palm oil. This study has the potential to serve as a model for studies that will contribute to the advancement of conscious consumption and circular production in palm oil sustainability certification parameters. Preliminary results revealed that producers and consumers are aware of sustainability. Moreover, sustainability certification might positively affect brand recognition and purchase tendency for palm oil products. Developing efficient marketing and informative advertising based on scientific facts is crucial to maintaining and expanding awareness of the sustainability concept of palm oil and its products.

INTRODUCTION

Palm oil, widely used in food, cosmetics, and biofuels, has become a critical subject in environmental and health discussions due to its association with deforestation, biodiversity loss, and greenhouse gas emissions (Wiebe et al., 2019). To mitigate these concerns, sustainability certifications such as the Roundtable on Sustainable Palm Oil (RSPO) have been developed, aiming to promote environmentally responsible palm oil production (RSPO, 2021). Upon the demand of consumers for the use of certified palm oil; the Indonesian government introduced the Indonesian Sustainable Palm Oil (ISPO) standards in 2011, and the Malaysian government introduced the Malaysian Sustainable Palm Oil (MSPO) standards in 2015. In this direction, the establishment of well-planned and traceable palm oil production has a great determination on the protection of the environmental ecosystem in safe and healthy production (Ivancic and Koh, 2016). As of December 31, 2019, MSPO has become mandatory for all Malaysian palm oil producers and 639,407.61 hectares were registered as independent smallholders' plantations, 444,872.36 hectares were registered as organized smallholders' plantations,

and 3,600,781.46 hectares were registered as total certified plantations. (MPOCC, 2024a). Making MSPO mandatory ensures that producers adopt a standard; therefore, a rapid increase has been achieved. The main objectives of the MSPO certification include; management commitment and responsibility, health worker safety and employment conditions, compliance with legal requirements, development of new plantations, and transparency (MPOCC, 2024b).

However, the success of these initiatives largely depends on consumer awareness and understanding of the sustainability issues surrounding palm oil (Taufique and Vaithianathan, 2018). Research has shown that socio-demographic factors like education and income significantly influence consumer behavior towards sustainable products (Diamantopoulos et al., 2003; Zhao et al., 2014). Moreover, gender differences have also been observed, with women generally displaying higher environmental awareness than men (Hunter et al., 2004; Zelezny et al., 2000).
Consumers' understanding of sustainability certifications plays a vital role in shaping purchasing behavior. While some studies indicate that sustainability labels influence purchasing decisions, knowledge gaps regarding these certifications persist, limiting their effectiveness. (Grankvist et al., 2004; Hartmann and Apaolaza-Ibáñez, 2012). Younger and middle-aged adults, typically more engaged in sustainability issues, are key drivers of demand for eco-friendly products.
This study seeks to assess consumer awareness and perceptions of palm oil, focusing on the acceptability of palm oil, knowledge of its uses, and the role of sustainability certifications in shaping purchasing decisions. By surveying a diverse sample, the study provides insights into consumer behavior and the factors influencing sustainable purchasing trends.

MATERIAL AND METHODS

This study employed a cross-sectional survey design to collect data on consumer awareness, perceptions, and behaviors related to palm oil and sustainability certification awareness. The survey was distributed online and person to person, simultaneously to a diverse sample of 104 participants across various demographics. The questionnaire included sections on general awareness of palm oil (e.g., familiarity with palm oil used in products), understanding of sustainable palm oil (e.g., knowledge of RSPO certification), and purchasing behavior (e.g., the influence of sustainability claims on buying decisions). Participants were also asked to rate the quality of palm oil-containing products and to identify differences between palm oil and other related oils. All responses were examined and presented as percentages on graphs.

RESULTS AND DISCUSSION

The results of the survey are shown in graphs in Figures. According to the findings given in Figure 1, the survey's participant profile, predominantly female (68.26%) and concentrated in the 25-44 age range, reflects a well-educated and relatively affluent group, with 52.88% holding a master's degree or higher and 40.38% reporting high income. This demographic, often linked to greater environmental awareness and sustainable consumption behaviors, likely contributed to the high levels of awareness and preference for sustainable palm oil observed in the survey, indicating a group well-positioned to make informed, eco-conscious purchasing decisions.

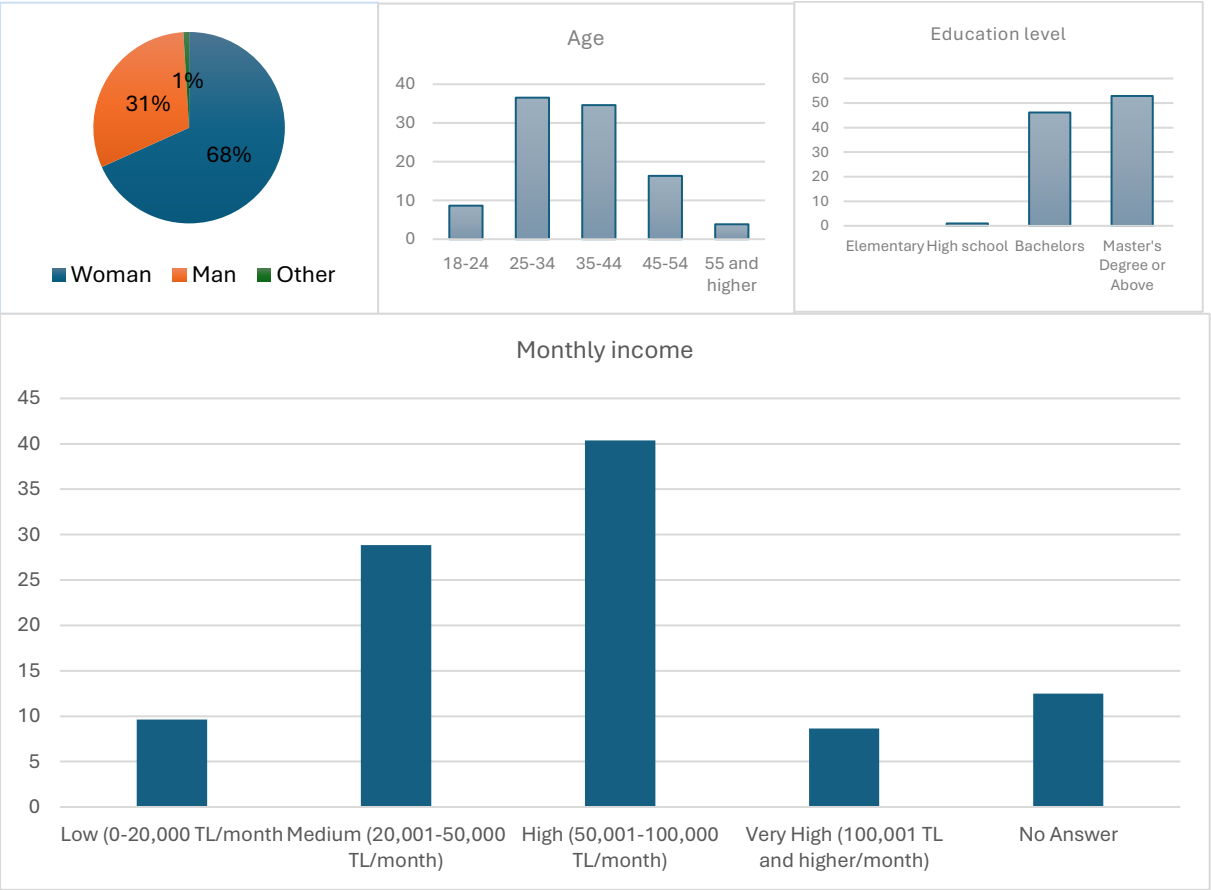

Figure 1. General information about the survey participants

The demographic profile of the survey participants, including their higher education levels, income, and gender distribution, aligns with findings from prior studies that associate these factors with increased environmental awareness and sustainable consumption behaviors. For instance some previous studies highlight that higher education and income are strong predictors of environmental concern and the likelihood of purchasing sustainable products (Diamantopoulos et al., 2003; Zhao et al., 2014). Additionally, in some previous research, it was reported that women tend to show greater environmental awareness, which could explain the higher proportion of female respondents in the survey (Hunter et al., 2004; Zelezny et al., 2000). The predominance of young to middle-aged adults, who are typically more engaged in sustainability issues, further supports the survey's findings of heightened awareness and preference for sustainable palm oil among this demographic (Liere and Dunlap, 1980).

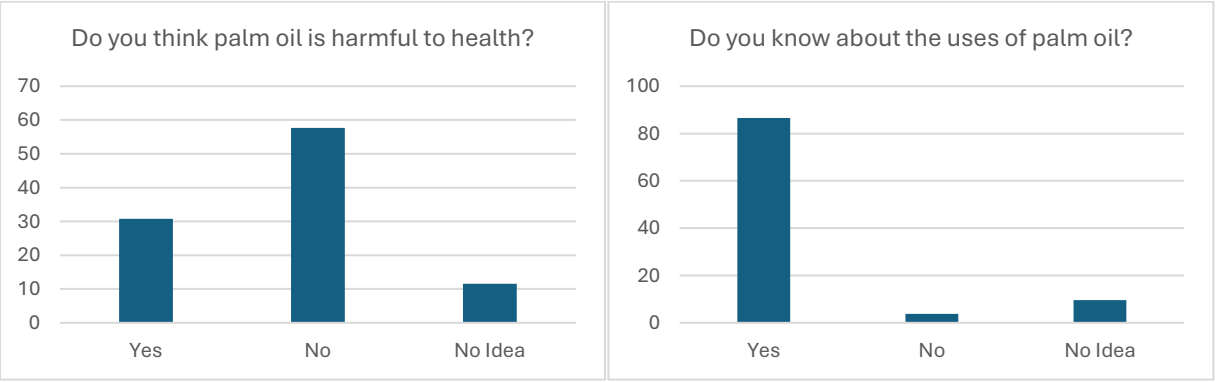

Figure 2. Palm oil acceptability of the customers

The results on palm oil acceptability among customers reveal a complex perception of this ingredient, which is influenced by various factors such as health concerns, awareness of its uses, and sustainability issues (Figure 2). While a significant portion of respondents (57.69%) do not believe palm oil is harmful to health, there is still a notable minority (30.76%) who perceive it as harmful, reflecting ongoing public debates and mixed information about palm oil's health impacts. Awareness of palm oil's uses is high, with 86.53% of respondents familiar with its applications, indicating that most consumers are well-informed about the prevalence of palm oil in products. However, there is ambiguity regarding the differences between palm oil and other oils, such as "hurma" oil, with respondents almost evenly split on this question, suggesting some confusion or lack of detailed knowledge about different types of oils. This response actually conflicts with the previous findings about how consumers are aware of palm oil, and its uses since "hurma oil" and "palm oil" technically address the same oil. Accordingly, it has been observed that a significant portion of consumers think that they have detailed information about palm oil and its uses, but even this simple difference in expression misleads consumers. This suggests that some marketing-oriented initiatives, such as advertising campaigns, significantly impact consumer perceptions.

These perceptions are crucial because they directly affect consumer behavior. For instance, while many consumers know palm oil and its uses, their health concerns and understanding of its sustainability aspects play a significant role in their purchasing decisions. The survey also shows that environmental sustainability (35.29%) and healthy living (32.35%) are key factors influencing purchasing decisions for products containing palm oil, reflecting a growing consumer trend toward products that align with personal health and environmental values. This aligns with previous studies, highlighting health and sustainability's increasing importance in consumer decision-making processes (Hartmann and Apaolaza-Ibáñez, 2012). Overall, the results suggest that while many consumers generally accept palm oil, its

acceptability is contingent upon ongoing concerns and knowledge gaps related to health and sustainability, which can significantly influence consumer preferences and behavior.
Although environmental issues positively affect the consumption of products containing palm oil, it has been seen that it is necessary to evaluate the price factor, which has a share in the consumption phenomenon, together with the product quality. When examined in this direction, the findings indicate a balance between the price and quality of palm oil products. The cost reduction in the prices of palm oil products has not been reflected in their quality at the same rate, and it has been determined that the effect of quality is moderate. The reason for this finding can be shown as the compatibility of palm oil processing methods with sustainability principles (Panapanaan et al., 2009; Gassler and Spiller, 2018).

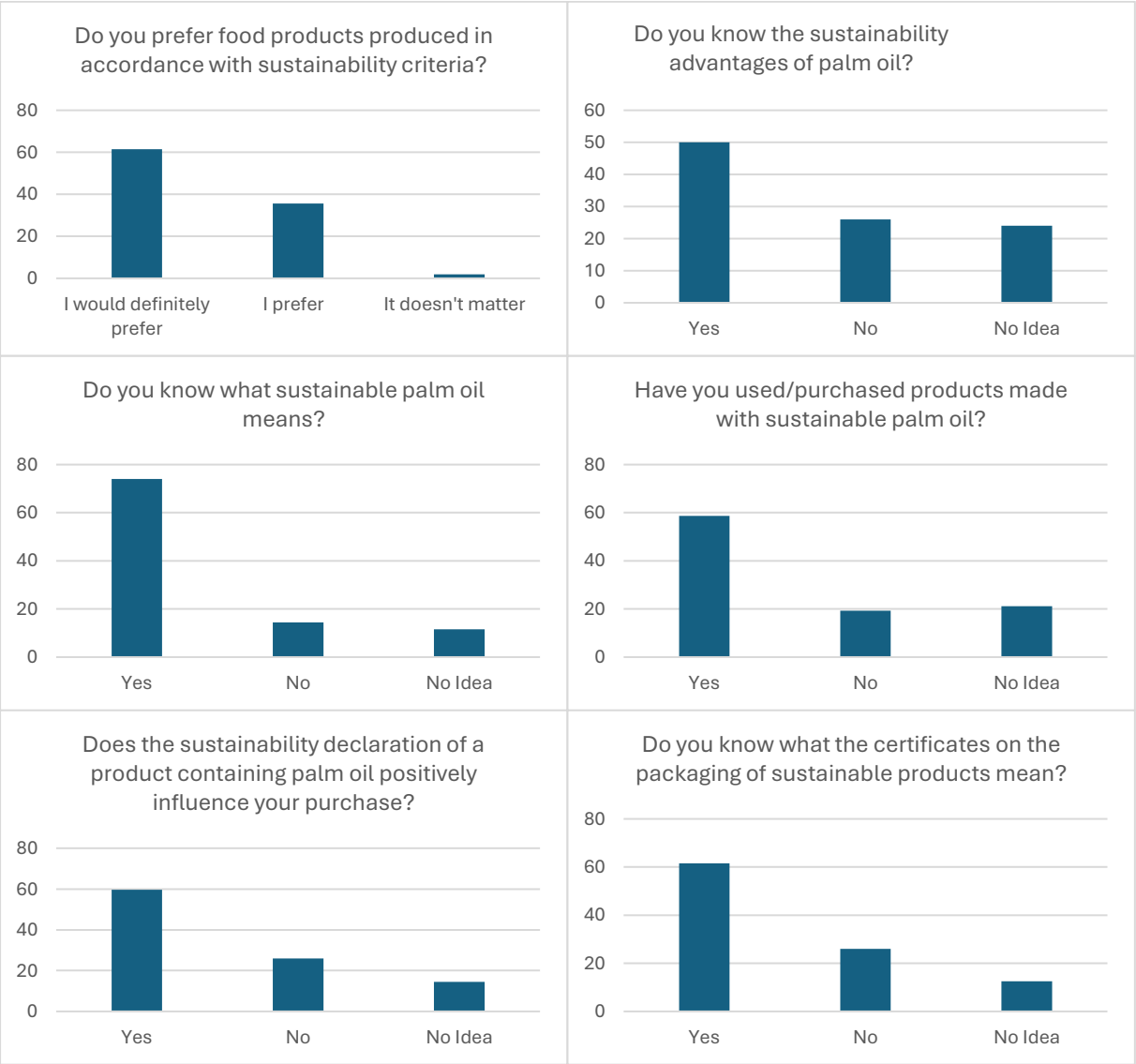

Figure 3. Sustainability awareness and sustainability certification on palm oil

-The results on sustainability awareness and palm oil sustainability certification indicate a relatively high level of consumer engagement with sustainability issues, though there are notable gaps in understanding (Figure 3). A significant majority of respondents (74.03%) reported knowing what sustainable palm oil means, and 50% were aware of the specific sustainability advantages associated with palm oil. This level of awareness reflects a growing consumer consciousness around sustainability, particularly in relation to palm oil, which has been widely discussed in environmental and ethical contexts due to its impact on deforestation and biodiversity.
However, despite this awareness, there is still a considerable portion of the population that is either unaware or unsure about the specifics of sustainability certifications. For instance, 25.96% of

respondents admitted they do not know what the certifications on product packaging imply, and 24.03% are unaware of the sustainability advantages of palm oil. This suggests that while consumers are increasingly aware of sustainability as a concept, their understanding of the detailed implications of certifications and specific sustainability benefits is incomplete.

The influence of sustainability certifications on purchasing behavior is also evident, with 61.53% of respondents indicating that such certifications positively influence their buying decisions. This aligns with findings from previous research, which demonstrates that sustainability labels and certifications can significantly affect consumer behavior, especially among those already inclined towards environmentally friendly practices (Taufique and Vaithianathan, 2018). Nonetheless, the knowledge gaps identified suggest that for certifications to be fully effective in driving consumer behavior, further efforts are needed to educate consumers on what these certifications represent and how they contribute to sustainable practices. Overall, the results highlight the critical role of informed consumer education in enhancing the impact of sustainability certifications on purchasing decisions.

CONCLUSIONS

The results highlighted the significant role of sustainability certifications in shaping consumer awareness and purchasing behaviors related to palm oil products. The findings indicate that while there is a high level of general awareness about sustainability, gaps remain in understanding specific certifications and their implications. Consumers, particularly those with higher education levels and incomes, prefer products aligned with environmental and health values. However, misconceptions and incomplete knowledge about palm oil and related products persist, suggesting more targeted educational efforts are needed. Future research should focus on exploring the long-term impact of sustainability education on consumer behavior and how marketing strategies can more effectively communicate the benefits of sustainable palm oil. Additionally, examining the role of pricing and quality perception in sustainable product choices could provide deeper insights into consumer decision-making processes.

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EFFECT OF POROSITY RATIO ON SOUND ABSORPTION IN STRUCTURES PRODUCED WITH 3D PRINTING

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ABSTRACT

Today, studies carried out for the purpose of noise control and increasing sound comfort bring the importance of acoustic insulation to the forefront more and more each passing day. In this context, porous materials used to reduce or absorb sound energy in various engineering applications offer efficient solutions. In addition, the development of layered manufacturing (3D printing) technologies in recent years has made it possible to design materials with controllable acoustic properties and customized internal structures. In this study, the sound absorption performances of samples produced with 3D printing technology using PLA (Polylactic Acid) material with different volumetric porosity ratios were comparatively examined. Sound was given to the input end of the experimental setup at fixed frequencies and sound pressure levels were measured in dB at the output end. The effect of the change in porosity ratio on sound absorption was evaluated.

Keywords: Acoustic performance, Sound insulation, Porous material, 3D printing, PLA

INTRODUCTION

With increasing urbanization, industrialization, and technological advancements, environmental noise pollution has become a significant problem. According to the World Health Organization (WHO), long-term exposure to loud noise can lead to many health problems, such as hearing loss, sleep disorders, stress, lack of concentration, and various heart diseases (WHO, 2018). This highlights the need to ensure acoustic comfort in many areas, particularly residences, workplaces, transportation systems, and industrial facilities. Therefore, efforts to control sound insulation and sound are gaining increasing importance every day.

In this context, porous materials are widely used for sound absorption and damping, based on the principle that sound waves disperse within the material's internal structures and lose their energy. The acoustic performance of such materials depends on many parameters, such as pore geometry, porosity, and density (Islam et al., 2023). In particular, volumetric porosity directly affects the sound absorption capacity of a material, as it determines the amount of voids within the material's internal structure. In recent years, the development of additive manufacturing (3D printing) technologies has made it possible to design much more complex and customized internal structures compared to traditional production methods. This allows the acoustic performance of porous structures to be examined in a controlled manner, and materials with desired properties can be produced. In particular, the use of biodegradable and environmentally friendly thermoplastics such as polylactic acid (PLA) provides sustainable solutions both environmentally and economically. Some studies on the subject have investigated the acoustic performance of porous structures with different geometries produced using composite materials

such as PLA and various natural fibers (corkwood, hemp, coffee waste, etc.) using 3D printing technology (Kang et al., 2025; Lyu et al., 2025; Rezaieyan et al., 2024). Studies have shown that materials exhibit better acoustic insulation properties as the porosity ratio increases.

In pipe acoustics, the wave equation is obtained using the one-dimensional wave equation:

$$\frac{\partial^2 p(x,t)}{\partial x^2} = \frac{1}{c^2}\frac{\partial^2 p(x,t)}{\partial t^2} \tag{1}$$

where p(x,t) is the acoustic pressure, c is the speed of sound, x is the position along the pipe axis, and t is time.

The behavior of acoustic waves within a pipe varies depending on the boundary conditions. In a pipe with both ends open, the fundamental resonant frequencies are expressed by wavelengths determined by the pipe length and are generally defined by the following equation:

$$f_n = \frac{nc}{2L}, (n = 1,2,3, \dots) \tag{2}$$

where c is the speed of sound, L is the pipe length, and n is the mode number. In pipes with one end closed, resonance occurs only at odd-numbered harmonics, and the fundamental frequency and upper harmonics are:

$$f_n = \frac{(2n-1)c}{4L}, (n = 1,2,3, \dots) \tag{3}$$

These equations determine the frequencies at which sound waves within the pipe exhibit significant amplitude increases. In acoustic material testing, these governing equations are used as a reference for experimental observations and play an important role in evaluating the sound absorption or damping performance of different materials placed within the pipe.

In this study, PLA samples with different porosity levels were produced using 3D printing technology, and their sound absorption performance was experimentally investigated. In the developed experimental setup, a sound signal generated at specific frequencies was applied to the inlet end of the pipe, and the sound pressure levels at the outlet end were measured in dB. The decibel difference between the inlet and outlet was analyzed to evaluate the effect of changes in porosity on acoustic performance. The results provide guidance for the design of customizable acoustic materials using additive manufacturing.

MATERIAL AND METHODS

The test samples used in this study were designed as cylindrical shapes with varying volumetric porosity levels. The samples had an outer diameter of 45.2 mm and a thickness of 25 mm. Technical drawings of the samples are shown in Figure 1. Porosity was controlled by reducing the fill ratio of the sample's internal volume. They were produced in seven different groups with volumetric porosity ratios of 10%, 20%, 30%, 40%, 50%, 60%, and 70%, respectively. The designed samples and their nomenclature are given in Figure 1.

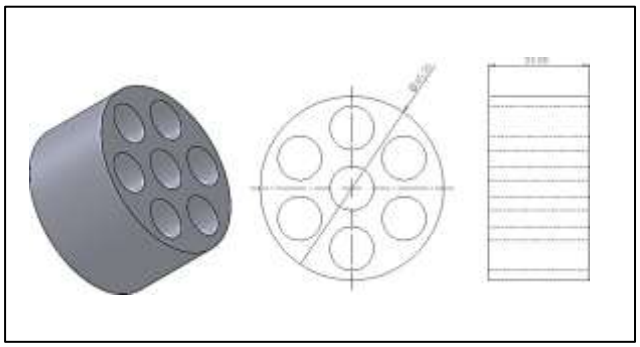


Figure 1. Sample dimensions

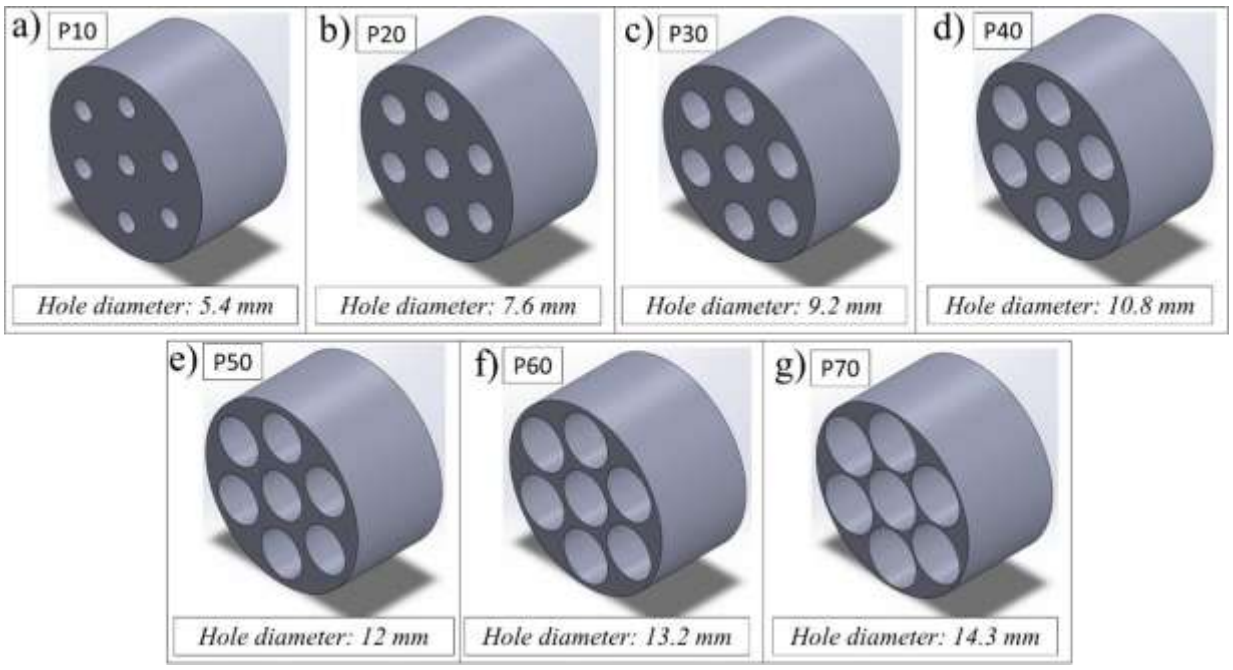


Figure 2. Samples with porosity ratio by volume a) 10%, b) 20%, c) 30%, d) 40%, d) 50%, e) 60% and f) 70%

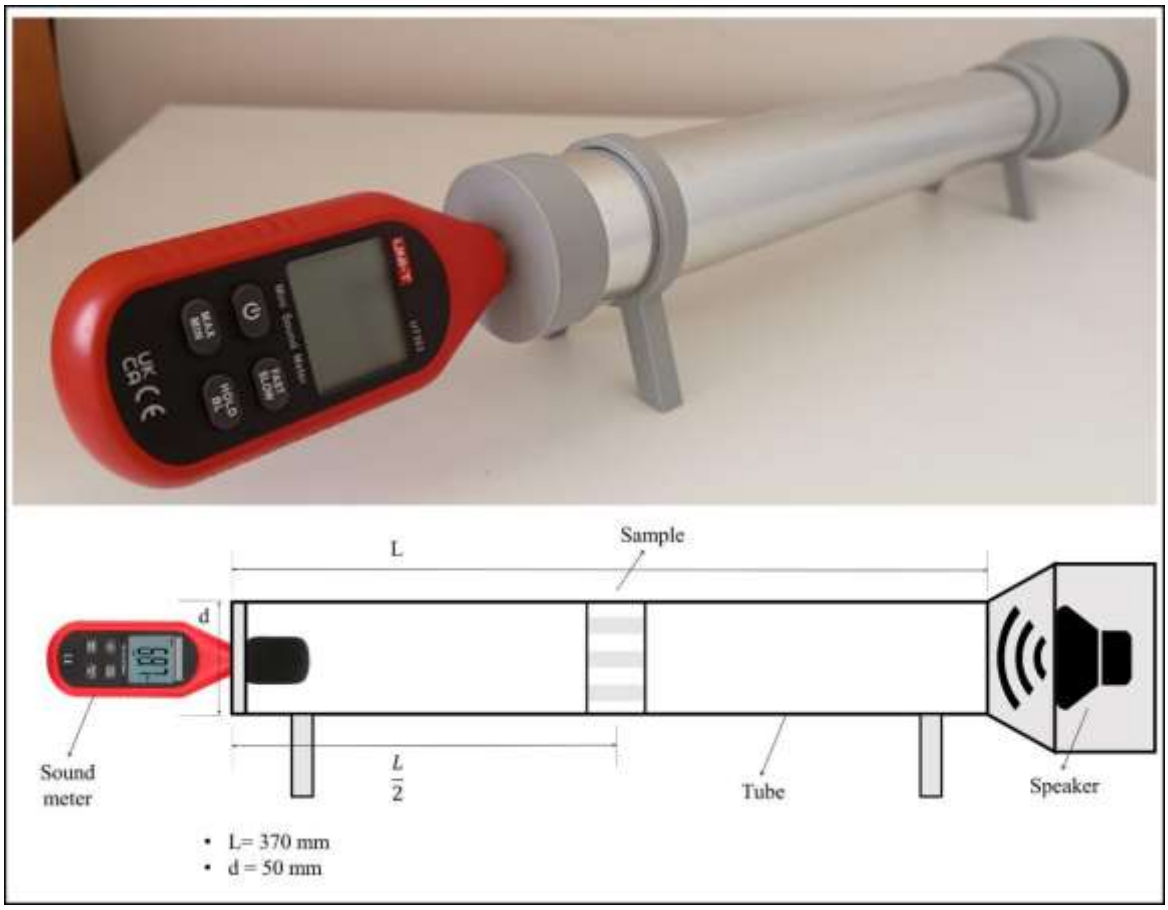
A FDM (Fused Deposition Modeling)-based 3D printer was used for sample production. The material used for sample production was commercially available biodegradable PLA filament. PLA is a frequently preferred thermoplastic in 3D printing applications due to its environmentally friendly structure, low melting temperature (~180–220 °C), and high print quality. PLA filament with a diameter of 1.75 mm was used in this study. Some printing and printer parameters used in sample production are listed in Table 1.

Table 1. Some printing and printer parameters.

Parameter	**Value**
Nozzle diameter	0.4 mm
Printing temperature	220 ˚C
Table temperature	50 ˚C
Printing speed	60 mm/s
Layer hight	0.2 mm
Infill density	%30

The experimental setup and its schematic representation designed to evaluate the sound absorption performance of the samples are given in Figure 3. The samples were placed in a tight fit inside a cylindrical tube. Measurements were made by placing all samples inside the tube individually and then sequentially (from 10% to 70% (P10-P70) and from 70% to 10% (P70-P10)). During the experiment, a loudspeaker was fixed to one end of the tube, and sound was introduced into the tube at fixed frequencies (100 Hz, 200 Hz, 300 Hz, 400 Hz, 500 Hz, 1000 Hz, 2000 Hz, 3000 Hz, 4000 Hz, 5000 Hz, respectively). A dB meter was placed at the other end to compare the sound attenuation performance of samples with different porosity levels. Each measurement was repeated three times, and the average values were taken. For each measurement, sound pressure levels were measured both inside the empty pipe and after the sample was placed inside. The difference between these values was used to calculate the sound loss in

Figure 3. Experimental setup and schematic representation



RESULTS AND DISCUSSION

In this study, the sound absorption performance of PLA samples with different volumetric porosity ratios was evaluated using a pipe-type test setup. Measurements were made at a total of 10 different frequencies between 100 Hz and 5000 Hz. This wide frequency range is important for observing the acoustic behavior of porous materials at different frequencies. The output SPL values of the samples based on their porosity ratios are given in Table 2. The dB losses calculated based on the initial SPL are presented in Table 3, and their graphs are shown in Figure 4.

When the values in the tables and graphs are examined, it is generally seen that the sound pressure levels measured at the output end decreased as the porosity ratio decrease demonstrating that samples with lower porosity ratios exhibited higher sound absorption performance. The highest dB difference was measured at 2000 Hz for the P10 sample, 21.6 dB. However, the dB difference was not the same at all frequencies. For example, at 500 Hz, a 9.6 dB decrease was observed in the P10 sample, while a 2 dB decrease was observed in the P70 sample. At 100 Hz, a 1.3 dB decrease was observed in the P10 sample, while a 2.2 dB decrease was observed in the P70 sample. This demonstrates that material-sound interaction is frequency-dependent and that different porosity ratios should be optimized for different frequency bands. Furthermore, the increase in dB losses is generally more consistent with increasing frequency. This supports the possibility of porous structures being used as effective absorbers, particularly for mid- to high-frequency sounds. In this context, it was concluded that special-purpose acoustic panels can be produced by designing porosity appropriate to the targeted frequency range.

Analysis of the measurement results revealed that sound absorption was limited at low frequencies (100–200 Hz) at all porosity ratios. This may be attributed to the longer wavelengths of low-frequency sounds, which, in turn, interact less effectively compared to the material thickness. Experimental measurements have shown that higher sound pressure levels are achieved at the exit end of a pipe compared to an empty pipe at certain frequencies. While this might initially appear to increase the sound level, contrary to expectations, it can be explained physically by considering the effects of resonance, reflection, and impedance within acoustic systems. When a sample is placed inside a pipe, the internal acoustic environment of the pipe changes, creating resonant conditions at certain frequencies. In this case, sound waves accumulate within the pipe, creating a higher pressure level at the exit end. This effect is particularly pronounced in short pipes and at certain resonant frequencies. Furthermore, some samples with low porosity exhibit reflective surface properties, reflecting sound energy back, which can cause phase overlap within the system, leading to an increase in the dB value measured at the exit end.

Arranging the samples in different orders also significantly affected the system's acoustic response. The P70–P10 and P10–P70 measurements represent cases where all samples were arranged consecutively within the pipe without leaving any gaps. These configurations demonstrate the sound attenuation performance of multilayer porous material systems. At certain frequencies, these combinations yielded more effective results. For example, at 1000 Hz, a 10.3 dB increase was observed for the P10 sample, a 2.7 dB increase for the P70 sample, and a 7.6 dB decrease for the P70-P10 array. At 400 Hz, the dB difference between the P70- P10 and P10-P70 arrays was greater than that observed in individual measurements. This demonstrates that such combinations can be highly effective in acoustic panel design.

Table 2. Sound pressure levels measured in various frequency bands of samples with different porosity ratios

Frequency (Hz)	*Sound pressure level measured in empty pipe (dB(A))*	*Sound pressure level measured at the pipe outlet for samples with different porosity ratios (dB(A))*								
		P10	**P20**	**P30**	**P40**	**P50**	**P60**	**P70**	**P70 - P10**	**P10 - P70**
100	71,2	69,9	68,5	69,7	70,2	70,8	69,7	69,0	71,8	71,4
200	87,0	89,7	85,6	85,4	84,7	86,6	85,7	85,2	95,8	96,5
300	101,7	95,3	106,9	107,8	103,0	103,3	102,0	100,6	93,0	89,7
400	109,5	94,1	98,5	101,4	104,9	105,3	105,8	106,1	93,5	90,1
500	106,4	96,8	99,8	101,8	103,0	104,4	104,3	104,3	97,2	92,7
1000	106,0	116,3	115,0	112,4	109,9	108,8	108,6	108,7	98,4	107,8
2000	96,7	75,1	80,8	84,9	88,1	90,0	93,1	94,0	76,4	84,9
3000	89,1	75,0	80,7	84,0	86,0	87,9	89,0	89,3	88,0	78,4
4000	97,4	86,4	94,0	97,0	97,8	99,0	102,1	102,8	84,2	83,4
5000	83,7	71,0	75,7	79,2	80,1	81,4	82,9	84,3	79,8	77,9

Low freq.	Mid-range freq.	High freq.

Table 3. Differences in sound pressure levels at various frequency bands in samples with different porosity ratios

Frequency (Hz)	*dB Difference*								
	P10	**P20**	**P30**	**P40**	**P50**	**P60**	**P70**	**P70 - P10**	**P10 - P70**
100	-1,3	-2,7	-1,5	-1,0	-0,4	-1,5	-2,2	0,6	0,2
200	2,7	-1,4	-1,6	-2,3	-0,4	-1,3	-1,8	8,8	9,6
300	-6,4	5,3	6,2	1,4	1,6	0,3	-1,1	-8,7	-12,0
400	-15,4	-10,9	-8,1	-4,5	-4,1	-3,6	-3,4	-16,0	-19,4
500	-9,6	-6,6	-4,6	-3,3	-2,0	-2,0	-2,0	-9,2	-13,7
1000	10,3	8,9	6,4	3,9	2,8	2,6	2,7	-7,6	1,7
2000	-21,6	-15,9	-11,8	-8,6	-6,7	-3,5	-2,6	-20,3	-11,7
3000	-14,0	-8,4	-5,1	-3,1	-1,2	-0,1	0,2	-1,1	-10,6
4000	-11,0	-3,5	-0,4	0,4	1,6	4,6	5,4	-13,2	-14,0
5000	-12,7	-8,0	-4,5	-3,6	-2,3	-0,8	0,6	-3,9	-5,8

Low freq.	Mid-range freq.	High freq.

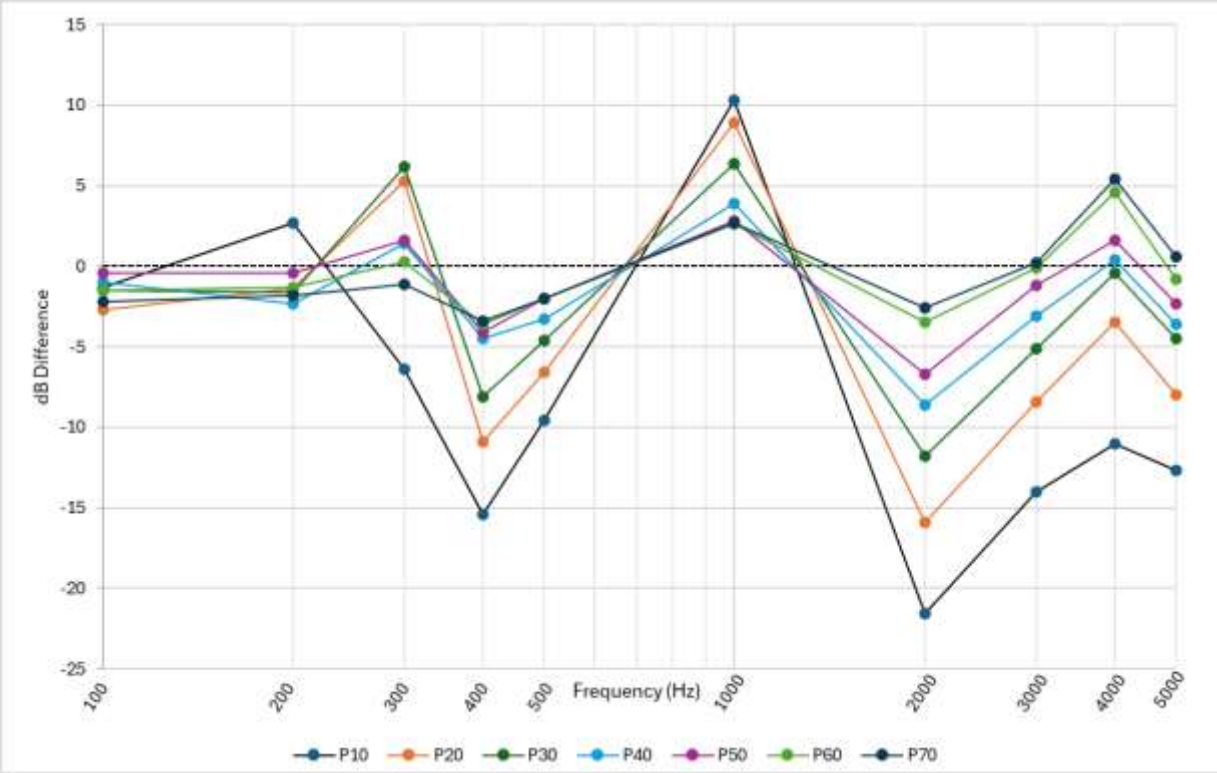


Figure 4. Graph of the differences in sound pressure levels (dB) at various frequency values in samples with different porosity ratios

CONCLUSION

In this study, the sound attenuation performance of samples with different porosity levels produced from PLA material using 3D printing was experimentally investigated. By comparing measurements at different frequencies and the orientation of the arrays, the effects of material structure and placement order on acoustic performance were evaluated. The key findings of the study are summarized below:

- As the porosity level in PLA samples increased, sound transmission through the material became easier and sound absorption decreased.
- Samples with lower porosity showed more effective sound attenuation performance, particularly at mid- and high frequencies.
- The highest attenuation was observed in the frequency range of 2000–3000 Hz. In this range, the P10 sample significantly reduced the output dB level.
- Sample orientation had a significant impact on sound absorption. The high-porosity to low-porosity (P70–P10) arrangement yielded more effective results at mid- and high frequencies.
- PLA structures produced with 3D printing can offer effective and customizable solutions in acoustic applications by optimizing the porosity ratio and array direction

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PLANT BY-PRODUCTS AS BIOACTIVE GOLDMINES: INSIGHTS FROM SUNFLOWER SEED HUSKS AND POMEGRANATE PEELS

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ABSTRACT

Plant by-products like sunflower seed husks and pomegranate peels are valuable sources of bioactive compounds, with significant potential for applications in food, pharmaceutical, and cosmetic industries. This study examines their biochemical profiles and bioactivities to promote sustainable resource utilization. Proximate analysis revealed distinct compositions: sunflower seed husks, with notably low moisture content, contrast sharply with the high-moisture pomegranate peels (over 80% water). Sunflower seed husks contain a higher lipid percentage, while pomegranate peels have minimal lipids. Both by-products exhibit comparable protein levels and total soluble sugars, highlighting their nutritional value. Antioxidant activity, assessed via DPPH scavenging, was significantly stronger in sunflower seed husks (approximately five times higher than pomegranate peels). However, pomegranate peels offered a substantially higher extraction yield, nearly four times that of sunflower seed husks. HPLC analysis identified diverse phenolic profiles: pomegranate peels were rich in catechin, ellagic acid, and rutin, while sunflower seed husks contained elevated levels of resorcinol and sinapic acid. Both demonstrated antimicrobial activity against Staphylococcus aureus, with sunflower seed husks showing roughly double the inhibition of pomegranate peels. These findings underscore the unique bioactive potential of each by-product, suggesting their valorization could drive innovation in functional products, reduce waste, and support circular economy goals. Further exploration of their industrial applications is highly promising.

Keywords*: Biological activities, Nutritional composition, Phenolics, Waste valorization.*

INTRODUCTION

The agro-industrial processing of sunflower (Helianthus annuus L.) and pomegranate (Punica granatum L.) generates substantial amounts of by-products, notably sunflower husks and pomegranate peels, which are often discarded or used as low-value animal feed (Gulya, 2004; Hasnaoui et al., 2014). This represents not only an environmental burden but also a lost opportunity to recover valuable compounds that could be exploited for food, pharmaceutical, and cosmetic applications. In the context of increasing global interest in sustainable resource utilization, the valorization of such by-products has become an important research priority (Mejri et al., 2025).

Plant by-products, such as sunflower husks and pomegranate peels, are considered promising reservoirs of bioactive molecules, particularly phenolic compounds, which are known for their antioxidant, antimicrobial, and anti-inflammatory properties. However, while pomegranate

peels have received some attention due to their high polyphenol content, studies remain fragmented, and sunflower husks in particular have been largely overlooked despite representing 21–30% of the total seed weight (Gulya, 2004). Recent investigations have further demonstrated the wellness-promoting potential of other underutilized plant matrices: Mejri et al. (2025) optimized the extraction of phenolic-rich fractions from date seed husks , spikelets, and perianths, reporting significant antioxidant and antimicrobial properties, while Ben Mansour et al. (2023, 2025) highlighted squash by-products, especially peels, as valuable sources of phenolics with strong bioactivities and developed encapsulation strategies to enhance their stability and applicability.

Among the most attractive bioactives, phenolic antioxidants stand out due to their role in mitigating oxidative stress, which is linked to the onset of chronic diseases such as cardiovascular disorders, diabetes, and cancer (Mansour et al., 2023). Antioxidants from plant sources also contribute to food preservation by delaying lipid oxidation, thereby extending shelf-life and improving product stability (Falleh et al., 2020). In parallel, antimicrobial properties of phenolic-rich extracts have gained increasing attention as natural alternatives to synthetic preservatives, especially against foodborne pathogens such as Staphylococcus aureus (Falleh et al., 2020; Ben Jemaa et al., 2022). Such dual antioxidant–antimicrobial functionality underscores the relevance of valorizing underutilized by-products like sunflower husks and pomegranate peels.

Despite their potential, systematic studies comparing the biochemical composition and bioactivities of sunflower husks and pomegranate peels are rare. Most available reports have focused on individual fractions, such as pomegranate peel tannins or sunflower oilseed husks, without addressing the broader valorization of husks and peels as bioactive-rich materials. This lack of comparative data limits our understanding of their respective advantages and possible complementary roles in functional product development. Addressing this gap is critical to advancing waste-to-resource strategies and supporting circular economy models.

In this study, we investigated the nutritional composition, phenolic profile, antioxidant activity, and antimicrobial potential of sunflower husks and pomegranate peels. High-performance liquid chromatography (HPLC) was employed to identify individual phenolic compounds, while radical scavenging and bacterial growth inhibition assays were used to evaluate bioactivity. By characterizing and comparing these two agro-industrial by-products, our work aims to highlight their potential as natural sources of bioactive compounds and to contribute to the growing body of research on sustainable by-product valorization.

State of the Art

The valorization of agro-industrial by-products has gained strong momentum in the last decades, reflecting global sustainability agendas and the increasing demand for clean-label, natural alternatives in the food, cosmetic, and pharmaceutical industries, with materials such as sunflower husks (hulls), pomegranate peels, grape pomace, olive residues, and coffee husks now considered valuable bioresources for functional ingredients rather than waste. This paradigm shift is underpinned by advances in green extraction technologies, encapsulation strategies, and an expanding body of evidence on health-promoting bioactivities. Modern green extraction methods have transformed the recovery of bioactive compounds, as conventional solvent extractions are increasingly replaced by environmentally friendly techniques that maximize yields and minimize ecological impact. Ultrasound-assisted extraction, microwave-assisted extraction, pressurized liquid extraction, supercritical CO_2, and deep eutectic solvents have all been employed to recover polyphenols, flavonoids, and dietary fibers, demonstrating clear advantages over conventional techniques in both efficiency and sustainability (Mejri et al., 2025). Ultrasound treatment accelerates solvent penetration into plant matrices, microwaves enhance mass transfer by disrupting cellular walls, and supercritical CO_2 provides residue-free,

selective extraction, while deep eutectic solvents offer tunable polarity and biodegradability, making them particularly attractive for food-grade applications. Applied to olive pomace, grape marc, coffee husks, and cereal brans, these approaches yield extracts rich in antioxidants, fibers, and flavonoids, while similar techniques have significantly increased polyphenolic recovery from pomegranate peels and improved extraction of phenolic acids from sunflower husks, which otherwise remain underutilized (Hasnaoui et al., 2014; Karunajeewa et al., 1989). Yet, beyond extraction, the stability and bioavailability of recovered compounds remain bottlenecks, prompting growing interest in encapsulation systems such as nanoemulsions, liposomes, and polysaccharide-based carriers. These delivery systems protect phenolics and flavonoids from degradation, improve solubility, and enhance controlled release, thereby extending their functional performance in food and nutraceutical contexts. Encapsulated grape pomace polyphenols have demonstrated prolonged antioxidant activity in beverages, while edible films enriched with pomegranate peel extracts extended the shelf life of perishable fruits, reinforcing their role in natural food preservation (López-Astorga et al., 2025; Vikrant et al., 2024). Functional integration of by-products into food matrices further supports their valorization: sunflower husk fibers incorporated into bakery products improved hydration, dough texture, and overall fiber content, producing breads with enhanced nutritional quality (Karunajeewa et al., 1989), whereas pomegranate peel powders enriched in dietary fiber fractions and phenolic compounds not only contributed to antioxidant activity but also slowed glucose diffusion, highlighting potential applications in glycemic control (Hasnaoui et al., 2014). Similar valorization pathways are evident across other agricultural residues, where olive pomace and grape marc extracts are used as additives in dairy and beverages, coffee husks yield caffeine- and antioxidant-rich fractions for nutraceuticals and cosmetics, and cereal brans provide arabinoxylans and β-glucans with prebiotic effects. However, despite technical advances, significant barriers remain to industrial translation, notably variability in raw materials due to cultivar, season, and environment, which complicates standardization and consistent product performance. For example, pomegranate peel fiber content can range from 33% to over 60% of dry matter depending on genotype and growing conditions, while sunflower husks exhibit fluctuating phenolic acid profiles shaped by cultivation and processing variables (Hasnaoui et al., 2014; Karunajeewa et al., 1989). Such heterogeneity requires robust quality-control frameworks, predictive models, and possibly blending strategies to ensure reproducibility. Scalability of green extraction technologies also presents hurdles, as methods such as ultrasound and microwave need adaptation to continuous, high-throughput industrial systems. On the regulatory and consumer side, the perception of “waste-derived” ingredients demands transparent labeling, rigorous safety assessments, and clinical validation to build confidence, while regulatory pathways must evolve to better accommodate by-product-derived bioactives (Mansour et al., 2023). Nevertheless, the research trajectory strongly aligns with the United Nations Sustainable Development Goals, particularly SDG 12 on responsible consumption and production and SDG 2 on zero hunger, by transforming residues into nutrient-rich resources while reducing waste streams. Moreover, replacing synthetic additives with natural preservatives from pomegranate peels or sunflower husks directly contributes to SDG 3 on good health and well-being by offering safer dietary options and lowering exposure to chemical preservatives. Large-scale collaborative programs provide encouraging examples: PRIMA-funded Mediterranean initiatives integrate local by-products into sustainable value chains by combining green extraction, encapsulation, and feed applications, fostering technology transfer, promoting rural economies, and strengthening resilience in agri-food systems.

Nutritional Composition of Sunflower Seed husks and Pomegranate Peels

The valorization of agro-industrial by-products such as sunflower husks (also referred to as hulls) and pomegranate peels has gained strong momentum in recent years due to their rich

nutritional profiles and their alignment with sustainable food system strategies and circular economy practices (Gulya, 2004; Hasnaoui et al., 2014). Detailed proximate analysis highlights distinct compositional differences between these two matrices, largely shaped by their botanical origins and the processing conditions from which they emerge. Sunflower seed husks typically exhibit very low moisture content, usually in the range of 4–5%. Such dryness makes them highly stable during long-term storage, transport, and handling, while also enhancing their suitability for subsequent extraction of bioactive compounds. This contrasts sharply with pomegranate peels, which are characterized by a very high water content, often exceeding 80%. The latter property makes them more prone to microbial spoilage if not rapidly dried, lyophilized, or otherwise stabilized. As a result, processing strategies for pomegranate peels often need to prioritize moisture reduction to ensure the preservation of functional components and to maintain bioactive potency (Figure 1).

Ash content in both by-products is relatively similar, generally around 3–4%. This value reflects their moderate but still nutritionally relevant mineral contribution, with elements such as potassium, magnesium, and calcium being particularly abundant. These minerals not only support general dietary requirements but also serve as cofactors in enzymatic pathways critical to both human and animal physiology.

The lipid profiles of the two materials diverge markedly. Sunflower seed husks contain approximately 2–3% lipids, with unsaturated fatty acids such as linoleic acid representing the dominant fraction (Gulya, 2004). This aligns with their role as a residual product of oilseed processing, providing potential for incorporation into formulations where essential fatty acids can deliver added health value. In contrast, pomegranate peels generally contain less than 1% lipids, underscoring their classification as a carbohydrate-dominant matrix with inherently lower caloric density. From a formulation perspective, this makes pomegranate peels more suitable for functional applications requiring bioactive polyphenols or polysaccharides rather than lipid enrichment (Hasnaoui et al., 2014).

Protein levels, surprisingly, display close similarity across both by-products, with concentrations generally reported between 13 and 15 mg/mL (Ben Jemaa et al., 2022). While not sufficient to position these matrices as primary protein sources, such levels nonetheless highlight their supplementary potential. They can thus be integrated into feed formulations to improve protein balance or into specialized food products where additional plant-based proteins are desirable. The amino acid composition, though less extensively studied, suggests that these proteins could contribute bio-functional properties, including antioxidant activity or foaming capacity, when isolated and characterized further.

Total soluble sugars represent another shared attribute. Both sunflower husks and pomegranate peels contain approximately 1.8–2.0 mg/g of soluble carbohydrates. These sugars, though present in modest quantities, are of interest for biotechnological valorization. They can act as precursors in fermentation-based processes, contribute to the development of natural sweeteners, or serve as substrates for microbial bioconversion into higher-value compounds. This equivalence in sugar content between the two by-products also indicates potential for combined or complementary use in integrated biorefinery concepts.

Overall, these compositional features position sunflower husks as lipid- and stability-oriented resources, with particular utility in feed, nutraceutical, or functional food applications where fatty acid enrichment and long-term preservation are prioritized. Pomegranate peels, conversely, emerge as water-rich, carbohydrate- and polyphenol-focused resources requiring immediate stabilization but offering high yields of soluble bioactives for applications in beverages, coatings, or extracts. Both matrices, in their respective domains, contribute meaningfully to the portfolio of agro-industrial by-products that can be harnessed for sustainable nutrition and health-oriented innovations.

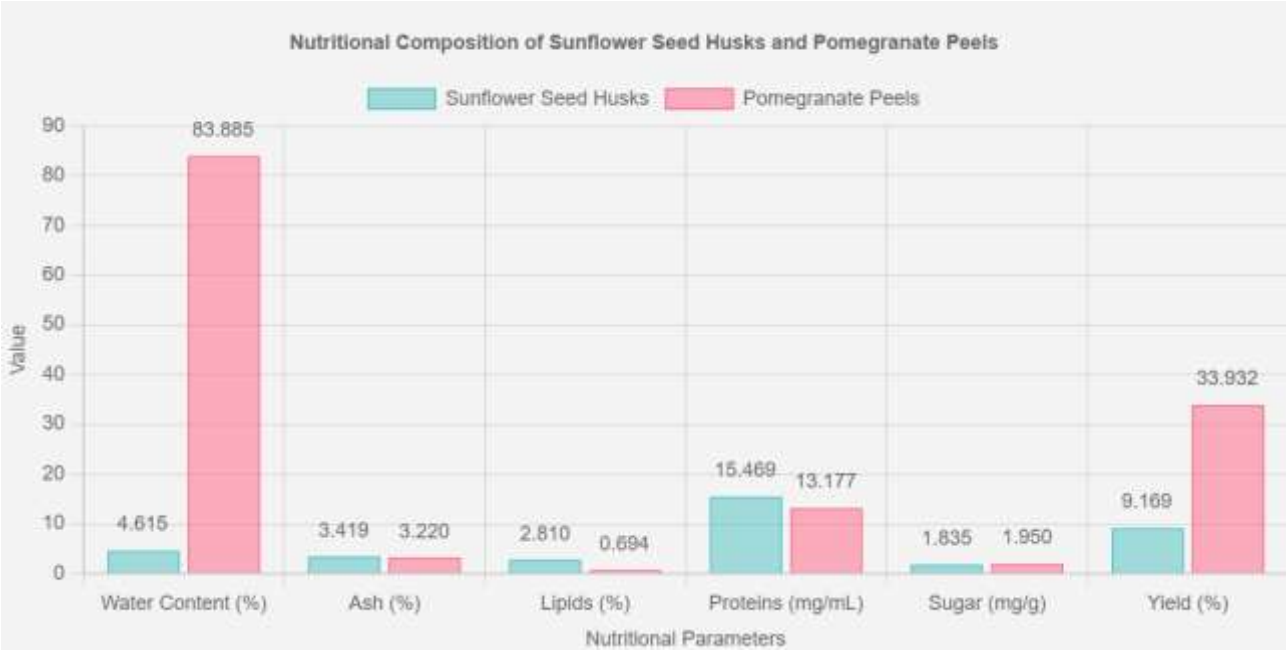

Figure 1. Nutritional Composition of Sunflower Seed husks and Pomegranate Peels.

Antioxidant Potential and Extraction Efficiency

Antioxidant activity has emerged as a central pillar in the valorization of agri-food by-products, reflecting both consumer and industrial demand for natural preservatives and health-enhancing compounds (Falleh et al., 2020). The search for plant-based alternatives to synthetic additives is particularly relevant in food, nutraceutical, and pharmaceutical sectors, where oxidative degradation limits product quality, safety, and shelf life. Consequently, by-products once regarded as waste are increasingly investigated as reservoirs of functional molecules, particularly polyphenols, flavonoids, and lipid-soluble antioxidants, capable of mitigating oxidative stress and associated chronic pathologies. Among the by-products recently studied, sunflower seed husks and pomegranate peels present contrasting yet complementary attributes. Evaluations using the DPPH radical scavenging assay demonstrate that sunflower seed husks possess a strong antioxidant capacity, reaching inhibition rates of nearly 40%. This level of activity is approximately fivefold higher than that of pomegranate peels, which display values closer to 9%. Such disparity is commonly attributed to the chemical nature of the antioxidant molecules present. Sunflower seed husks are especially rich in lipid-associated antioxidants, notably tocopherols and certain phenolic acids, that excel in neutralizing reactive oxygen species and delaying oxidative chain reactions in lipid systems (Mansour et al., 2025). These compounds confer a specific advantage in food matrices where lipid oxidation represents a primary degradation pathway. However, antioxidant capacity alone does not determine the overall feasibility of by-product recovery. Extraction yield plays a decisive role in defining economic viability. In this respect, pomegranate peels significantly outperform sunflower seed husks. Reported yields for pomegranate peels approach 34%, compared to approximately 9% for sunflower husks (Hasnaoui et al., 2014). This difference arises from structural and compositional factors. Pomegranate peels possess a porous cellular structure and high levels of water-soluble phenolic compounds, which allow solvents to penetrate more effectively and release target metabolites during extraction (Mansour et al., 2023). Sunflower husks, by contrast, contain more compact, lignocellulosic material that limits solvent accessibility, reducing extractable fractions despite their strong antioxidant potential per unit of dry matter. The tension between antioxidant strength and extraction yield is not unique to these two

matrices. Similar observations have been documented for date perianths and squash peels, where optimization strategies are required to maximize recovery efficiency without compromising bioactivity (Mejri et al., 2025; Mansour et al., 2025). Advanced statistical tools, particularly response surface methodology (RSM), have been applied to refine extraction parameters, balancing solvent polarity, time, and temperature to enhance both yield and functional activity. These approaches underscore the need for integrative optimization frameworks when scaling up by-product valorization processes. Beyond laboratory assays, the practical significance of these antioxidant-rich extracts lies in their wide-ranging applications. In food systems, sunflower seed husk extracts could serve as natural stabilizers for lipid-rich products such as edible oils, margarines, or bakery items, where they would delay rancidity and extend shelf life. Conversely, pomegranate peel extracts—with their high extraction yield and abundance of hydrophilic phenolics—may be more suitable for fortification of aqueous systems, including fruit juices, teas, or functional beverages. The cosmetic sector also presents an attractive avenue, as phenolic-rich extracts from pomegranate peels can be incorporated into creams or serums to mitigate oxidative skin damage and promote anti-aging effects. Health-promoting effects represent another crucial dimension. Dietary incorporation of these extracts may contribute to the prevention of chronic disorders linked to oxidative stress, including cardiovascular diseases, metabolic syndrome, and certain cancers (Falleh et al., 2020). This dual functionality—technological as food preservatives and biological as health-promoting agents—places sunflower husks and pomegranate peels at the crossroads of circular economy principles and functional food innovation.

Phenolic Profiles and Bioactive Diversity

High-performance liquid chromatography (HPLC) provides critical insights into the phenolic profiles of agri-food by-products, revealing the biochemical diversity that underpins their bioactivity. Sunflower seed husks and pomegranate peels, in particular, stand out as contrasting yet complementary reservoirs of phenolics, positioning them as “bioactive goldmines” for targeted applications in food, pharmaceutical, and cosmetic industries. Analytical results highlight the dominance of flavonoids and ellagitannins in pomegranate peels, consistent with their well-documented antioxidant and antimicrobial capacities. Key compounds quantified include catechin (336.58 µg/g), rutin (275.52 µg/g), and ellagic acid (153.08 µg/g), alongside moderate levels of chlorogenic acid (55.55 µg/g) and syringic acid (28.83 µg/g). These molecules are mechanistically linked to the modulation of oxidative pathways and the inhibition of microbial enzymes, supporting both anti-inflammatory activity and pathogen resistance (Mansour et al., 2023). Catechin, for example, is renowned for its free-radical scavenging ability, while ellagic acid contributes to DNA protection and anti-proliferative effects. Rutin adds vascular-protective benefits, making pomegranate peel extracts attractive for nutraceutical formulations. Sunflower seed husks, in contrast, exhibit a markedly different phenolic spectrum. Their profiles are dominated by phenolic acids, particularly resorcinol (856.70 µg/g) and sinapic acid (110.52 µg/g), with only minor contributions from catechin (47.07 µg/g). These compounds reflect the physiological role of husks in providing UV protection and maintaining structural integrity of oilseeds. Resorcinol, in particular, is a multifunctional molecule with antioxidant, antimicrobial, and dermatological relevance, often used in pharmaceutical and cosmetic formulations. Sinapic acid enhances oxidative stability in lipid systems, suggesting that sunflower husk extracts may be well-suited for preserving oils and fat-rich foods. Interestingly, chromatographic data also revealed unidentified peaks. In sunflower husks, a non-identified compound was detected at 4.567 min (NI, 266.19 µg/g), while pomegranate peels exhibited a distinct peak at 9.34 min (NI, 331.21 µg/g). These findings point to the presence of yet-uncharacterized bioactives that could broaden the functional repertoire of these by-products. Advanced structural elucidation through techniques such as LC-MS/MS

and NMR will be necessary to determine their identity and potential bioactivity. Such discoveries would not only expand the scientific understanding of phenolic diversity but could also reveal novel molecules of industrial significance. The phenolic heterogeneity observed here aligns with studies on other by-products such as date seed husks and squash peels, where compositional differences are shaped by varietal, environmental, and processing factors (Mejri et al., 2025; Mansour et al., 2023). This diversity underscores the possibility of tailoring extraction strategies to optimize the recovery of specific compounds with desired biological functions. For example, solvent polarity and extraction temperature can be fine-tuned to preferentially target flavonoids, ellagitannins, or phenolic acids, depending on the intended application. Moreover, combining extracts from sunflower husks and pomegranate peels could provide synergistic advantages. While sunflower husks contribute lipid-soluble stability via phenolic acids, pomegranate peels supply a rich arsenal of flavonoids and ellagitannins with water-soluble antioxidant and antimicrobial effects. Such complementary interactions may yield broad-spectrum formulations capable of enhancing oxidative stability in diverse food matrices. To preserve the integrity of these sensitive compounds, encapsulation technologies—such as nanoemulsions, liposomes, or biopolymer-based carriers—are increasingly advocated to enhance stability, bioavailability, and controlled release in functional foods or supplements (Mansour et al., 2023).

Table 1. Identification and quantification of phenolic compounds from sunflower seed husks and pomegranate peels. Values are presented as milligrams Gallic acid equivalent per gram of extract (mgGAE/g E).

RT (min)	Sunflower Seed Husks	Pomegranate Peels	Compound
4.567	266.19	-	NI eqAG
9.34	-	331.21	NI eqAG
10.67	-	55.55	Chlorogenic Acid
11.069	856.70	-	Resorcinol
11.85	-	336.58	Catechin
12.482	47.07	-	Catechin
14.91	-	28.83	Syringic Acid
16.34	-	275.52	Rutin
17.54	-	153.08	Ellagic Acid
18.955	110.52	-	Sinapic Acid

Antimicrobial Activity and Food Safety Implications

The antimicrobial potential of plant by-products is gaining traction as a response to the escalating threat of foodborne pathogens and antibiotic resistance (Falleh et al., 2020). Both sunflower seed husks and pomegranate peels exhibit inhibitory activity against *Staphylococcus aureus*, a frequent contaminant in dairy and meat products. Sunflower husks achieve approximately 29% growth inhibition—nearly double that of pomegranate peels (14%)—based on the methodology of Ben Jemaa et al. (2022). This stronger performance may be linked to

their resorcinol and sinapic acid content, known to compromise bacterial membranes and disrupt quorum sensing (Falleh et al., 2020). Pomegranate peels, although less potent, contribute ellagic acid and catechin, which act through phenolic–protein interactions to exert bacteriostatic effects against Gram-positive bacteria (Hasnaoui et al., 2014). Evidence from parallel research, including cedar essential oils and date by-products, underscores the utility of response surface methodology in enhancing antimicrobial activity (Ben Jemaa et al., 2022; Mejri et al., 2025). Applying similar optimization approaches to sunflower and pomegranate matrices could further maximize their bioactive potential. The antimicrobial relevance of these by-products extends beyond *S. aureus*. Pomegranate peel polyphenols have demonstrated inhibitory effects against *Escherichia coli*, where incorporation into chitosan-based edible films markedly increased inhibition zones (27.67 mm) compared to negligible activity in extract-free films (Kumar et al., 2021). The mechanism, as with *S. aureus*, involves membrane destabilization and quorum sensing interference, reducing pathogen virulence. In parallel, sunflower hull extracts have shown significant activity against *E. coli* O157:H7, *S. aureus*, *Bacillus cereus*, *Listeria monocytogenes*, and *Salmonella typhimurium*, with inhibition zones ranging from 10 to 22 mm (Taha et al., 2012). These findings reinforce the promise of sunflower husks and pomegranate peels as broad-spectrum antimicrobial agents with applications in food preservation. Their inclusion in food or feed systems could reduce reliance on synthetic additives, aligning with consumer demand for "clean-label" solutions. By extending shelf life and minimizing risks associated with chemical preservatives, they address both safety and sustainability goals (Falleh et al., 2020). From an agricultural perspective, their application is equally appealing. Pomegranate peel extracts, when integrated into fruit-derived antimicrobial systems, enhance post-harvest quality management, while sunflower husks can reinforce grain-based bio-preservation strategies.

Functional By-Products for Sustainable Ruminant Diets

The translation of laboratory evidence into practical applications in animal nutrition is steadily advancing, with particular emphasis on valorizing agricultural by-products as functional feed components. Within the framework of the PRIMA-MEDGOAT project, special attention has been directed toward pomegranate peel polyphenols, which are increasingly recognized as promising feed additives capable of enhancing livestock health and productivity. These compounds exhibit potent antioxidant properties that can mitigate oxidative stress, improve gut function, and strengthen immunity in ruminants such as calves, goats, and beef cattle (Safari et al., 2018; Varma et al., 2018).

Pomegranate peel is rich in phenolic compounds and hydrolyzable tannins, conferring multiple benefits when incorporated into ruminant diets. Its supplementation at levels of approximately 2–4% of feed has been associated with improved nutrient digestibility, enhanced rumen fermentation efficiency, and reduced oxidative biomarkers. In dairy systems, inclusion of pomegranate peel has been linked to increased milk yield and improved milk quality, suggesting a nutraceutical effect that extends beyond basic nutrition (El-Ashry et al., 2019; Safari et al., 2018). In young calves, dietary supplementation reduced the prevalence of intestinal parasites, reflecting the anthelmintic potential of peel-derived tannins. Meanwhile, across different livestock categories, blood antioxidant status improved significantly, indicating systemic protective effects against oxidative imbalance (Varma et al., 2018).

The versatility of pomegranate peel also supports diverse modes of use. It can be provided fresh, incorporated as a dried extract, or preserved in silage form to ensure longer storage and easier integration into feed rations. Such flexibility enhances its applicability across farming systems, whether intensive or semi-intensive. Notably, beyond improvements in health indicators, pomegranate peel supplementation has been shown to promote weight gain and optimize meat quality in small ruminants, providing added value for producers. These outcomes align with the

broader objectives of sustainable livestock management, where functional feed ingredients contribute simultaneously to animal health, productivity, and product quality.

In parallel, sunflower seed hulls represent another by-product with growing interest for ruminant feeding, though their contribution is primarily structural rather than bioactive. Composed of roughly 50% crude fiber, 7% protein, and with a dry matter digestibility of about 18%, sunflower hulls are best suited as a source of insoluble fiber (Alharthi et al., 2021; Alobre et al., 2021). When included at levels ranging from 15% to 35% of total dietary dry matter, and balanced with protein-rich feeds, they effectively support rumen function by stimulating chewing activity and saliva secretion, which are essential for buffering rumen pH. Their role as a bulky roughage source helps maintain gut motility and microbial balance.

Animal feeding trials indicate that sunflower hulls can be safely incorporated into the diets of goats, sheep, and cattle without negative impacts on growth performance. In fact, moderate inclusion has been associated with stable weight gain and overall improved digestive health. While they lack the bioactive potency of polyphenol-rich pomegranate peels, sunflower hulls serve as a cost-effective roughage alternative that contributes to diet formulation flexibility, particularly in regions where high-quality forage may be limited.

Socio-Economic and Environmental Perspectives

The valorization of agro-industrial by-products plays a pivotal role in advancing circular economy models by transforming waste streams into functional, marketable products. Sunflower husks and pomegranate peels, often discarded in bulk during processing, represent abundant raw materials that can be redirected into high-value sectors such as food, feed, nutraceuticals, and cosmetics. This shift not only reduces the volume of agricultural residues but also lessens the environmental footprint of agro-industrial chains. By diverting husks and peels from landfills or uncontrolled disposal, greenhouse gas emissions linked to decomposition are minimized, while risks of soil leachates and water contamination are mitigated. From an economic standpoint, valorization introduces new income opportunities for farmers, cooperatives, and small processors, particularly in Mediterranean regions where sunflower and pomegranate cultivation is widespread. Local industries stand to benefit from cost savings on waste management and from the creation of new product lines, thereby increasing competitiveness. Moreover, the integration of such by-products into feed or functional food systems supports rural development by strengthening agro-industrial linkages and reducing dependence on imported additives. Nevertheless, several challenges constrain large-scale adoption. Regulatory acceptance requires rigorous safety assessments and standardized quality controls, while consumer confidence hinges on transparent labeling and verified health claims. Cost-competitiveness with synthetic additives also remains a barrier, given that extraction, processing, and encapsulation technologies can be resource-intensive. Bridging these gaps demands coordinated strategies: targeted subsidies, supportive policies, and investment in green technologies to reduce production costs.

International collaborations offer a strong pathway forward. EU-funded PRIMA initiatives illustrate how transnational partnerships can catalyze technology transfer, foster knowledge exchange, and build capacity for sustainable bio-based industries. By integrating scientific advances with socio-economic incentives, such programs highlight the potential of agro-industrial by-products to drive both environmental protection and regional economic resilience.

Challenges and Future Prospects in By-Product Valorization

Despite the growing evidence supporting the bioactive potential of agro-industrial by-products, several obstacles limit their transition from laboratory to large-scale application. A major challenge arises from variability in chemical composition, which can differ significantly

depending on cultivar, growing conditions, harvest period, and storage practices. Seasonal and regional fluctuations alter the concentration of polyphenols, flavonoids, and tannins, thereby influencing both antioxidant activity and extraction yields. Such inconsistency complicates industrial standardization and requires robust quality-control protocols to ensure reproducibility (Mejri et al., 2025). Extraction efficiency represents another critical bottleneck. Conventional solvent-based techniques often suffer from low yields, high solvent consumption, and potential degradation of thermolabile compounds. To address these limitations, green extraction technologies—such as ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), and pressurized liquid extraction—are gaining traction. UAE, in particular, has shown promise in enhancing mass transfer, reducing solvent use, and improving the recovery of phenolic compounds without compromising their functional integrity (Mejri et al., 2025). The integration of such methods not only improves sustainability but also aligns with industrial demands for cost-effective and eco-friendly processing. Beyond technological hurdles, regulatory and consumer-related issues must also be carefully considered. Ingredients derived from "waste" streams may face skepticism, with consumers questioning their safety, quality, or nutritional value. Transparent labeling, coupled with rigorous toxicological evaluations and clinical validation of health benefits, is essential to build trust and ensure compliance with food safety regulations (Mansour et al., 2023). Clear communication strategies that emphasize sustainability and the valorization of natural resources can further enhance consumer acceptance. Future research directions should move toward interdisciplinary approaches that integrate analytical chemistry, food technology, and biomedical sciences. Stability studies are urgently needed to evaluate how processing, storage, and formulation affect the bioactivity of recovered compounds. In vivo trials, both preclinical and clinical, will be critical for confirming the physiological relevance of in vitro findings, particularly regarding antioxidant, antimicrobial, and anti-inflammatory effects (Falleh et al., 2020). Additionally, encapsulation technologies should be explored to enhance the stability and bioavailability of phenolic extracts, ensuring their efficacy in complex food or pharmaceutical matrices.

International collaborations provide a powerful mechanism to accelerate progress in this domain. Programs such as *MedGoat-H2020* demonstrate how transnational projects can facilitate technology transfer, foster harmonization of extraction protocols, and enable shared access to advanced infrastructure. Such initiatives are particularly relevant for the Mediterranean region, where large quantities of by-products like pomegranate peels and sunflower husks are generated annually. By linking scientific innovation with socio-economic priorities, these collaborations strengthen the value chain from farm to market. Ultimately, the valorization of by-products must be viewed not only as a technological challenge but also as a socio-environmental imperative. Aligning with the United Nations Sustainable Development Goals, particularly those targeting zero waste, responsible consumption, and food security, by-product utilization represents a pathway toward resilient, low-carbon, and resource-efficient food systems. With continued investment in green technologies, rigorous safety validation, and international cooperation, the full potential of these bioactive resources can be unlocked, transforming agricultural residues into drivers of sustainable development.

CONCLUSION

The comparative assessment of sunflower seed husks and pomegranate peels clearly demonstrates their remarkable potential as bioactive reservoirs. Both by-products exhibit valuable nutritional traits, distinct phenolic signatures, and complementary biological activities. Sunflower husks stand out for their strong antioxidant and antimicrobial effects, while pomegranate peels provide higher extraction yields and richer flavonoid content. This duality suggests that integrated valorization strategies, combining the strengths of both matrices, could

yield multifunctional formulations for food preservation, nutraceutical development, and cosmetic applications. The advances in green extraction and encapsulation technologies further support their transition from laboratory findings to industrial adoption. Beyond technical advantages, the valorization of these by-products contributes to sustainability and circular economy goals, reducing agro-industrial waste while generating economic opportunities in rural and processing communities. Yet, successful integration requires tackling challenges linked to raw material variability, regulatory approval, and consumer acceptance. In conclusion, sunflower husks and pomegranate peels should no longer be regarded as low-value residues but as strategic raw materials for the next generation of eco-innovative products. Their effective use aligns with global efforts to promote health, sustainability, and responsible resource management.

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Conflicts of Interest.
The authors declare no conflict of interest.

Using artificial intelligence (AI).
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APPLICATION OF SOUS-VIDE PROCESSING ON SELECTED FRUITS AND EVALUATION OF ITS EFFECTS ON QUALITY CHARACTERISTICS

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ABSTRACT

In this study, the aim was to determine the effects of sous-vide processing on the quality characteristics of apple, quince and pumpkin, which are widely consumed fruits in Turkey. The sous-vide method is a gentle cooking technique that uses low temperatures and long cooking times in vacuum-sealed packaging to preserve nutritional value, color, texture and sensory quality. In this research, sous-vide cooking was applied at predetermined temperature-time combinations and the results were compared with the conventional steaming method. Additionally, the changes during storage were also evaluated. The findings revealed that, compared to conventional cooking, sous-vide processing helped to better preserve the color and texture of fruits, reduced cooking losses, and improved consumer sensory acceptance.

Keywords: Sous-vide, fruit, color, texture, sensory analysis, storage

INTRODUCTION

In the food industry, it is becoming increasingly important to prepare products in line with consumer expectations while maintaining both healthy and quality characteristics. Sous-vide method is a heat treatment technology based on the principle of low temperature and prolonged cooking in vacuum packaging. This method contributes to the preservation of particularly heat-sensitive nutritional compounds, reducing textural losses and ensuring microbial safety. Fruits such as apples, quince and pumpkin are frequently cooked and consumed in different forms such as desserts and jams in our country. However, studies on cooking these products by sous-vide method are very limited.

In this context, in this study, quality parameters such as color, texture, sensory quality, moisture content, cooking efficiency and storage stability were evaluated by applying sous-vide process to apple, quince and pumpkin at specified temperatures and times. Comparative analyses with the traditional steaming method revealed the feasibility of sous-vide as an alternative cooking technology. This study aims to contribute to the development of fruit-based ready-to-eat products and to extend product shelf life.

LITERATURE SUMMARY

2.1. Sous-Vide Cooking Technique

Sous-vide means "under vacuum" in French and is a heat treatment method based on the long-term cooking of food in airtight plastic bags at controlled low temperatures (usually between 50-95 °C) (Baldwin, 2012). During this process, the food is first placed in a vacuum bag, vacuum-sealed to remove oxygen, and then cooked in a precisely temperature-controlled water bath or steam oven for the targeted time (Schellekens, 1996).

The main difference of the sous-vide technique is that the risk of overcooking at the targeted temperature is eliminated by precisely controlling the temperature. In this way, thermal reactions such as protein denaturation, starch gelatinization and enzyme inactivation take place in a controlled manner (Kathuria et al., 2022).

The method offers many advantages over conventional cooking methods in terms of color, texture, nutrient preservation and microbiological safety (Creed, 1998). Especially in vegetables and fruits, the loss of heat sensitive compounds such as vitamin C, phenolic compounds and carotenoids is minimized (Stea et al., 2007). Ghazala et al. (1996) reported that color preservation and soft texture were better achieved when apples were cooked by sous-vide method. Iborra-Bernad et al. (2015) observed less structural distortion and better texture integrity in vegetables such as potatoes and carrots.

Sous-vide method is also important for food safety. The absence of oxygen in the vacuum environment and precise temperature control are critical, especially for anaerobic pathogens such as Clostridium botulinum, and a safe product is obtained when the pasteurization time and temperature are set appropriately (Baldwin, 2012).

However, the method also has some limitations. Long cooking times can increase energy consumption. Furthermore, the microbial safety of the foodstuff depends on the quality of the vacuumization and the accuracy of the temperature control system. This control can be difficult to achieve in domestic applications (Singh et al., 2023).

In conclusion, the sous-vide method is a promising technology for vegetable and fruit products, especially for the preservation of sensory and nutritional properties. However, its applicability on an industrial scale needs to be optimized and product-based validation studies need to be continued.

Figure 1 Vestel Sous-vide Cooking Oven and 3D Drawing

2.2. Usage Areas of Sous-Vide Applications

While the sous-vide method is widely used especially in protein foods, in recent years it has also been investigated in vegetable and fruit groups to improve quality. Stanikowski and Michalak-Majewska (2021) compared sous-vide, boiling and steaming methods in root vegetables and reported that sous-vide was the most advantageous method in terms of color, texture and preservation of bioactive compounds.

In a comprehensive review summarizing the effects of sous-vide on vegetables and fruits, Kathuria et al. (2022) found that this method causes less cellular damage at low temperature and is superior in terms of sensory properties as well as microbial safety. It was emphasized that the sous-vide method created significant differences compared to traditional methods, especially in the preservation of color and nutritional values.

Yılmaz and İlyasoğlu (2023) reported that sous-vide technique positively affected both physical and sensory quality in vegetables such as green beans and pumpkin and provided a more stable structure in the storage process. Since products obtained by sous-vide cooking are cooked at lower temperatures, the cell walls are less damaged, which allows the textural integrity to be preserved. This is especially noticeable in hard textured fruits such as quince.

In a study conducted by Najman et al. (2021), it was found that sous-vide process contributed to the preservation of phenolic compounds and improved the functional properties of the product in various physical-chemical analyzes performed on quince samples.

Singh et al. (2023) emphasized that sous-vide technique is a process that improves food quality in general and evaluated it as an effective method in terms of both microbiological safety and stability of functional components. In the study, it was stated that low temperature applications had positive effects on sensory quality and bioactive content, especially in fruits and vegetables.

Roca and Brugués (2007) reported that products prepared by the sous-vide method can largely maintain their sensory parameters such as color and taste when stored at -18°C as long as microbial safety is ensured.

2.3. Comparison of Sous-Vide and Traditional Cooking Methods

The main differences between sous-vide and conventional cooking methods focus on critical parameters such as the type of heat treatment, temperature control, the amount of oxygen to which the product is exposed and nutrient losses. While traditional methods (boiling, baking, steaming, etc.) generally cook foods at high temperatures (100 °C and above) for short periods of time, sous-vide method is based on cooking at low temperatures (50-95 °C) for long periods of time. This difference has significant effects on the preservation of nutrients, color pigments, textural structures and volatile aromatic compounds (Creed, 1998; Stea et al., 2007).

One of the most obvious advantages of the sous-vide method over conventional cooking techniques is the minimization of the loss of heat-sensitive compounds. In a study conducted by Stea et al. (2007), it was reported that folate, carotenoid and α-tocopherol levels in vegetables were preserved to a higher extent when cooked by sous-vide method compared to boiling and steaming methods. Similarly, Iborra-Bernad et al. (2015) observed better color stability and less structural degradation in root vegetables such as potatoes and carrots after sous-vide cooking.

Significant differences were also observed in terms of color parameters. Ghazala et al. (1996) reported that sous-vide cooking of apple samples resulted in a smaller reduction in L* (lightness) than conventional boiling, which was favorable for consumer perception. Similarly, Stanikowski and Michalak-Majewska (2021) found more vibrant colors and higher levels of bioactive compounds in root vegetables cooked sous-vide.

In terms of texture, low-temperature cooking allows the cell walls to break down more slowly, resulting in a soft but not disintegrating structure. This is particularly evident in hard fruits/vegetables such as quince, apples and carrots (Singh et al., 2023). Samples cooked sous-vide can offer a more homogeneous texture and a lower firmness value compared to conventional cooking (Bourles et al., 2009).

In terms of aroma compounds, traditional methods cause more loss of volatile compounds, whereas in sous-vide applications, the aromatic profile is more intense and natural due to the retention of these compounds in the pouch (Kathuria et al., 2022).

However, both methods have their own advantages. While traditional methods offer shorter cooking times, the sous-vide method requires more time and equipment precision. In terms of energy consumption, the long cooking time may be a disadvantage for some applications.

2.4. Ready-to-eat Foods Produced with Sous-Vide and Storage Durability

Today, consumers' time constraints, search for practicality and healthy eating tendencies are increasing the demand for ready-to-eat foods. In this context, foods produced by sous-vide method constitute an important alternative for ready-to-eat products in terms of both minimizing nutrient losses and allowing the product to be cooked in the package and stored for a long time (Singh et al., 2023).

One of the most obvious advantages of products prepared by sous-vide method is that the risk of external contamination is eliminated by cooking the products in vacuum packaging. This packaging can be directly cooled, frozen, or short-term pasteurized without opening the product after cooking to ensure microbial safety (Baldwin, 2012). Reducing the oxygen level in the

vacuum environment also prevents oxidative deterioration and contributes to the preservation of color, aroma and fatty acid profiles (Creed, 1998).

In terms of storage stability, sous-vide products are very advantageous. Roca and Brugués (2007) showed that meat products cooked sous-vide can maintain microbiological safety and sensory quality for up to 28 days at +4 °C. Similarly, Yilmaz and Ilyasoglu (2023) reported that frozen storage at -18 °C after sous-vide cooking of vegetables such as green beans and pumpkin significantly preserved sensory characteristics for 30 days.

In order for sous-vide produced products to be sold on the shelves, the cold chain must be maintained, usually in combination with low-temperature pasteurization. These products are categorized as "chilled ready meals" and can have a shelf life of up to 21-30 days at a temperature range of +2/+4 °C, mostly under refrigerated storage conditions (Kathuria et al., 2022). When stored in frozen form, this period can be up to several months.

Sous-vide products are also advantageous in terms of functional food components, allowing the preservation of vitamins, phenolic compounds and volatile flavors that can break down at high temperatures, thus preserving both the health value and flavor profile of the product (Stea et al., 2007; Najman et al., 2021). In this way, healthier alternatives can be offered for both home consumption and industrial-scale ready-to-eat food production.

As a result, ready-to-eat foods produced by sous-vide method can outperform products produced by conventional methods in parameters such as high quality, long shelf life and microbiological safety. However, critical control points such as chain cooling, hygienic vacuuming and temperature monitoring need to be carefully implemented.

2.5. Apple (Malus domestica Borkh.)

Apple is one of the most produced and consumed fruits worldwide. According to FAO data for 2023, world apple production exceeds 90 million tons, while Turkey is among the top 5 producing countries with a production of 4.5 million tons. Apples contain approximately 84-86% water, 10-13% carbohydrates, 0.3% protein and 0.2% fat. It is also rich in vitamin C, potassium, polyphenols and fiber (Boyer & Liu, 2004).

Apple has a high antioxidant capacity thanks to phenolic compounds and flavonoids concentrated especially in the peel. In this respect, apple is considered as a functional food with protective effects against heart diseases, diabetes and some types of cancer (Hyson, 2011). Apple is also a fruit resistant to cooking. Sous-vide cooking practices have been determined to provide minimal loss of nutrients by preserving the textural integrity of apples (Silva et al., 2021).

2.6. Quince (Cydonia oblonga Mill.)

Quince is a stone fruit belonging to the Rosaceae family and is generally consumed cooked rather than fresh. Turkey ranks first in quince production, accounting for approximately 25% of the world quince production (TurkStat, 2023). Quince is rich in pectin, tannins, organic acids (malic acid, citric acid), potassium and vitamin C (Rather et al., 2020).

Quince fruit has an astringent taste due to its high tannin content and is usually consumed in dessert form by cooking with flavorings such as sugar, cloves and cinnamon. With the softening of the cell walls during the cooking process, quince gains a characteristic aroma and reddish color (Sharma et al., 2011). Sous-vide cooking allows this transformation to take place in a controlled manner, resulting in a more homogeneous texture and stable color than traditional methods (Ghazala et al., 1996).

2.7. Pumpkin (Cucurbita moschata Duchesne ex Poir.)

Pumpkin is a fruit rich in beta-carotene, vitamin C and potassium, which is widely used in products such as desserts and soups during the winter months. This fruit, which has a high fiber content, is also preferred in healthy nutrition programs thanks to its low calorie structure. 100 grams of pumpkin contains about 26 kcal of energy, 9 mg of vitamin C and 1.3 g of fiber (USDA, 2022).

Pumpkin is suitable for pureeing due to its structure that softens when cooked. However, traditional boiling and baking methods can cause dull color and nutrient losses. In studies conducted with sous-vide cooking, it was reported that pumpkin retained brighter color, reduced beta-carotene loss and better preservation of starch structure as a result of prolonged cooking at low temperature (Stea et al., 2007).

MATERIALS AND METHODS

3.1. Material

Quince, apple and pumpkin fruits used in this study were obtained from a local market in Izmir. The fruits were stored at +4°C until use. Sous-vide cooking was carried out in a sous-vide oven provided by Vestel A.Ş. Color, texture and moisture analyses were carried out with the devices in the pilot plant of Ege University Food Engineering Department.

3.2. Determination of Temperature and Time

In this section, examples of sous-vide and conventional cooking temperatures and times reported in the literature for apple, quince and pumpkin are presented. The combinations used in the studies and the results obtained are compiled in tabular form and the temperature-duration profiles in our study are determined. While determining these profiles, it is aimed that the product outputs we will get are in line with the findings in the literature and the expected results.

Table 1 Examples of sous-vide cooking reported in the literature (temperature/time)

Source	Temperature (°C)	Duration (minutes)	Product and Findings
Ghazala et al. (1996)	65, 85	60, 20	Apple; color and hardness analysis
Ortiz-Catalan et al. (2017)	60-90	30-90	Apple; pectin enzyme activity and texture
Najman et al. (2021)	85	45	Quince; phenolic and antioxidant content
Kathuria et al. (2021)	75-95	30-90	Quince and vegetables; general quality parameters
Yilmaz & Ilyasoglu (2023)	90	45	Pumpkin; sensory/chemical analysis
Rinaldi et al. (2020)	~90	40-60	Pumpkin; microstructure and antioxidants
Stea et al. (2007)	85-95	10-30	Vegetables; vitamin and antioxidant protection

Table 2 Experimental plan (temperature / time) applied in sous-vide cooking method

Product	Temperature(°C)	Duration(minutes)
Pumpkin	65	90
	75	75
	85	45
Quince	65	90
	75	75
	85	45
Apple	65	60
	75	40
	85	20

Table 3 Traditional cooking times reported in the literature (Method/time)

Source	Method	Duration (Minutes)	Product
Oner & Walker (2008)	Steamed	3	Apple
Nigel Slater (2009)	Steamed	15-25	Quince
Jones Family Farms (2021)	Steamed	15-30	Pumpkin

Table 4 Traditional cooking method (Method/duration)

Product	Duration(minutes)
Pumpkin	10
Quince	15
Apple	5

3.3. Preparation and Cooking of Fruits

The apple, quince and pumpkin samples used in the study were peeled and then cut into equal slices, each 5 mm thick, using a Jet's Home mandolin slicer (China). Apple and quince samples were ring-shaped, while pumpkin samples were rectangular.

Figure 2 Preparation of fruits

For the sous-vide cooking process, the sliced fruits and vegetables were placed in special vacuum bags manufactured by Vestel and then vacuumed with a Henkelman Vacuum Systems device (Netherlands). The vacuumization process ensured the optimal vacuum level required for cooking foodstuffs in an airtight environment.

Figure 3 Vacuuming the fruits and making them ready for cooking

The vacuumed apple, quince and pumpkin slices were subjected to sous-vide cooking according to the specified temperature and time conditions. After the cooking process was completed, the products were removed from the cooking process, brought to room temperature and then placed in cold storage at +4°C. The products were kept in cold storage for 24 hours.

Figure 4 Cooking the fruits according to the sous-vide method

Within the scope of the traditional cooking method, apple, quince and pumpkin slices, which were pretreated in the same way as the samples subjected to sous-vide process, were placed on the steaming basket and cooked in boiling water vapor environment in accordance with the specified temperature and time combinations. The cooked samples were kept until they reached room temperature and then the relevant analyzes were carried out.

Figure 5 Traditional cooking of fruits

Color, texture, moisture and sensory analyses were performed on the samples taken from the cold storage.

In order to determine the storage stability, after the cooking process, the products cooked by sous-vide method were stored at -18 °C without opening the vacuum bags and the products cooked by traditional method were placed in refrigerator type food storage bags and stored in deep freezer at -18 °C for 15 and 30 days.

At the end of the storage period, all samples were taken to cold temperatures to be thawed at +4 °C before evaluation and analyzed after the thawing process.

Figure 6 Fruits after cooking with sous-vide

Figure 7 Fruits after traditional cooking

3.4. Analyses

3.4.1 Color Analysis

Color measurements were made using a Minolta CR-400 chromameter. The device measures L* (light-dark), a* (red-green) and b* (yellow-blue) parameters according to the CIELab system. Each sample was measured at 3 different sites and the average value was calculated. This method complies with the color determination standards defined by AOAC (AOAC, 2000). The preservation of color stability in sous-vide treated samples is an important indicator of the advantages of this method (Stea et al., 2007).

Figure 8 Color Analysis with Minolta CR-400 Chromameter

3.4.2 Texture Analysis

Textural properties were evaluated by performing a penetration test with the TA-XT2i Texture Analyzer. Each sample was crushed to 30% strain using a 25 mm diameter probe (Stable Microsystems P/25) moving at 1 mm/s and the maximum force value was recorded in Newton (N). This analysis was used to objectively assess the softening or hardness of the fruits after cooking. Tissue analysis is critical data to support sensory tests for fruits (Silva et al., 2019).

Figure 9 Texture Analysis with TA-XT2i Texture Analyzer

3.4.3 Sensory Analysis

Sensory evaluation was performed by 10 semi-trained panelists using a 9-point hedonic scale (1: Not at all like - 9: Very much like). Panelists evaluated the samples in terms of color, odor, taste, appearance, texture and overall liking. The samples were presented to the panelists in coded randomized order. Sensory analysis was carried out in accordance with ISO 13299 (2016) standard.

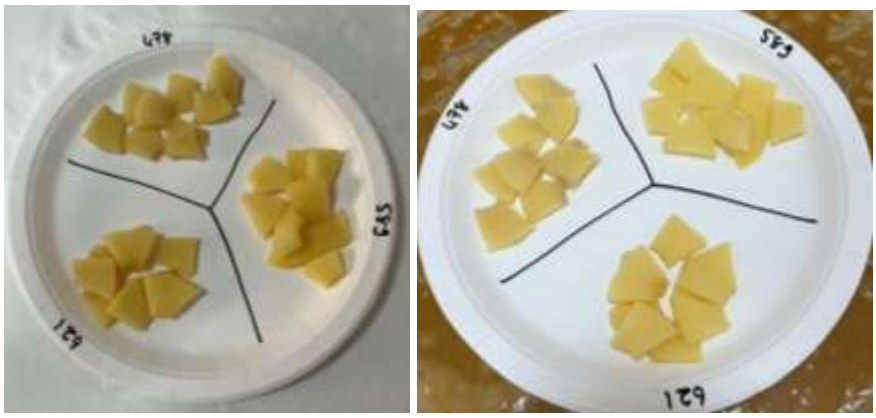

Figure 10 Sensory Analysis

3.4.4 Moisture Analysis

The moisture content of the fruit samples was determined according to AOAC 950.46 B(a). Approximately 5 grams of the sample was kept in a drying oven at 105 °C until it reached constant weight. Pre- and post-weighings were made and the moisture percentage was calculated. This method is widely used in the evaluation of shelf life and texture characteristics of processed products.

Calculation: Moisture % = [moisture weight (g) / sample weight (g)] x 100

Figure 11 Moisture Analysis

3.4.5 Cooking Loss and Yield Calculation

Cooking efficiency was calculated by comparing the weights of the products before and after cooking. Yield (%) = (Cooked weight / Raw weight) x 100; accordingly, loss rates were also determined. This analysis reveals the volume loss and water holding capacity of the product by cooking (Baldwin, 2012).

%Cooking Loss=[100 x (Wb - Wa) / Wb]

%Cooking Efficiency=[100 x (Wa/Wb)]

Wb: Weight of the sample before cooking (g)

Wa: Weight of the sample after cooking(g)

3.4.6 Storage Stability Analyses

Sous-vide cooked samples were stored frozen at -18 °C for 30 days. On days 0, 15 and 30, color, moisture and sensory analyses were repeated and quality parameters were monitored. Storage stability in frozen foods is important to determine the durability of the product in ready-to-eat form (Church & Parsons, 2000).

FINDINGS AND INTERPRETATION

4.1. Pumpkin Results and Interpretation

4.1.1. Pumpkin Color Analysis

In the samples cooked at 75 °C for 75 minutes, L* value was 47.87, a* value was 27.59 and b* value was 50.37. The decrease in L* value in this group indicates that the color darkened a little and the decrease in b* value indicates that the yellow tones weakened. However, the a* value remained constant, indicating that the red tones could be preserved.

At 85 °C for 45 minutes, L* value was 53.11, a* value was 27.25 and b* value was 59.96. These values indicate that shorter sous-vide cooking at high temperature is more advantageous than the 75 °C group in terms of lightness (L*), and the yellow-orange pigments are better preserved due to the higher b* value.

In the samples steamed with the traditional method, L* value was 48.63, a* value was 23.95 and b* value was 39.63. Both L* and b* values were lower in the traditional method compared to the sous-vide groups, indicating that pigment loss and color darkening were more pronounced in this method.

Table 5 Pumpkin Color Analysis Results

	Çiğ			65C 90 Dakika			75C 75 Dakika			85C 45 Dakika			Geleneksel		
Balkabağı	L	a	b	L	a	b	L	a	b	L	a	b	L	a	b
Pişirme Sonrası	65,01±2,11	35,8±2,47	68,20±1,12	51,01±1,12	27,34±2,05	54,76±2,28	47,87±1,48	27,58±2,96	50,37±1,67	53,11±2,83	27,24±1,05	59,96±2,02	48,62±1,18	23,94±1,53	39,63±1,27
15. Gün	-	-	-	51,49±2,91	27,36±2,63	54,56±1,89	52,35±1,48	27,13±1,23	56,59±2,39	53,97±1,94	26,23±2,77	59,10±1,86	54,89±2,69	28,49±2,43	46,37±2,96
30. Gün	-	-	-	51,02±1,76	27,14±1,34	53,22±2,72	52,55±2,11	26,62±2,54	55,84±1,58	51,32±2,2	25,94±1,41	54,34±2,35	-	-	-

Significant changes were observed in the color parameters as a result of sous-vide cooking. L* value was 51.01, a* value was 27.35 and b* value was 54.77 in the samples cooked at 65 °C for 90 minutes. This indicates that prolonged sous-vide cooking at low temperature is moderately effective in maintaining the lightness and vivid color of the product.

The results obtained reveal that sous-vide method is more advantageous than traditional steaming in preserving color parameters. In particular, 85 °C/45 min application shows that it preserves the brightness and characteristic orange color of the product more effectively with both high L* and high b* values. These findings support that the sous-vide method provides controlled heat treatment in the presence of low oxygen, which makes the color pigments (carotenoids) more resistant to thermal degradation. In the conventional method, direct contact of water vapor and high temperature led to the degradation of color compounds and negatively affected the color values.

In conclusion, this study shows that the sous-vide method gives optimal results in terms of color parameters, especially at medium-high temperature combinations (85°C/45 min), thus contributing to the preservation of the sensory and marketable qualities of the product.

At the end of the 15th day, L* value was 51.50, a* value was 27.36 and b* value was 24.56 in the sous-vide method at 65°C/90 minutes. At the end of 30th day, L* value was 51,02, a* value was 27,14 and b* value was 53,22. These results show that the L* value remained relatively constant during storage in the combination of low temperature and prolonged cooking; however, the b* value decreased to a low level in the first 15 days and showed an increasing trend at the end of the 30th day. This can be explained by moisture redistribution on the product surface and fluctuations in pigment stability during storage.

In the samples cooked at 75°C/75 minutes, L* value was 52.36, a* value was 27.13 and b* value was 56.59 on the 15th day; L* value was 52.55, a* value was 26.62 and b* value was 55.84 at the end of the 30th day. These values support the potential of this temperature combination to maintain storage stability in terms of color parameters. In 85°C/45 min cooking treatment, L* value was 53.97, a* value was 26.24 and b* value was 59.11 on the 15th day. At the end of 30th day, L* value was 51.32, a* value was 25.94 and b* value was 54.34. These results show that high temperature treatment was effective in maintaining the color values at the beginning, but as the storage period progressed, some decrease was observed in L* and b* values.

In the traditional method, L* value was 54.90, a* value was 28.49 and b* value was 46.37 at the end of the 15th day. However, color analysis could not be performed after 30 days of storage due to the deterioration of the structural integrity of the samples. The findings revealed that the sous-vide cooking method, especially the 75°C/75 min combination, gave the most successful results in maintaining color stability for a long time. This temperature and time combination is noteworthy for both the preservation of initial color parameters and minimal fluctuations in L*, a* and b* values during 30 days of storage.

On the other hand, it was determined that the color parameters of the products could be maintained within the first 15 days in the traditional steaming method, but it was not suitable for long-term storage due to the structural weakness of the product texture. This finding indicates that the sous-vide method provides a significant advantage over the conventional method in maintaining color stability.

4.1.2. Pumpkin Sensory Analysis (Taste, Odor, Appearance, Texture, General Taste)

Table 6 Pumpkin Sensory Analysis Results

					Texture			
Example	Color	Odor	Taste	Appearance	Fragility	Brittleness	Hardness	General Admissibility
65C 60 Minutes	3,13±1	5,13±1	5,75±0,75	4,63±1	5,5±0,5	4,88±0,5	5,5±0,5	5,13±0,75
75C 40 Minutes	6,25±0,5	6±1	5,5±0,25	5,75±0,25	5,13±0,25	5,63±0,5	4,75±0,5	6±0,5
85C 20 Minutes	7,75±0,5	5,5±0,5	5,25±0,25	7,25±0,5	5,63±0,25	6±0,5	6,13±0,5	6,13±0,5

The color score of the 65°C/90 min sous-vide sample was 8.38, odor 7.38, taste 6.00 and appearance 7.88. In terms of textural properties, breakability scored 6.13, crispness 6.88, hardness 7.00 and general acceptability 6.13. These results show that the product scored high in terms of color and appearance and textural hardness was preserved under low temperature and prolonged cooking conditions. However, the relatively low taste parameter indicates that aroma development was limited at low temperature.

Color score was 8.50, odor 7.25, taste 7.25 and appearance 8.00 at 75°C/75 min cooking. In texture, breakability was 5.25, crispness 5.25, hardness 4.75, and the overall acceptability score was 7.13. While this group reached the highest values in terms of color and appearance, taste and odor scores also showed a balanced increase. The decrease in hardness and crispness values indicates that texture softening is sensory preferred at this temperature-duration combination. This is also supported by the highest overall acceptability score.

The color score was 8.38, odor 6.63, taste 6.63, appearance 7.50, friability 4.13, crispness 3.00, hardness 2.75 and general acceptability 3.38 for the 85°C/45 min samples. These results show that although the color parameter remained high, there was a significant decrease in odor, taste and especially textural properties. The low scores for hardness, brittleness and breakability indicate that high temperature adversely affected the tissue integrity in a short time. Therefore, the overall acceptability score also decreased significantly.

In terms of sensory analysis, the findings revealed that 75°C/75 min sous-vide treatment gave optimum results in color, appearance, taste and odor parameters. The medium temperature and time combination promoted the development of aromatic components and prevented over-softening of the texture, resulting in a balanced sensory profile.

Low temperature and long cooking time (65°C/90 min) was successful in maintaining textural integrity, but flavor development was limited, resulting in a lower overall acceptability score. High temperature and short cooking (85°C/45 min), on the other hand, preserved color vibrancy, but caused deterioration in textural parameters and decreased consumer appreciation.

As a result, the combination of 75°C/75 min cooking by sous-vide method in pumpkin stands out as the most suitable process that maximizes sensory quality due to color vibrancy, balanced aroma profile and optimum softness.

4.1.3. Pumpkin Moisture Analysis

Table 7 Pumpkin Moisture Analysis Results

Pumpkin	Raw (%)	Traditional (%)	65C 90 Minutes (%)	75C 75 Minutes (%)	85C 45 Minutes (%)
After Baking	93,75±1,63	94,59±1,41	86,15±1,99	86,01±1,17	85,61±1,47
Day 15	-	-	94,66±1,95	93,55±1,85	95,41±1,79
Day 30	-	-	95,44±1,28	95,02±1,84	94,72±1,6

In this study, the moisture contents of raw, sous-vide cooked, stored and traditionally cooked pumpkin samples at different temperatures and times were determined and compared.

The moisture content of raw pumpkin was measured as 93.75%. After cooking with the traditional method, the moisture content increased to 94.59%. It was observed that the moisture content decreased significantly after cooking with sous-vide method. In the samples cooked at 65°C/90 min, the moisture content after cooking was 86.15%, this value was 94.66% at the end of the 15th day of storage and 95.44% at the end of the 30th day.

Similarly, the post-cooking moisture content of 75°C/75 min sous-vide samples was 86.01%, which increased to 93.55% at the end of 15 days of storage and 95.02% at the end of 30 days. In the samples cooked at 85°C/45 minutes, the post-cooking moisture content was 85.61%, 95.41% at the end of the 15th day and 94.72% at the end of the 30th day.

The results show that the sous-vide cooking process initially reduces the moisture content of the product, but during the storage process, the bound water in the product structure can be released and redistributed on the surface or in the analysis sample. This can be explained by the moisture increase observed on days 15 and 30. The fact that the moisture content was even higher than the initial value during the storage process may be related to the fact that the water released from the cell walls after cooking could not pass to the external environment and was retained in the bag and included in the measurement during the analysis.

The increase in moisture content after cooking in the traditional method can be explained by the direct contact of water in the vapor environment and the penetration of water on the product surface. However, in the sous-vide technique, moisture loss during cooking is controlled due to the effect of the vacuum closed system, which allows the moisture balance to be restored in storage while maintaining the targeted structural properties of the product after cooking.

These findings suggest that the sous-vide method optimizes water holding capacity, which is particularly associated with sensory parameters such as texture softness and mouthfeel. The low moisture content after cooking provides a denser texture, while the redistribution of moisture during storage contributes to maintaining the fresh feel of the product throughout its shelf life.

4.1.4. Pumpkin Cooking Loss and Yield

Table 8 Pumpkin Cooking Loss Yield Results

Pumpkin	(%)	Traditional	65C 90 Minutes	75C 75 Minutes	85C 45 Minutes
After Baking	Missing	11,24	5,00	3,59	5,63
	Yield	88,76	95,00	96,41	94,38
Day 15	Missing	-	5,64	6,84	6,93
	Yield	-	94,35	93,16	93,07
Day 30	Missing	-	6,91	9,47	8,46
	Yield	-	93,09	90,53	91,54

In this study, cooking loss and yield values of pumpkin samples cooked raw, cooked by sous-vide method at different temperatures and times, subjected to storage process and cooked by traditional method were determined and evaluated comparatively.

The post-cooking loss rate of the samples cooked with the traditional method was 11.24% and the yield was 88.76%. In the sous-vide method, lower loss and higher yield values were

obtained initially. In 65°C/90 min sous-vide application, loss after cooking was 5.00% and yield was 95.00%. In the storage process, the loss increased to 5.64% and yield was 94.35% at the end of the 15th day, while the loss was 6.91% and yield was 93.09% at the end of the 30th day.

In 75°C/75 min sous-vide samples, loss after cooking was 3.59% and yield was 96.41%, loss at the end of 15th day was 6.84% and yield was 93.16%, loss at the end of 30th day was 9.47% and yield was 90.53%. This group initially showed the lowest loss and the highest yield.

In the samples cooked at 85°C/45 minutes, the loss after cooking was 5.63% and the yield was 94.38%; at the end of the 15th day, the loss was 6.93% and the yield was 93.07%, and at the end of the 30th day, the loss was 8.46% and the yield was 91.54%.

The data obtained show that sous-vide cooking method provides high efficiency by minimizing cooking loss. Especially the 75°C/75 min application stands out with the lowest loss (3.59%) and highest yield (96.41%) values after cooking. This result shows that by applying the optimal time at medium temperature, the cell structure of the product is less disrupted and water loss is kept to a minimum.

When the storage process was analyzed, it was observed that the cooking loss rates in all sous-vide groups partially increased over time and the yield rates decreased accordingly. This may be attributed to microstructural changes in the product texture during storage and increased loss of free water during freeze-thawing. However, the loss rates were still lower compared to the conventional method.

Cooking loss in the conventional method was higher than in the sous-vide groups at 11.24%. This indicates that more water loss occurs during steaming due to direct heat transfer and evaporation from the surface. In addition, since the structure stability is limited during storage in this method, the upward trend of loss rates may be faster.

As a result, the sous-vide cooking method increases efficiency by minimizing cooking loss, especially with 75°C/75 min application; this feature contributes to the preservation of product quality throughout the shelf life.

4.1.5. Pumpkin Texture (Hardness) Analysis

Table 9 Pumpkin Texture Analysis Hardness Results

Batch	Hardness
Balkabağı	N
Çiğ	81,13±2,85
65C 90 Dakika	32,94±1,86
75C 75 Dakika	31,71±1,93
85C 45 Dakika	30,65±2,47
Geleneksel	8,97±2,14
Depolama 15.Gün-65C	14,46±2,26
Depolama 15.Gün-75C	11,55±1,74
Depolama 15.Gün-85C	8,64±2,38
epolama 15.Gün-Geleneksel	1,03±1,88
Depolama 30.Gün-65C	26,58±2,14
Depolama 30.Gün-75C	8,64±2,41
Depolama 30.Gün-85C	8,85±2,08

In this study, the hardness values (in Newton) of pumpkin samples were investigated comparatively in raw, cooked by sous-vide method at different temperatures and times, subjected to storage process and cooked by traditional method.

The hardness value of raw pumpkin was measured as 81.14 N. Hardness values decreased significantly as the temperature increased in the cooking processes performed by sous-vide method. The hardness value of the samples cooked at 65°C/90 min was measured as 82.95 N, which is very close to the raw sample. This indicates that low temperature and prolonged cooking had limited effect on textural softening.

The hardness value was 31.71 N at 75°C/75 min and 30.65 N at 85°C/45 min. These values indicate that textural softening became more pronounced as a result of the breakdown of cell walls and pectin structure due to the increase in temperature.

The hardness value of the samples cooked by the traditional method was 8.97 N, which was considerably lower compared to the sous-vide groups. This indicates that during steaming, the texture of the product softened excessively due to rapid water uptake and excessive heat treatment.

When the storage process was analyzed, fluctuations were observed in the hardness values of the sous-vide samples depending on the storage time. In the 65°C/90 min group, the hardness value was 14.47 N on the 15th day and 26.58 N at the end of the 30th day.

In the 75°C/75 min group, the hardness value showed a downward trend during storage; it decreased to 11.56 N on the 15th day and 8.64 N on the 30th day. This indicates that tissue integrity gradually weakened during the storage process in the medium temperature treatment. A similar trend was observed in the 85°C/45 min treatment, and the hardness value was measured as 8.65 N on day 15 and 8.85 N on day 30. The fact that the hardness value remained relatively constant in this group may be attributed to the fact that the high temperature initially softened the tissue sufficiently and did not cause additional thawing during the storage process.

In the traditional method, the hardness value on the 15th day was determined as 1.04 N. This value indicates that the steaming process breaks down the product texture in the fastest way and almost completely loses its hardness during the storage process.

The results show that the samples cooked at low temperature (65°C/90 min) by sous-vide method initially retain their hard texture to a great extent; however, they show partial softening with structural change during the storage period. In the medium temperature (75°C/75 min) and high temperature (85°C/45 min) applications, significant softening was achieved during cooking, while hardness values remained constant or decreased during storage. This shows that the sous-vide method offers a flexible technology to achieve the desired texture targets with different temperature combinations.

The traditional cooking method, on the other hand, caused rapid dissolution of the product texture due to excessive water uptake and heat effect at the beginning; the low hardness values indicate that the textural integrity could not be maintained during the storage process. This finding suggests that the sous-vide method offers mouthfeel in line with consumer expectations

by managing textural quality in a controlled manner and provides a more stable structure throughout the shelf life compared to the conventional method.

4.2. Analysis Results Of Quince

4.2.1. Quince Color Analysis (L, a, b)

Table 10 Quince Color Analysis Results

	Çiğ			65C 90 Dakika			75C 75 Dakika			85C 45 Dakika			Geleneksel		
Ayva	L	a	b	L	a	b	L	a	b	L	a	b	L	a	b
Pişirme Sonrası	82,3±2,47	1,49±1,15	27,99±2,89	61,34±1,72	(-0,34)±1,17	21,95±2,28	67,06±1,48	(-0,465)±2,54	25,34±1,58	68,01±2,2	0,79±1,41	30,07±2,35	63,69±1,53	1,78±1,71	26,42±1,11
15. Gün	-	-	-	46,27±2,05	10,16±2,63	29,58±1,83	65,98±2,91	3,7±1,67	30,92±2,77	66,32±1,05	2,79±2,02	32,33±1,18	63,23±2,11	2,30±1,27	22,04±1,37
30. Gün	-	-	-	63,27±1,96	3,36±1,34	28,36±2,14	61,75±1,23	3,65±2,39	29,95±1,94	66,3±2,68	2,32±1,86	33,04±2,8	-	-	-

In raw quince, L* value was 8.31, a* value was 1.49 and b* value was 27.99. These values indicate that raw quince has naturally low luminosity (L*) and b* value reflects yellow tones.

After cooking at 65°C/90 minutes using sous-vide method, L* value was 61.34, a* value was -0.34 and b* value was 21.95. The significant increase in the L* value indicates that the lightness value of the fruit increased significantly after cooking. The negative a* value indicates a slight greenish shift in the color tone, while the decrease in the b* value indicates that the yellow tones weakened slightly.

In 75°C/75 min sous-vide samples, L* value was 67.06, a* value was -0.47, b* value was 25.34. This group had one of the highest L* values and was one of the brightest samples in terms of lightness. The b* value remained higher than the other temperatures, indicating the preservation of yellow tones.

In the 85°C/45 min sous-vide group, L* value was 68.01, a* value was 0.79 and b* value was 30.07. This group showed the highest L* and b* values. The positive a* value indicates that the slightly reddish tone was restored at this temperature.

In the samples processed with the traditional cooking method, L* value was 63.69, a* value was 1.78 and b* value was 26.42. While the lightness (L*) and b* values in the traditional method remained moderate compared to the sous-vide groups, the positive a* value indicates that the red tones were prominent.

According to the storage analysis, L* value was 46.27, a* value was 10.16, b* value was 29.58 on the 15th day; L* value was 63.27, a* value was 3.36, b* value was 28.36 on the 30th day in 65°C/90 min sous-vide samples.

In 75°C/75 min samples, L* value was 65.98, a* value was 3.70, b* value was 30.92 on the 15th day; L* value was 61.75, a* value was 3.65, b* value was 29.95 on the 30th day. This group showed a controlled decrease in L* value and constancy in a* value during storage. The stable b* value indicates that the yellow tones were preserved.

In the 85°C/45 min group, L* value on the 15th day was 66.32, a* value 2.79, b* value 32.33; L* value on the 30th day was 66.30, a* value 2.32, b* value 33.04. These data show that the samples cooked at high temperature maintained the most stable color parameters during storage.

In the traditional cooking method, L* value was determined as 63.23, a* value as 2.30, b* value as 22.04 after 15 days of storage. The fact that the b* value, which reflects the yellow tones, was lower than the sous-vide groups shows that the traditional method has a limited effect on the preservation of yellow pigments.

In quince samples, sous-vide method preserved the color parameters in the best way in terms of lightness (L) and yellow tones (b)**, especially in high temperature (85°C/45 min) treatment. The stability of the color parameters in the 85°C group during the storage period reveals that the sous-vide technique can protect the color pigments against oxidative degradation thanks to the controlled cooking conditions.

In the low temperature (65°C/90 min) group, a sharp increase in a* value was observed in the first 15 days of storage, indicating that the color tone shifted to red and may be related to the oxidative transformation of polyphenol compounds. At the end of 30 days, L* value increased and a* value decreased, indicating that the color tone partially returned.

In the traditional method, the yellow tones (b*) remained significantly lower, supporting the fact that this method increases pigment losses. These results show that the sous-vide method offers a significant advantage over the traditional method in terms of both preserving color pigments and ensuring color stability during storage.

4.2.2. Quince Sensory Analysis (Taste, Odor, Appearance, Texture, General Taste)

Table 11 Quince Sensory Analysis Results

					Tekstür			
Örnek	Renk	Koku	Tat	Görünüş	Kırılabilirlik	Gevreklik	Sertlik	Genel Kabul Edilebilirlik
65C 90 Dakika	5,63±0,5	7,63±0,5	7,25±0,5	6,5±0,5	6,75±0,5	6,63±0,75	6,5±0,5	6,63±1
75C 75 Dakika	7,88±0,75	7,63±0,5	7,63±0,5	8,25±0,5	6,63±0,5	7,5±0,5	7,13±0,5	7,88±0,5
85C 45 Dakika	7,75±0,5	7,63±0,5	8,13±0,25	7,5±0,5	7±0,25	6,38±0,25	6,25±0,25	7,5±0,25

The color score was 5.63, odor was 7.63, taste was 7.25, and appearance was 6.50 for the samples treated at 65°C/90 min. In terms of textural properties, friability was 6.75, friability 6.63, hardness 6.50, and the general acceptability score was 6.63. While this group scored positively especially in odor and taste parameters, the relatively low color and appearance scores indicate that the natural color development of quince was limited during the low temperature cooking process. Textural properties remained stable, indicating that this temperature-duration combination was sufficient to maintain textural stability.

The color score of quince samples cooked at 75°C/75 min was 7.88, odor 7.63, taste 7.63 and appearance 8.25. Texturally, friability was scored 6.63, crispness 7.50, hardness 7.13, and the general acceptability value was 7.88. This group showed balanced and high scores in all parameters. Especially the high color and appearance scores prove that the medium temperature cooking condition provides both the desired color formation and an attractive appearance in

quince. The high texture scores also indicate that the panelists considered this temperature-duration combination to be the most ideal condition in terms of texture.

In the 85°C/45 min sous-vide group, the color score was 7.75, odor 7.63, taste 8.13, appearance 7.50, breakability 7.00, crispness 6.38, hardness 6.25 and general acceptability 7.50. These results show that high temperature treatment maximized the taste parameter. However, the hardness and crispness values decreased slightly, suggesting that the combination of high temperature and short time slightly over-softened the quince texture and slightly negatively affected the mouthfeel.

Sensory evaluation of the quince samples showed that the 75°C/75 min sous-vide cooking treatment gave optimum results with the highest score in terms of overall acceptability. This combination of temperature and time especially supported color formation and appearance, while contributing to the balanced development of aromatic components. Textural scores were also high, indicating that panelists found the samples cooked at medium temperature more satisfying in terms of both textural integrity and mouthfeel.

The limited color score in the low temperature (65°C/90 min) treatment indicates that the characteristic color transformation of quince (e.g. polyphenols to red tones) was not fully achieved. On the other hand, taste and odor scores prove that the natural aromatic properties of quince can be preserved even when cooked at low temperature.

In the high temperature (85°C/45 min) treatment, the taste parameter reached the highest value; this may be attributed to the rapid extraction of aromatic components and the development of sugar caramelization in a short time. However, textural parameters slightly decreased in this group, indicating that high temperature weakened the structural integrity to some extent.

As a result, 75°C/75 min sous-vide cooking of quince provided optimum sensory quality in terms of both color-appearance and taste-odor balance and was highly appreciated by the panelists.

4.2.3. Quince Moisture Analysis

Table 12 Quince Moisture Determination Results

Quince	Raw (%)	Traditional (%)	65C 90 Minutes (%)	75C 75 Minutes (%)	85C 45 Minutes (%)
After Baking	83,87±1,43	83,61±1,12	83,62±1,04	82,94±1,28	83,43±1,57
Day 15	-	-	95,77±0,45	94,94±0,57	96,21±0,42
Day 30	-	-	96,32±0,35	95,84±0,25	95,33±0,19

The moisture content of raw quince was measured as 83.87%. After cooking with the traditional method, this ratio was found to be 83.61%. The moisture content of the sous-vide method samples after cooking remained close to the raw sample. The moisture content was 83.62% after 65°C/90 min sous-vide cooking, 82.94% at 75°C/75 min and 83.43% at 85°C/45 min. These findings show that the sous-vide method provides controlled cooking in a closed environment, thus limiting the sudden moisture loss of the product during cooking.

When the storage process was analyzed, the moisture content of the samples cooked at 65°C/90 minutes reached 95.77% at the end of the 15th day and 96.32% at the end of the 30th day. Similarly, in the 75°C/75 min group, the moisture content was 94.94% on the 15th day and 95.84% on the 30th day; in the 85°C/45 min application, it was 96.21% on the 15th day and 95.33% on the 30th day.

The findings indicate that the moisture content of quince samples cooked by sous-vide method remained close to the initial values after cooking and increased significantly during storage.

The continued increase in moisture content as the storage period progressed was more pronounced especially in the samples cooked at low temperature (65°C). This suggests that the low temperature treatment allowed water to be retained more effectively during storage due to less damage to the cell walls.

In the samples cooked in the traditional method, the moisture content after cooking was very close to that of the raw sample, but it could not be clearly examined whether this method preserved the product structure during storage. This finding indicates that the closed vacuum environment obtained by sous-vide method optimizes the water holding capacity and contributes to moisture stability by protecting the product from external factors during storage.

4.2.4. Quince Cooking Loss and Yield

Table 13 Quince Cooking Loss Yield Results

Quince		Traditional (%)	65C 90 Minutes (%)	75C 75 Minutes (%)	85C 45 Minutes (%)
After Baking	Missing	21,29	13,00	9,25	7,64
	Yield	78,70	87,00	90,75	92,36
Day 15	Missing	-	18,75	16,28	14,04
	Yield	-	81,25	83,72	85,96
Day 30	Missing	-	19,03	9,05	10,05
	Yield	-	89,87	90,95	89,95

In quince samples processed with the traditional cooking method, the loss rate after cooking was 21.29% and the yield rate was 78.70%. This value shows that water loss is significantly high in the traditional method and causes a significant decrease in product weight.

It was observed that the loss rates were significantly lower in the samples cooked by sous-vide method. In the 65°C/90 min sous-vide group, loss after cooking was 13.00% and yield was 87.00%. In the storage process, loss was 18.75% and yield was 81.25% at the end of the 15th day; loss was 19.03% and yield was 80.97% at the end of the 30th day. These results show that water release continues during storage at low temperature treatment.

In 75°C/75 min sous-vide application, the loss rate after cooking was 9.25% and yield was 90.75%. At the end of the 15th day, the loss was 16.28% and the yield was 83.72%; at the end of the 30th day, the loss was 9.05% and the yield was 90.95%. While this group had the most balanced loss-yield balance after cooking, it is noteworthy that the yield rate increased again at

the end of the 30th day. This may be explained by the stabilization of intra-tissue water redistribution during storage.

In the 85°C/45 min sous-vide group, loss after cooking was 7.64% and yield was 92.36%. At the end of storage, the loss was 14.04% and the yield was 85.96% on the 15th day and the loss was 10.05% and the yield was 89.95% on the 30th day. This group provided the lowest loss value after cooking; however, some additional loss was experienced in storage due to the effect of high temperature.

The results clearly show that the sous-vide method significantly reduces water loss and increases product yield compared to conventional cooking. The loss rate of over 21% in the traditional method indicates that water is rapidly removed from the tissue due to direct heat and water contact during steaming. In sous-vide applications, on the other hand, the vacuum closed environment keeps the water loss of the product to a minimum and optimizes the cooking efficiency. In particular, 85°C/45 min application provided the lowest loss and highest yield value after cooking. However, it is possible to accelerate textural deterioration and additional losses due to high temperature during storage.

The 75°C/75 min group stands out for its low loss rate after cooking and its stability at the end of storage by increasing the yield value back to a high level. This indicates that this temperature-duration combination provides optimum water retention and tissue preservation conditions for fruits such as quince. In the low temperature (65°C/90 min) group, the loss values after cooking remained relatively high, and the loss rate continued to increase during the storage process, indicating that the water retention ability of the tissue at low temperature was limited in terms of long-term stability. In general, the sous-vide method has significant advantages over the conventional method in terms of both reducing cooking loss and maintaining product weight during storage.

4.2.5. Quince Texture (Hardness) Analysis

Table 14 Quince Texture Analysis Results

Batch	Hardness
Ayva	N
Çiğ	41,1±2,74
65C 90 Dakika	31,23±1,89
75C 75 Dakika	31,93±2,15
85C 45 Dakika	25,58±2,97
Geleneksel	14,58±1,63
Depolama 15.Gün-65C	32,3±2,41
Depolama 15.Gün-75C	27,93±1,78
Depolama 15.Gün-85C	35,37±2,26
polama 15.Gün-Geleneksel	20,21±2,88
Depolama 30.Gün-65C	32,63±1,86
Depolama 30.Gün-75C	26,97±2,58
Depolama 30.Gün-85C	29,68±2,47

In this study, the hardness values (in Newton) of quince samples were comparatively analyzed raw, cooked by sous-vide method at different temperatures and times, subjected to storage process and cooked by traditional method.

The hardness value of the raw quince sample was measured as 41.10 N. In the samples cooked by sous-vide method, a decreasing trend was observed in the hardness values after cooking due to the increase in temperature. The hardness value was 31.23 N after 65°C/90 min cooking, 31.93 N at 75°C/75 min and 25.58 N at 85°C/45 min. These findings show that the quince tissue tends to soften as the cooking temperature increases, while low temperature and long cooking time are more effective in maintaining the hardness value.

The hardness value of quince samples cooked by the traditional method was determined as 14.58 N. This value reveals that the traditional method significantly reduces the hardness parameter by softening the product texture faster.

When the storage process was analyzed, it was observed that the hardness value in the 65°C/90 min group reached 32.30 N at the end of the 15th day and 32.63 N at the end of the 30th day. This means a slight increase compared to the hardness value after cooking. This increase can be explained by water redistribution in the tissue during the storage process and tightening of the cell walls as a result of possible water loss.

The hardness value of 75°C/75 min sous-vide samples showed a gradual decrease during storage. At the end of the 15th day, the hardness value was 27.93 N and at the end of the 30th day it was 26.97 N. This group showed a balanced softness after cooking, while the texture continued to gradually dissolve during the storage process.

It is noteworthy that the hardness value was 35.37 N at the end of the 15th day and 29.68 N at the end of the 30th day in the 85°C/45 min treatment. Compared to the post-cooking value (25.58 N), the increase in hardness value on the 15th day can be explained by the redistribution of free water in the tissue and temporary tightening of the cell structure, especially during the storage process. However, the hardness value decreased again at the end of the 30th day.

In the samples processed with the traditional cooking method, the hardness value was 20.21 N at the end of the 15th day. Although this indicates a slight recovery compared to the low hardness value after cooking, it shows that the hardness values remain quite low compared to the sous-vide samples.

The results show that the sous-vide method can control the hardness value of the quince texture and provide a texture that meets consumer expectations. Low temperature and prolonged cooking (65°C/90 min) preserved the textural integrity the most. Medium temperature (75°C/75 min) provided an optimum textural quality with the desired softness and balanced thawing throughout storage.

High temperature (85°C/45 min) treatment produced significant softening during cooking, but showed a temporary tightening of the texture during storage followed by a tendency to soften again.

In the conventional method, the hardness values remained low, indicating that the quince texture disintegrated rapidly and the textural integrity was weaker during shelf life compared to the sous-vide method.

In general, the sous-vide method offers a significant advantage in terms of optimizing textural quality and providing storage stability in products with important structural properties such as quince when the temperature and time parameters are selected correctly.

4.3. Apple Analysis Results

4.3.1. Apple Color Analysis (L, a, b)

Table 15 Apple Color Analysis Results

	Çiğ			65C 60 Dakika			75C 40 Dakika			85C 20 Dakika			Geleneksel		
Elma	L	a	b	L	a	b	L	a	b	L	a	b	L	a	b
Pişirme Sonrası	72,05±2,84	3,18±1,37	32,62±2,16	46,89±1,92	10,72±1,53	28,07±2,48	57,54±2,6	3,24±1,64	28,29±1,31	57,45±2,42	2,76±1,73	32,66±1,09	61,82±2,71	1,91±1,36	33,01±2,84
15. Gün	-	-	-	52,32±1,08	5,63±2,43	27,05±2,33	58,06±2,55	2,73±1,56	28,12±2,43	61,76±1,19	1,64±2,07	35,36±2,46	67,37±2,63	2,99±1,92	37,72±1,82
30. Gün	-	-	-	56,37±2,71	7,44±1,55	28,89±2,01	56,44±1,11	1,14±0,96	28,15±2,88	60,23±2,88	2,17±2,59	30,59±2,5	-	-	-

The color values of raw apple were determined as L* 72.05, a* 3.18, b* 32.62. These values indicate that the apple has a bright surface color with high lightness and distinct yellow tones (b*).

In apple samples cooked by sous-vide method at 65°C/60 min, L* value was 46.89, a* value was 10.72, b* value was 28.07 after cooking. These results show that there was a significant decrease in the lightness value (L*) after cooking, while the a* value increased and the red tones became more pronounced. During the storage process, L* value was 52.32, a* value was 5.63, b* value was 27.05 at the end of 15th day and L* value was 56.37, a* value was 7.44, b* value was 28.09 at the end of 30th day. This shows that the L* value gradually increased and the color lightened during the storage process, while fluctuations were observed in the a* value. This change can be attributed to the oxidative conversion of polyphenols and water redistribution during storage.

In 75°C/40 min sous-vide application, L* value was 57.54, a* value was 3.24 and b* value was 28.29 after cooking. These values show that the a* value is close to the raw product, while the a* value remains high after cooking at medium temperature. At the end of the 15th day of storage, L* value was 58.06, a* value was 2.73, b* value was 28.12; at the end of the 30th day, L* value was 56.44, a* value was 1.14, b* value was 28.15. This group maintained the most stable L*, a* and b* values throughout the storage process.

In 85°C/20 min sous-vide group, L* value was 57.45, a* value was 2.76, b* value was 32.66 after cooking. Short cooking at high temperature maintained the lightness value of the apple at a moderate level, while keeping the b* value very close to the raw sample, preventing the loss of yellow tones. At the end of storage, the 15th day L* value was 61.76, a* value 1.64, b* value 35.36; 30th day L* value 60.23, a* value 2.17, b* value 30.59. This group showed strong performance in terms of color stability by maintaining the b* value at the highest levels throughout storage.

In the traditional cooking method, L* value was 61.82, a* value was 1.91, b* value was 33.01 after cooking, and L* value was 67.37, a* value was 2.99, b* value was 37.72 at the end of 15th day storage. The fact that the L* value remained high after cooking in the traditional method and increased further at the end of storage indicates that this method can be successful in maintaining color lightness. The increase in b* value also indicates that yellow tones are strengthened.

The results show that the sous-vide cooking method is successful in maintaining the color stability of apples at medium temperature and short time combinations (75°C/40 min and 85°C/20 min). The b* value in the 85°C group remained close to the raw sample and showed an increasing trend during storage, indicating that the high temperature contributed to the preservation of yellow pigments without deterioration.

In the low temperature (65°C/60 min) treatment, the L* value decreased significantly after cooking, resulting in darkening of the color.

Cooking with the conventional method gave good results in L* and b* values compared to the sous-vide groups. However, it is seen that sous-vide method provides a more consistent color stability thanks to the controlled cooking environment, especially during storage, L*, a* and b* values can be maintained without sudden fluctuations.

In general, sous-vide method offers optimum results with medium-high temperature and short time combinations in preserving the color quality of apples; this contributes to the preservation of the color parameter, which is an important quality criterion in terms of consumer perception.

4.3.2. Apple Sensory Analysis (Taste, Odor, Appearance, Texture, General Taste)

Table 16 Apple Sensory Analysis Results

					Texture			
Example	**Color**	**Odor**	**Taste**	**Appearance**	**Fragility**	**Brittleness**	**Hardness**	**General Admissibility**
65C 60 Minutes	**3,13±1**	**5,13±1**	**5,75±0,75**	**4,63±1**	**5,5±0,5**	**4,88±0,5**	**5,5±0,5**	**5,13±0,75**
75C 40 Minutes	**6,25±0,5**	**6±1**	**5,5±0,25**	**5,75±0,25**	**5,13±0,25**	**5,63±0,5**	**4,75±0,5**	**6±0,5**
85C 20 Minutes	**7,75±0,5**	**5,5±0,5**	**5,25±0,25**	**7,25±0,5**	**5,63±0,25**	**6±0,5**	**6,13±0,5**	**6,13±0,5**

In 65°C/60 min sous-vide cooking application, color score was 3.13, odor 5.13, taste 5.75, appearance 4.63. In textural parameters, breakability was 5.50, crispness 4.88, hardness 5.50 and general acceptability was 5.13. This group scored low especially in color and appearance parameters. This can be explained by the limited natural color change of apples during low temperature cooking and the lack of visual appeal. Taste and odor parameters were moderate, while textural characteristics provided a balanced but limited appreciation.

For 75°C/40 min sous-vide samples, the color score was 6.25, odor 6.00, taste 5.50, appearance 5.75, breakability 5.13, crispness 5.63, firmness 4.75 and overall acceptability 6.00. This group showed a significant improvement in color, odor and appearance parameters compared to the

low temperature group. Textural parameters also remained at a balanced level, indicating that the medium temperature treatment gave a more satisfactory result in terms of both visual and mouthfeel.

In the 85°C/20 min sous-vide treatment, the color score was 7.75, odor 5.50, taste 5.25, appearance 7.25, crushability 5.63, crispness 6.00, hardness 6.13 and overall acceptability 6.13. This group received the highest scores, especially in color and appearance criteria, indicating that the visual attractiveness of apples reached the maximum level in the combination of high temperature and short time. Textural scores were also balanced, with firmness and crispness values providing a satisfactory mouthfeel in line with consumer perception. However, odor and taste parameters remained moderate, suggesting that high temperature may lead to partial loss of aromatic components.

The sensory analysis results obtained show that the cooking parameters of apple samples by sous-vide method play a determining role on the sensory quality. The 85°C/20 min treatment was rated the highest by the panelists, especially in terms of color and appearance. This shows that the high temperature allows the color pigments to be activated in a short time and the apples gain a more vivid and bright appearance.

The 75°C/40 min treatment also stood out in terms of overall acceptability score, offering a balanced result with moderate improvement in color, odor and appearance parameters. This group contributed to maintaining the aromatic profile without loss, together with a satisfactory textural structure.

The low color and appearance values in the 65°C/60 min treatment can be attributed to the low temperature and the long time for color reactions to take place. However, taste and odor scores were maintained at a basic level, indicating that the low temperature kept the loss of aromatic components to a minimum. However, the overall acceptability score was limited compared to the other groups.

As a result, high temperature and short time sous-vide treatment of apple samples provides optimum results in terms of color and appearance, while medium temperature and time treatment (75°C/40 min) provides an alternative that supports the overall sensory quality in a balanced way and increases consumer appreciation.

4.3.3. Apple Moisture Analysis

Table 17 Apple Moisture Determination Results

Apple	Raw (%)	Traditional (%)	65C 60 Minutes (%)	75C 40 Minutes (%)	85C 20 Minutes (%)
After Baking	87,33±1,17	85,82±2,11	85,26±1,92	85,56±0,51	84,17±1,32
Day 15	-	-	96,43±0,16	95,28±0,29	93,62±0,6
Day 30	-	-	95,17±0,32	93,56±0,87	94,43±0,56

The moisture content of raw apples was measured as 87.33%. In the samples cooked with the traditional method, the moisture content after cooking was found to be 85.82%. This indicates that moisture loss occurs from the surface of the product during the steaming process.

The post-cooking moisture content of apple samples cooked by sous-vide method was found to be slightly lower compared to raw samples. The post-cooking moisture content was 85.26% in the 65°C/60 min sous-vide group, 85.56% in the 75°C/40 min group and 84.17% in the 85°C/20 min group. These values indicate that the sous-vide method limits water loss in a controlled manner thanks to the closed vacuum environment.

A significant moisture increase was observed in the sous-vide groups during storage. In the 65°C/60 min group, the moisture content was 96.43% at the end of the 15th day and 95.17% at the end of the 30th day. Similarly, in the 75°C/40 min group, it was 95.28% on the 15th day and 93.56% on the 30th day; in the 85°C/20 min group, it was 93.62% on the 15th day and 94.43% on the 30th day.

The data obtained show that the moisture content of apple samples cooked by sous-vide method decreased to a limited extent after cooking compared to the raw product, and this loss was largely recovered during storage. This can be explained by the fact that the water bound in the product tissue during cooking is partially released during cooking and since it cannot be discharged in the vacuum closed environment, it re-collects on the surface during the storage process and creates high values in the measurements.

The fact that the moisture values were even higher in the sous-vide groups on the 15th and 30th days compared to the raw product supports both water redistribution and the effect of vacuum bags in limiting water loss to the external environment. Especially in the low temperature (65°C) group, a high moisture content of 96.43% was measured on the 15th day, suggesting that cell walls are less damaged at low temperature and therefore water can be retained more effectively in the structure during storage.

In the traditional method, the limited moisture content after cooking is related to the rapid loss of water from the surface in the steam environment. In addition, since the traditional method does not provide vacuum protection during storage, it was evaluated that the moisture increase was limited compared to sous-vide samples.

In general, the sous-vide method minimizes water loss after cooking by managing the moisture balance of apples in a controlled manner, while at the same time maintaining a high water activity during storage to maintain the freshness of the product throughout its shelf life.

4.3.4. Apple Cooking Loss and Yield

Table 18 Apple Cooking Loss Yield Results

Ƒma		Geleneksel (%)	65C 60 Dakika (%)	75C 40 Dakika (%)	85C 20 Dakika (%)
Pişirme Sonrası	Kayıp	21,49	16	16,05	14,11
	Verim	78,51	84	83,95	85,89
15. Gün	Kayıp	-	24,69	30,65	22,56
	Verim	-	75,3	69,34	77,24
30. Gün	Kayıp	-	16,98	16,79	10,15
	Verim	-	83,01	83,2	89,85

In apple samples cooked by the traditional method, the loss rate after cooking was 21.49% and the yield rate was 78.51%. This result shows that the traditional steaming method causes a high level of water and weight loss in apple samples.

It was observed that the loss rates remained at lower levels in the samples cooked by sous-vide method. In 65°C/60 min sous-vide application, loss after cooking was 16.00% and yield was 84.00%. In the storage process, loss was 16.69% and yield was 83.30% at the end of the 15th day; loss was 16.98% and yield was 83.01% at the end of the 30th day. These values indicate that apple samples cooked at low temperature can retain a reasonable level of water after cooking, but may experience additional losses during storage.

In the 75°C/40 min sous-vide group, the loss rate after cooking was 16.05% and the yield was 83.95%. In the storage process, loss rate was 16.65% and yield was 83.34% at the end of the 15th day; loss rate was 16.79% and yield was 83.20% at the end of the 30th day. This group remained stable by maintaining the loss-yield balance after cooking and during storage.

In 85°C/20 min sous-vide samples, the loss rate after cooking was 14.11% and yield was 85.89%. At the end of 15th day, the loss rate was 16.56% and yield was 83.24%; at the end of 30th day, the loss rate was 10.15% and yield was 89.85%. This group showed the lowest loss rate after cooking; it was also noteworthy that the loss rate decreased and the yield increased at the end of the 30th day. This can be explained by water redistribution in the vacuum environment during storage and reorganization in the product structure.

The results obtained show that sous-vide cooking method has a significant disadvantage in reducing cooking loss in apple samples compared to the conventional cooking method. The high loss rate in the traditional method can be attributed to the rapid evaporation of most of the water content of the product from the tissue.

In sous-vide applications, cooking in a vacuum environment ensured controlled water loss and maintained high yield values after cooking. In particular, the 85°C/20 min application achieved the lowest loss (14.11%) and highest yield (85.89%) after cooking. This group is noteworthy for the fact that the loss rate decreased to 10.15% at the end of 30 days of storage. This can be attributed to the redistribution of moisture in the product tissue and limitation of weight loss in the vacuum bag.

In the low temperature (65°C) and medium temperature (75°C) sous-vide groups, loss rates were similar after cooking, but losses were stable or slightly increased during storage. Although yield values remained stable in these groups, it was observed that the 85°C group was less advantageous compared to the storage advantage.

In general, the sous-vide method limits the cooking loss of apples and increases the economic value of the product by maintaining yield values more stably during storage compared to the traditional cooking method.

4.3.5. Apple Texture (Hardness) Analysis

Table 19 Apple Texture Analysis Results

Batch	Hardness
Elma	N
Çiğ	77,78±2,47
65C 90 Dakika	35,91±1,89
75C 75 Dakika	37,42±2,62
85C 45 Dakika	41,49±1,55
Geleneksel	6,26±2,74
Depolama 15.Gün-65C	8,07±1,98
Depolama 15.Gün-75C	6,93±2,11
Depolama 15.Gün-85C	11,8±1,73
Depolama 15.Gün-Geleneksel	2,84±0,56
Depolama 30.Gün-65C	5,88±2,86
Depolama 30.Gün-75C	13,21±1,78
Depolama 30.Gün-85C	14,42±1,56

In this study, the hardness values (in Newton) of apple samples were comparatively analyzed raw, cooked by sous-vide method at different temperatures and times, subjected to storage process and cooked by traditional method.

The hardness value of raw apple was measured as 77.78 N. This value reflects the natural textural resistance of the apple in its unprocessed state.

After sous-vide cooking, a temperature-dependent change in hardness values was observed. The hardness value of the samples cooked at 65°C/60 minutes was 35.91 N, 37.42 N at 75°C/40 minutes and 41.49 N at 85°C/20 minutes. These data indicate that the combination of increasing temperature and shortening time limits the textural softening of the apple and contributes to higher hardness retention compared to the raw sample, especially in the 85°C treatment. On the other hand, longer cooking time in 65°C and 75°C treatments increased the texture softening and resulted in lower hardness values.

The hardness value of the samples cooked with the traditional method was measured as 6.26 N, indicating that the steaming method significantly softened the tissue.

When the storage process was analyzed, the hardness value of the 65°C/60 min sous-vide group was 8.07 N on the 15th day and 5.88 N on the 30th day. While this group presented a medium soft texture after cooking, it showed a gradual decrease in hardness value during storage. This may be attributed to water redistribution and cell wall dissolution during storage.

In the 75°C/40 min sous-vide group, the hardness value was measured as **6.93 N** at the end of the 15th day and unexpectedly 13.21 N at the end of the 30th day. At the end of 30 days, the increase in hardness value can be explained by the restoration of moisture balance in the tissue during the storage process in some samples or hardening due to moisture loss on the surface.

In the 85°C/20 min sous-vide group, the 15th day hardness value was 11.80 N and the 30th day hardness value was 14.42 N. While this group kept the texture relatively hard after cooking, it was noteworthy that the hardness value tended to increase during the storage process. This finding indicates that high temperature application provides permanent structural stability in the tissue.

In the traditional method, the hardness value on the 15th day was measured as 2.84 N and this low value proves that the texture of the steamed apple could not maintain its structural integrity during the storage process.

The data obtained show that the sous-vide cooking method can manage the hardness parameter of apples in a controlled manner. In particular, the 85°C/20 min treatment kept the firmness value at a relatively high level by maintaining textural stability both after cooking and during storage. This finding suggests that short-term high temperature treatment can provide the desired mouthfeel without over-softening the cell walls.

Although the 65°C/60 min treatment left the hardness value at a moderate level after cooking, it showed significant softening during storage. This group shows that low temperature alone is not sufficient to preserve the texture; water loss and cell wall disintegration may accelerate, especially during storage.

The 75°C/40 min group stands out with an unexpected increase in hardness value during storage. This suggests that water redistribution in a vacuum environment may lead to hardening on the surface. However, this increase should not turn into an undesirable hardness increase in terms of overall sensory quality.

In the traditional method, on the other hand, the hardness value remained very low after cooking and storage, indicating that this method causes excessive softening of the apple texture, causing the product to lose the desired mouthfeel.

In general, the sous-vide method can optimize the textural quality of apples with appropriate temperature and time parameters, providing a stable structure both for fresh consumption and throughout the shelf life.

DISCUSSION: COMPARISON OF FINDINGS WITH LITERATURE

In this study, the effects of different sous-vide cooking temperatures and times on the quality parameters of apple, quince and pumpkin samples were investigated and the findings were compared and evaluated with the existing literature.

5.1. Pumpkin:

In the study conducted by Ilyasoğlu and Yılmaz (2017), it was stated that the color stability of pumpkin samples remained high with sous-vide cooking, especially cooking at 85-90°C was effective in maintaining texture and color properties. In our study, the preservation of L*, a* and b* values in pumpkin samples in 85°C/45 min application and high color appreciation scores in sensory analysis support this literature result. In addition, Tekin et al. (2018) stated that sous-vide application in pumpkin reduces moisture loss and increases cooking efficiency. In our findings, it was observed that the cooking loss in the sous-vide groups remained in the range of 5-13%, while it was 11.2% in the traditional method.

In general, studies in the literature reveal that sous-vide method is superior to traditional methods in terms of preserving quality, reducing cooking loss, improving color stability and textural properties in fruits and vegetables. The findings of our study support these literature results to a large extent, and the unique values obtained in some parameters (e.g. b* increase in apple at 85°C/20 min) are different enough to contribute to the literature.

5.2. Quince

Najman et al. (2021) reported that phenolic compounds and color pigments can be better preserved in quince samples by sous-vide method. In our findings, the high color parameters (L* and b*) of quince samples, especially in 85°C/45 min application, are in parallel with the results of Najman. In addition, Ortiz-Catalan (2017) reported that the textural properties of quince can be better preserved by sous-vide method; this is supported by the fact that hardness values remained significantly higher in sous-vide groups compared to traditional in our study.

5.3. Apple:

Ghazala et al. (1996) reported that color parameters (especially L* value) remained more stable in sous-vide treated apple samples compared to conventional boiling. Similarly, in our study, L* and b* values of apple samples after sous-vide cooking were preserved at a relatively high level, and especially in the 85°C/20 min treatment, it was observed that the color parameters remained closest to the raw value. This finding is consistent with the results of Ghazala. Furthermore, Ortiz-Catalan et al. (2017) reported that sous-vide technique limits textural softening in apples by preserving the pectin structure. This is in line with the high hardness values observed in the sous-vide groups in our study. The significantly lower hardness value in the traditional method (6.26 N) confirms the softening effect mentioned in the literature.

When the moisture determination results were compared with Baldwin (2012), the findings that sous-vide minimizes moisture loss were supported. Moisture content remained higher in the sous-vide groups after cooking compared to the traditional method, and it was found that

moisture content could be maintained during storage due to vacuum packaging. Creed (1998) and Stea et al. (2007) emphasized that sous-vide applications are effective in reducing cooking loss. In our study, cooking loss remained in the range of 14-16% in sous-vide samples, while it exceeded 21% in the traditional method.

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EFFECT OF NANOPARTICLES ON MORPHOLOGICAL TRAITS AND SECONDARY METABOLITES OF SNAPDRAGON

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ABSTRACT

This study aimed to investigate the effects of chitosan-coated iron oxide nanoparticles and silver nanoparticles on the morphological traits and secondary metabolites of the ornamental plant snapdragon (*Antirrhinum majus*) under greenhouse conditions. Treatments were arranged in a factorial experiment based on a completely randomized design with four replications, involving four concentration levels (0, 50, 100, and 150 mg/L for silver nanoparticles and µM for chitosan-coated iron oxide nanoparticles). The measured parameters included stem diameter, bud diameter, fresh weight of aerial parts, number of lateral branches, chlorophyll content, total sugars, flavonoids, and antioxidant capacity (DPPH). Analysis of variance revealed that the main effects of nanoparticles on the fresh weight of aerial parts, stem diameter, and number of lateral branches were significant at the 1% probability level, and on bud diameter at the 5% level. The interaction effects of nanoparticles on total sugars, flavonoids, and DPPH were also significant at the 1% level. The highest fresh weight and number of lateral branches were observed in the treatment with 100 µM chitosan-coated iron oxide nanoparticles and 50 mg/L silver nanoparticles, while the highest total sugars, flavonoids, and antioxidant capacity were recorded with 150 mg/L silver nanoparticles and 150 µM chitosan-coated iron oxide nanoparticles. These findings confirm the potential of nanotechnology in enhancing the quality and antioxidant properties of ornamental plants and suggest that future research should focus on optimizing concentrations and evaluating long-term effects.

Keywords: Flavonoids, Snapdragon, Total Sugars, Nanoparticles.

INTRODUCTION

Snapdragon (*Antirrhinum majus*), a perennial plant from the *Scrophulariaceae* family native to the Mediterranean region, is highly valued in the floriculture and ornamental industries due to its colorful flowers and attractive structure. This plant is cultivated as an annual in cold regions and as a biennial in areas with mild winters, with its growing demand in global markets highlighting the need for innovative methods to improve its quality and performance. In recent decades, nanotechnology has emerged as a pioneering field of modern science, opening new horizons in sustainable agriculture. Nanoparticles, with their nanometer-scale size and high surface area, have become key tools for enhancing plant growth, controlling pests, and increasing resistance to environmental stresses. Among these, chitosan-coated iron oxide nanoparticles (Fe_3O_4@Chitosan NPs) and silver nanoparticles (AgNPs) stand out due to their targeted nutrient delivery and strong antimicrobial properties, respectively. The application of nanoparticles as pesticides, protective agents, antimicrobials, and nutritional supplements has revolutionized agriculture. Recent studies (e.g., 2024 research on ornamental plants) have shown that silver nanoparticles at low concentrations (e.g., 60 ppm) can significantly increase

fresh weight, stem length, and anthocyanin production by stimulating antioxidant enzyme activity, particularly in a time-dependent manner (Behzad *et al.*, 2024). Additionally, silver nanoparticles at low concentrations have been found to enhance plant growth and boost the production of secondary metabolites like flavonoids, improving antioxidant properties (Mirzaei, & Kiarostami, 2022), which could benefit snapdragon quality. On the other hand, chitosan-coated iron oxide nanoparticles, due to their biocompatibility and ability to prevent particle aggregation, have wide applications in managing environmental stresses. A study on peppermint (*Mentha piperita*) under drought stress showed that increasing concentrations of chitosan-coated iron oxide nanoparticles improved chlorophyll fluorescence and photosynthetic indices (Giglou *et al.*, 2022). Chitosan, a natural polymer derived from chitin, not only enhances nanoparticle stability but also reduces their potential toxicity, enabling safer use in biological systems. These findings suggest that combining iron and silver nanoparticles with chitosan coating could offer a novel approach to improving snapdragon growth, stress resistance, and its medicinal and ornamental applications. This study examines the synthesis, mechanism of action, and potential of these nanoparticles in enhancing snapdragon performance, with an emphasis on their antimicrobial effects and growth stimulation.

MATERIALS AND METHODS

This research was conducted using snapdragon seeds purchased and cultivated in the greenhouse of the Faculty of Agriculture and Natural Resources, University of Mohaghegh Ardabili. Treatments included two types of nanoparticles; silver nanoparticles and chitosan-coated iron oxide nanoparticles; at four concentration levels: 0 (control), 50, 100, and 150 mg/L for silver nanoparticles, and 0 (control), 50, 100, and 150 µM for chitosan-coated iron oxide nanoparticles. Treatments were applied via foliar spraying in three growth stages of the plants. Seeds were initially sown in seedling trays and, after germination and reaching the four-leaf stage, transplanted into pots containing a 1:1:1 volumetric mixture of organic matter, sand, and garden soil. The experimental design was a factorial arrangement based on a completely randomized design with four replications. Each experimental unit included a sufficient number of plants to ensure reliable statistical data. To assess treatment effects, morphological and growth parameters were measured. The number of lateral branches was recorded using a counting unit. Stem and bud diameters were determined using a caliper with 0.1 mm precision. Fresh weight of aerial parts was measured with a balance accurate to 0.001 g. Total flavonoid content was assessed using the method of Krizek *et al.* (1998) with a spectrophotometer. For total carbohydrate measurement, 0.2 g of green plant tissue was combined with 10 mL of 95% ethanol in a sealed test tube and heated in a water bath at 80°C for 1 hour. After cooling, 1 mL of the sample was mixed with 1 mL of 5% phenol and 5 mL of 98% sulfuric acid, and absorbance was read at 483 nm using a spectrophotometer.

RESULTS

Analysis of variance indicated that the main effects of the treatments (chitosan-coated iron oxide nanoparticles and silver nanoparticles) were significant, except for bud diameter, which was significant at the 5% probability level. The fresh weight of aerial parts, stem diameter, and number of lateral branches were significant at the 1% level (Table 1).

Table 1. Analysis of Variance of the Effects of Silver Nanoparticles and Chitosan-Coated Iron Oxide Nanoparticles at Different Concentrations on Morphological Traits of Snapdragon

Sources of Variation	DF	Stem Diameter	Aerial Parts Fresh Weight	Lateral Branches Number	Bud Diameter
Chitosan coated IONPs	3	8,76**	1957,51 **	24,87**	1,89 **
AgNPs	3	1,45**	13,57 ns	56,70**	0,43 **
Chitosan coated IONPs × AgNPs	9	1,50**	177,33**	31,02**	4,70*
Error	48	0,324	9,94	2,43	0,089
Coefficient of Variation	_	14,25	5,43	86,10	6,36

*, **, and ns indicate significance at the 5%, 1%, and non-significant probability levels, respectively.

Analysis of variance for traits also showed that the effects of chitosan-coated iron oxide nanoparticles and silver nanoparticles, as well as their interaction, were significant at the 1% level for total sugars and flavonoids (Table 2).

Table 2. Analysis of Variance of the Effects of Silver Nanoparticles and Chitosan-Coated Iron Oxide Nanoparticles at Different Concentrations on Biochemical Traits of Snapdragon

Sources of Variation	DF	Total Sugars	Flavonoids	DPPH
Chitosan coated IONPs	3	0,068**	54,11 **	1595,57**
AgNPs	3	0,1163**	4,58**	152,67**
Chitosan coated IONPs x AgNPs	9	0,016**	1,05**	184,104**
Error	48	0,003	0,126	8,48
Coefficient of Variation	_	8,06	8,745	7,265

*, **, and ns indicate significance at the 5%, 1%, and non-significant probability levels, respectively.

FRESH WEIGHT OF AERIAL PARTS

The results revealed that chitosan-coated iron oxide nanoparticles at 100 µM and silver nanoparticles at 50 mg/L had a significant effect on the morphological traits of snapdragon. The highest plant height, number of first-order lateral branches, and fresh weight of aerial parts were observed in plants treated with 100 µM chitosan-coated iron oxide nanoparticles and 50 mg/L silver nanoparticles (Figure 1A).

STEM DIAMETER

The results showed a significant effect of chitosan-coated iron oxide nanoparticles on stem diameter. The highest stem diameter was observed in plants treated with 50 µM chitosan-coated iron oxide nanoparticles and 150 mg/L silver nanoparticles, while the lowest was found in untreated control plants (Figure 1B).

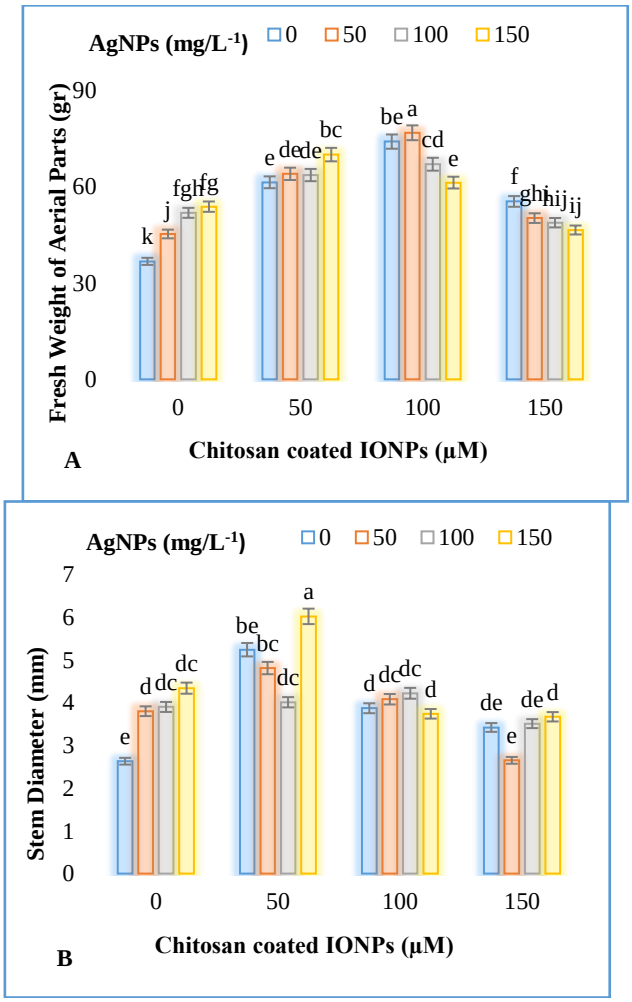

Figure 1. Interaction effects of chitosan Coated IONPs and AgNPs on Fresh Weight of Aerial Parts (A) and Stem Diameter (B) of Snapdragon.

BUD DIAMETER

Comparative analysis of morphological traits indicated that chitosan-coated iron oxide nanoparticles and silver nanoparticles had a significant effect on bud diameter. The highest bud diameter was observed in plants treated with 150 mg/L silver nanoparticles and 100 µM chitosan-coated iron oxide nanoparticles, while the lowest was found in untreated control plants (Figure 2A).

NUMBER OF LATERAL BRANCHES

The analysis of the effects of chitosan-coated iron oxide nanoparticles and silver nanoparticles on the number of lateral branches showed that the highest number was achieved in the treatment with 100 µM chitosan-coated iron oxide nanoparticles and 50 mg/L silver nanoparticles (Figure 2B).

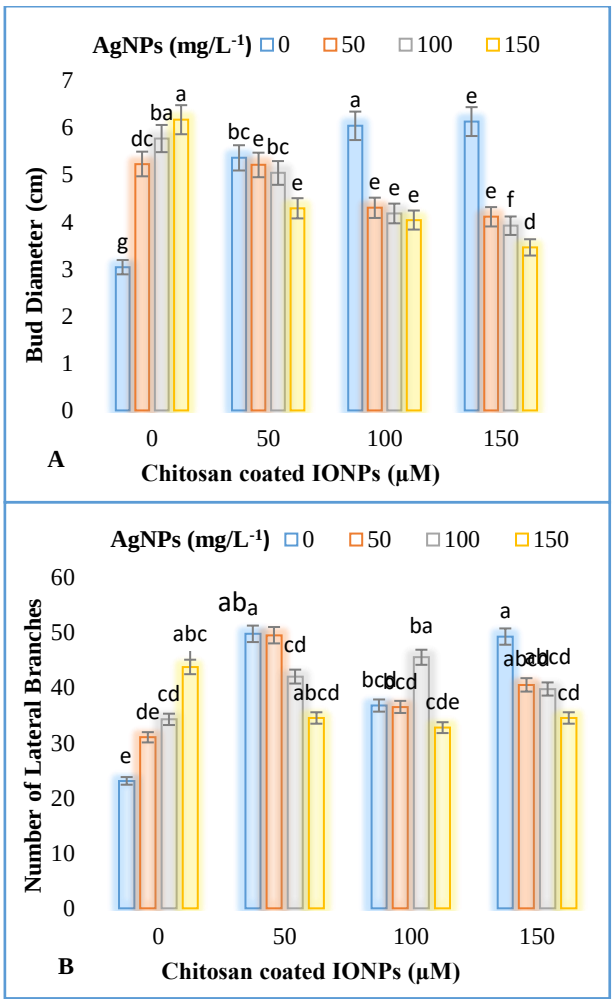

Figure 2. Interaction effects of chitosan Coated IONPs and AgNPs on Bud Diameter (A) Number of Lateral Branches (B) of Snapdragon.

TOTAL SUGARS

The analysis indicated that total sugar content in snapdragon varied with increasing concentrations of silver nanoparticles and chitosan-coated iron oxide nanoparticles. The lowest value was observed in the control treatment (0 mg/L silver nanoparticles and 0 µM chitosan-coated iron oxide nanoparticles), while the highest was recorded with 150 mg/L silver nanoparticles and 150 µM chitosan-coated iron oxide nanoparticles. Intermediate concentrations, such as 50 mg/L silver nanoparticles with 100 µM chitosan-coated iron oxide nanoparticles, also showed an increase in total sugars (Figure 3A).

FLAVONOIDS

The results showed that flavonoid content in snapdragon was influenced by varying nanoparticle concentrations in treatments. The lowest flavonoid content was observed in the control (0 mg/L silver nanoparticles and 0 µM chitosan-coated iron oxide nanoparticles), while the highest was achieved with 150 mg/L silver nanoparticles and 150 µM chitosan-coated iron oxide nanoparticles. Intermediate treatments, such as 50 mg/L silver nanoparticles with 100 µM chitosan-coated iron oxide nanoparticles, exhibited a significant increase in flavonoids, whereas 150 µM chitosan-coated iron oxide nanoparticles without silver nanoparticles resulted in a decrease, highlighting the importance of balanced concentrations (Figure 3B).

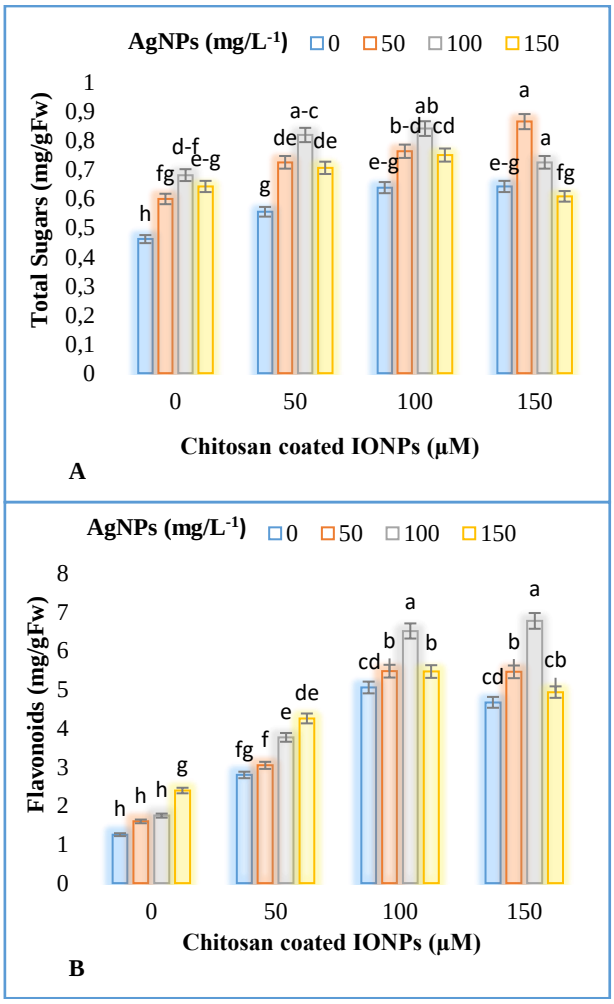

Figure 3. Interaction effects of chitosan Coated IONPs and AgNPs on Total Sugars (A) Flavonoids (B) of Snapdragon.

DPPH

The comparative analysis of mean data revealed that increasing concentrations of chitosan-coated iron oxide nanoparticles generally enhanced antioxidant capacity. The greatest treatment effect was observed from 0 to 150 µM chitosan-coated iron oxide nanoparticles combined with 50 mg/L silver nanoparticles. Additionally, the interaction of both treatments at 100 µM chitosan-coated iron oxide nanoparticles and 100 mg/L silver nanoparticles yielded the highest antioxidant capacity (Figure 4).

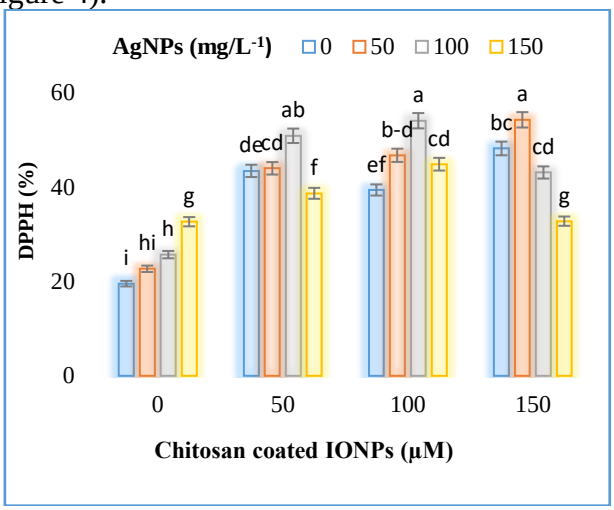

Figure 4. Interaction effects of chitosan Coated IONPs and AgNPs on DPPH.

DISCUSSION

The results of this study demonstrated that the application of chitosan-coated iron oxide nanoparticles and silver nanoparticles can significantly affect the morphological and biochemical traits of snapdragon. These findings align with previous reports on the efficacy of nanoparticles in improving growth characteristics and quality of ornamental plants (Anushi *et al.*, 2023; Behzad *et al.*, 2024). Silver nanoparticles, particularly at low concentrations such as 50 ppm, promoted growth, increased the number of lateral branches, and enhanced the fresh weight of aerial parts, likely due to their role in stimulating antioxidant enzyme activity and reducing oxidative stress (Liu, *et al.*, 2020). These nanoparticles may also influence plant signaling pathways by regulating the expression of growth-related genes (Rasheed *et al.*, 2022). Conversely, chitosan-coated iron oxide nanoparticles, with their biocompatible properties and gradual iron release, particularly at 100 µM, contributed to increased chlorophyll, stem diameter, and fresh weight. This could be attributed to improved iron availability, a key element in chlorophyll synthesis and plant metabolism (Giglou *et al.*, 2022). The chitosan coating enhances absorption and reduces potential nanoparticle toxicity, playing a significant role in their effectiveness (Shi *et al.*, 2020). The significant increase in total sugars and flavonoids in combined treatments with high nanoparticle concentrations indicates their interactive effect on secondary metabolite pathways. Flavonoids, important defensive compounds in plant systems, are influenced by environmental factors and stimulants like nanoparticles, potentially through the activation of phenylpropanoid pathways or increased activity of PAL and CHS enzymes (Selvakesavan *et al.*, 2023). These results are consistent with findings from another study on snapdragon by Pourbeyrami Hir and Sardari (2025), which reported significant improvements in vegetative growth, flower diameter, number of lateral branches, and total phenol content with combined use of chitosan-coated iron oxide and silver nanoparticles at concentrations of 100-150 µM and 50-150 mg/L, respectively. Overall, these findings not only confirm the potential

of nanoparticles in enhancing ornamental plant quality but also open new avenues for sustainable production, reduced chemical fertilizer use, and increased medicinal value of plants. Given the growing adoption of nanotechnology in precision agriculture, future research should focus on targeted, safe, and cost-effective nanoparticle applications.

CONCLUSION

This study demonstrated that the combined application of chitosan-coated iron oxide and silver nanoparticles, particularly at moderate to high concentrations, can effectively improve growth and physiological traits in Snapdragon. These nanoparticles enhanced plant height, branching, and flowering while also increasing chlorophyll fluorescence and phenolic compounds.

These findings support the strategic use of biocompatible nanoparticles in ornamental plant management as a promising approach for boosting performance and resilience, particularly within sustainable and eco-friendly agricultural systems.

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EVALUATION OF THE ADHESION STRENGTH OF BIOCERAMIC COATINGS ON LIGHTWEIGHT METALS

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ABSTRACT

Lightweight metals, particularly magnesium and aluminum, are of great interest in biomedical, automotive, and aerospace industries due to their low density and high specific strength. However, their limited corrosion resistance and insufficient surface mechanical properties under demanding conditions restrict their applications. Therefore, improving these properties through surface coating techniques is of critical importance. In this study, the adhesion strength of bioceramic coatings on magnesium and aluminum alloys was investigated. The bonding characteristics at the coating–substrate interface were evaluated using pull-off, scratch, and delamination tests, and compared in terms of different surface pre-treatments, coating materials, and processing parameters. The findings indicate that bioceramic coatings not only enhance surface performance but also provide strong adhesion, thereby improving long-term reliability.

Keywords: Magnesium, Aluminum, Bioceramic Coating, Adhesion Strength, Corrosion Resistance

INTRODUCTION

Lightweight metals, particularly magnesium (Mg) and aluminum (Al), are widely used in the biomedical, automotive, and aerospace industries due to their low density and high specific strength (Sivamaran, 2022). These metals enhance energy efficiency and offer design flexibility owing to their weight advantage. However, their limited corrosion resistance and insufficient surface mechanical properties adversely affect their long-term performance and restrict their application areas.

To address these limitations, various surface modification and coating technologies have been developed in recent years. Among them, bioceramic coatings stand out due to their high biocompatibility, wear resistance, and strong bonding potential with the substrate; hydroxyapatite (HAp), tricalcium phosphate (TCP), and zirconia are particularly preferred in implant applications (Sonowal, Gautam, Mambiri, & Depan, 2025).

The success of coatings largely depends on the adhesion strength at the coating–substrate interface; insufficient adhesion may lead to peeling, delamination, and performance degradation (PCI Magazine, 2015; Hong et al., 2024; Cloutier et al., 2016). Since the effectiveness of adhesion mechanisms relies on the combined action of surface energy, mechanical interlocking, and chemical bonding, both the surface preparation method (e.g., sandblasting, anodization, laser texturing) and the applied coating technique (sol-gel, electrophoretic deposition, thermal spraying, etc.) directly determine the adhesion strength achieved at the interface (Bharti, 2018).

In the literature, numerous studies have focused on enhancing the adhesion performance of bioceramic coatings on Mg, Al, and Ti alloys. In particular, the use of intermediate layers (e.g., anodization or plasma electrolytic oxidation) has been shown to improve the adhesion of HAp coatings, while composite coatings containing HAp–chitosan or bioactive glass reduce porosity, prevent crack formation, and provide higher adhesion strength values.

In this context, a systematic investigation of the adhesion strength of bioceramic coatings is of critical importance for improving reliability in both engineering and biomedical applications.

AIM AND SCOPE

The aim of this study is to systematically investigate the factors affecting adhesion strength in bioceramic coatings applied to lightweight metals, particularly magnesium (Mg), aluminum (Al), and titanium (Ti).

The following parameters were evaluated:

- Surface pretreatments: Anodization/MAO, laser texturing, sandblasting, silanization
- Coating materials: Hydroxyapatite (HAp), Ca–P phases (OCP, β-TCP), bioactive glasses, composite coatings
- Coating techniques: Sol-gel, electrophoretic deposition (EPD), hydrothermal method, chemical conversion, pulsed electrodeposition, thermal spraying
- Processing parameters: pH, coating thickness, porosity, heat treatment/densification, use of interlayers (TiO_2, ZrO_2, MgF_2)

Adhesion strength data were primarily assessed based on pull-off tests, and the findings were correlated with ISO 13779-4 and ASTM C633/D4541 standards. Results were expressed in MPa whenever possible, considering substrate type, surface preparation, coating method, coating thickness, adhesive used, and failure mode (adhesive/cohesive/mixed).

Within this scope, the study sought to answer the following questions:

- Which pretreatment + coating combinations reliably achieve the ≥15 MPa adhesion threshold required for clinical use?
- To what extent do composite or densified coatings (e.g., HAp–chitosan, HAp reinforced with bioactive glass) improve adhesion strength?
- What ranges of processing parameters such as pH, heat treatment, thickness, and porosity yield optimum results?
- How do different testing standards and specimen preparation conditions affect the comparability of data?
- While targeting high adhesion values, how can potential trade-offs with corrosion resistance and biocompatibility be balanced?

The outcomes of this study are summarized as:

(i) a standards-based data table,

(ii) recommended adhesion ranges and processing windows for specific substrate–coating–method combinations, and

(iii) design recommendations for orthopedic/dental as well as automotive/aerospace applications.

MATERIALS AND METHODS

In this study, literature published between 2007 and 2025 was reviewed, and adhesion strength data of bioceramic coatings applied on magnesium (Mg), aluminum (Al), and titanium (Ti) alloys were compiled. The coatings considered include hydroxyapatite (HAp), calcium phosphate phases (OCP, β-TCP), bioactive glasses, and composite systems. The aim is to

comparatively evaluate the adhesion strength values reported in different studies and to reveal common trends.

The article selection criteria were as follows:

- Adhesion strength must be directly reported in MPa or based on ISO/ASTM standardized pull-off testing.
- Substrate alloy, coating method, and processing parameters must be clearly specified.
- Data must be verifiable through full texts or reliable secondary sources (reviews/standards).

For comparability, only pull-off test results are presented in the table, while scratch, indentation, or delamination tests are included only as qualitative supporting information.

According to ISO 13779-2/-4, the clinically accepted adhesion criterion for HAp coatings is an average of ≥15 MPa, with no specimen falling below 10 MPa. Values below this threshold are considered insufficient for biomedical use.

The findings obtained from the selected literature are systematically presented in Table 1, which includes substrate type, coating method, processing parameters, and the resulting adhesion strengths.

STANDARDS AND TEST METHODS

The adhesion strength of bioceramic coatings is evaluated using various standardized test methods. In this study, particular attention was given to ISO 13779-4 and ASTM C633/D4541 standards.

ISO 13779-4 (Adhesion test for hydroxyapatite coatings): This standard was developed to determine the adhesion strength of hydroxyapatite (HAp) coatings used in medical implants. It specifies the specimen geometry, counter-part conditions, and adhesive properties in detail. For reliable measurement, the adhesive must exhibit a strength at least 5 MPa higher than the expected adhesion of the coating. In addition, for porous coatings, special precautions are described to prevent adhesive penetration into the interface (ISO, 2018).

ASTM C633 (Pull-off test for thermally sprayed coatings): Widely used to determine the tensile bond strength of ceramic and metallic coatings. However, the measured value reflects not only the coating–substrate interface but also the adhesive, surface preparation, and fixture conditions. Therefore, the influence of the testing setup must always be considered when interpreting results (ASTM International, 2017; Pawlowski, 2008).

ASTM D4541 (Portable adhesion testers): This method enables the measurement of pull-off strength using portable devices. While advantageous in field applications, it involves higher uncertainty and variability compared to laboratory-based ISO or ASTM C633 tests. Inter-laboratory repeatability has been reported to vary between 23–76%, indicating that results should be interpreted cautiously in safety and standard evaluations (Sherwin-Williams, n.d.). Moreover, differences of 25–58% have been observed depending on device type, environmental conditions, and adhesive (DeFelsko Corporation, n.d.). Studies on large-scale pipes also showed that ASTM D4541 exhibits higher standard deviation depending on environmental and technical conditions, potentially leading to uncertainty in applications requiring high precision (Croll, Siripirom, & Keil, 2014).

ISO 4624 (Pull-off test for paints and varnishes): An international standard used to measure the adhesion strength of coatings to substrates. In this test, small metal dollies bonded to the coating are pulled perpendicularly under controlled load until detachment occurs, determining the pull-off strength. The method reveals adhesion or internal cohesion of the coating and is used to evaluate coating performance (ISO, 2023).

The differences among these standards complicate the direct comparison of adhesion values reported in the literature. Therefore, in this study, only results obtained using the same test methods were compared numerically, while other methods were considered as supporting information.

RESULTS

The data obtained from the literature are summarized in Table 1, which systematically presents different substrates, coating types, pretreatments, methods, and test results.

Interlayer usage: In Mg alloys, intermediate layers such as anodization significantly enhance the adhesion strength of HAp coatings. For example, in the AZ31–HAp system produced by the CST method, adhesion strength increased from 6.92 MPa to 14.70 MPa with the addition of an anodization interlayer.

Process conditions: The pH of the coating solution plays a critical role in adhesion. In the AZ31–HAp system, an adhesion strength of 6.7 ± 0.5 MPa was reported at pH = 7.5, while in the WE43–HAp system the optimum value of 8.6 MPa was obtained.

Composite coatings: For Mg alloys, HAp–chitosan coatings produced by aerosol deposition reached 24.6–27.7 MPa, HAp/GO coatings prepared by the MAO/PEO method achieved 40.7 MPa, and HAp–MWCNT reinforced Ti coatings reached 32.9 MPa. In addition, 45S5 bioglass coatings subjected to microwave hybrid heating achieved 25–26 MPa. These findings demonstrate that composite systems provide higher adhesion compared to single-phase coatings.

Conversion coatings: In Mg alloys, Ca–P conversion films exceeded 20 MPa, while MgF_2 conversion coatings achieved the highest value of 43.2 ± 5.1 MPa. These results indicate that chemical compatibility and low solubility strengthen adhesion.

Aluminum alloys: Although ceramic oxide layers obtained by PEO/MAO are not strictly bioceramic, they exhibited very high adhesion strengths in the range of 33–70 MPa. When combined with laser micro-texturing, MAO treatments reached values up to 52 MPa.

Titanium alloys: Sol–gel TiO_2–SiO_2 coatings showed adhesion strengths of 36.6 MPa, while dental porcelain coatings reached 30.89 MPa. In contrast, porous wollastonite coatings achieved only 8.06 MPa according to ISO 4624, highlighting the importance of coating continuity and interface morphology.

The survey of the literature clearly demonstrates that the clinically accepted adhesion threshold of ≥15 MPa for bioceramic coatings is reliably attainable across a wide range of systems. Composite architectures and conversion coatings consistently exceed this limit, while MgF_2 interlayers and TiO_2–SiO_2 sol–gel coatings exhibit the most remarkable values. The superior adhesion strengths reported for Al alloys underscore their suitability for demanding industrial applications, whereas Ti alloys, with their inherent biocompatibility and sufficient adhesion, remain highly attractive for orthopedic and dental implant applications.

Table 1. Adhesion strength (MPa) of bioceramic coatings on Mg, Al, and Ti alloys

Metal	**Alloy**	**Coating (Bioceramic)**	**Method / Pretreatment**	**Adhesion Strength (MPa)**	**Test / Standard**	**Reference**
Mg	AZ31	HAp	CST + Anodization interlayer	14.70	Pull-off	Ngo, Do Chi, Pham, Pham, & Duong, 2024
Mg	AZ31	HAp	CST, no interlayer	6.92	Pull-off	Ngo et al., 2024
Mg	AZ31	HAp	CST, pH = 7.5	6.7 ± 0.5	Pull-off	Ngo, 2020
Mg	AZ31	OCP	CST, pH ≈ 5.5–6.2	≈4.2	Pull-off	Zhou, Chen, Xu, Tao, Song, & Zhang, 2021
Mg	AZ31B	Ca–P/oxide (MAO)	Micro-arc oxidation	43.5 ± 0.7	Pull-off	Wen, Zhan, Huang, Xu, Luo, & Xia, 2017
Mg	AZ31B	HA/GO composite (MAO)	One-step (HA/GO added into phosphate electrolyte)	40.7 ± 0.5	Pull-off	Wen et al., 2017
Mg	AZ31	HAp/chitosan	Aerosol deposition	24.6–27.7	Pull-off	Hahn et al., 2011
Mg	Mg alloy	HAp	Ultrasonic aqueous synthesis	18.1 ± 2.2	Pull-off	Sun, Chen, Pan, Wang, & Song, 2019
Mg	Mg alloy	Bioglass 45S5	Press + microwave hybrid heating	25.8 ± 2.6	Pull-off	Shen, Cai, Xu, & Zhang, 2015
Mg	AZ31	Ca–P conversion film	Chemical conversion coating	>20.2	Pull-off	Tan, Wang, Geng, Xi, Qiu, & Yang, 2010
Mg	Pure Mg / AZ31	MgF_2–$MgHPO_4$ (duplex)	Chemical conversion	13.46	Pull-off	Sathyaraj, Ravichandran, & Sankara Narayanan, 2022
Mg	AZ31B	MgF_2 conversion coating	Chemical conversion	≥43.2	—	Yan, Tan, Zhang, & Yang, 2014
Mg	Mg	β-TCP	Chemical conversion / Thermal spraying	16.1 ± 3.4	Pull-off	Geng, Tan, Jin, Yang, & Yang, 2009
Al	AA series	PEO/MAO (Al_2O_3)	Plasma electrolytic oxidation	70	Pull-off	Zhu, Zhang, Liu, Liu, Wang, & Qiao, 2022
Mg	AZ31	HAp	Pulsed ED vs. conventional ED	16.5 / 11.5	Pull-off	Mohajernia, Mazinani, Javidi, & Javid, 2015

Mg	AZ31	HAp	Sol-gel (dip-coating, sintering at 300–500 °C)	22 (300 °C), 28 (400 °C), 35 (500 °C)	Pull-off (ASTM C633 modified)	Niu, Shi, Wei, Li, & Chen, 2016
Al	5052	Ceramic oxide (Al_2O_3, MAO/PEO)	Laser micro-texturing + MAO	33–52	Pull-off (tensile)	Fan, Wang, Yin, Huang, Da, Jiang, Cao, Gai, & Zhang, 2023
Al	—	Al_2O_3 (PEO single layer)	PEO, "chain-like" discharge regime	~70	Pull-off (on epoxy)	Zhu et al., 2022
Ti	Pure Ti	TiO_2–SiO_2 (sol-gel)	Sol-gel method, dopant optimization	36.6	Pull-off (not specified)	Mumjitha & Raj, 2015
Ti	Commercially pure Ti	Wollastonite–silicate	Laser cladding + surface porosification	8.06	ISO 4624 (pull-off)	Zhevtun et al., 2020
Ti	Ti (unspecified)	HAp + 1% MWCNT	Sol-gel multilayer coating	32.9	Interfacial shear test	Jaafar, Hecker, Árki, & Joseph, 2020
Ti	Ti-6Al-4V	Dental porcelain	Sandblasting + firing	30.89	—	Dikova, Anchev, Dunchev, Gagov, & Dzhendov, 2022

The data in the table reveal that the adhesion strength of bioceramic coatings varies significantly depending on surface pretreatments and coating types. The addition of an anodization interlayer nearly doubled the adhesion strength, while composite coatings (HAp–chitosan, bioactive glass) achieved values of **≥25 MPa**. MgF_2 conversion coatings provided the highest adhesion, reaching approximately **43 MPa**. For Al alloys, PEO/MAO-based coatings exhibited strong adhesion in the range of **33–70 MPa**, whereas Ti alloys demonstrated high adhesion levels of **30–36 MPa** with sol–gel and dental porcelain coatings.

DISCUSSION

The literature findings indicate that the adhesion strength of bioceramic coatings is largely dependent on interface engineering. Pretreatments such as anodization, plasma electrolytic oxidation (PEO), and laser surface texturing significantly enhance coating–substrate interactions by improving surface wettability and facilitating mechanical interlocking.

Composite coatings (e.g., HAp–chitosan, bioactive glass-reinforced systems) reduce porosity, lower the risk of crack initiation, and provide more balanced load transfer. Such designs have enabled adhesion strengths of ≥25 MPa.

Conversion coatings (Ca–P, MgF_2) achieve high adhesion due to their chemical compatibility and low solubility. However, excessive coating thickness or uncontrolled internal stresses may lead to peeling and delamination.

When comparing results, the influence of experimental variables such as test method, adhesive selection, and specimen preparation must be considered. Therefore, direct comparison of adhesion values reported in the literature has limited reliability.

Overall, the clinically accepted adhesion threshold of 15 MPa can be achieved in bioceramic coatings. Nevertheless, this value alone is not sufficient; it must be evaluated in conjunction with other critical properties such as corrosion resistance, biocompatibility, and fatigue performance.

CONCLUSION

This study systematically examined the factors influencing the adhesion strength of bioceramic coatings on magnesium (Mg), aluminum (Al), and titanium (Ti) alloys. The findings demonstrate that surface pretreatments, coating techniques, and processing parameters play decisive roles in adhesion performance. With proper interface design and optimized parameters, adhesion strengths of ≥15 MPa can be achieved, and in some systems, values far exceeding this threshold have been reported.

Key findings:

- Adhesion strength increases significantly when surface cleanliness, roughness, and chemical compatibility are improved simultaneously.
- Mechanical roughening alone is often insufficient; the use of interlayers (e.g., anodization, MgF_2) markedly enhances adhesion.
- Excessively thick or insufficiently densified coatings raise residual stresses, increasing the risk of delamination.
- Composite and dense coatings (e.g., HAp–chitosan, bioactive glass-reinforced systems) reduce porosity, prevent crack initiation, and provide more stable adhesion.
- Adhesion values reported in the literature are influenced by different test standards and experimental variables; therefore, measurement conditions must always be considered when interpreting results.

Practical recommendations:

- In clinical and engineering applications, adhesion strength should be evaluated together with corrosion resistance, biocompatibility, and fatigue performance.
- Failure mode (adhesive, cohesive, or mixed) should always be reported.
- Whenever possible, cross-validation with different test methods (e.g., pull-off, scratch) should be performed.

Future directions:

- Development of strategies to mitigate thermal expansion mismatch between coating and substrate.
- Advancement of low-temperature coating techniques.

In conclusion, achieving the target adhesion strength in bioceramic coatings is feasible; however, ensuring the reliability of these values requires reproducible testing under standardized conditions.

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DEVELOPMENTAL NEUROTOXICITY OF CHLORPYRIFOS IN VIVO: STUDY OF CHOLINESTERASE ACTIVITY AS A BIOLOGICAL INDICATOR

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ABSTRACT

Acute poisoning by organophosphates chlorpyrifos is responsible for heavy mortality worldwide, especially in developing countries with high agricultural potential. These products are responsible for poisoning of varying severity depending on the nature of the compound and feed intake. The present work focuses on the study of the effect of toxicity on cholinesterase activity and some parameters of liver function in male rats “Wistar” gavaged with insecticide «chlorpyrifos ". Adult rats received three months chlorpyrifos by gavage at dose DL50/10. At the end of the experiment blood is recovered to determine the activity of butyrylcholinesterase by the colorimetric method of Ellman, and liver function parameters. The brain is taken for the activity of the brain butyrylcholinesterase and proteins contents. Our results show that chlorpyrifos induces increased levels of transaminase activities and alkaline phosphatase reflecting a malfunction of the liver function. Furthermore, inhibition of blood and brain cholinesterases, with an increase of protein levels in brain reflecting neurotoxicity in rats following exposure to chlorpyrifos. These data suggest that organophosphate insecticide administered at DL50/10 doses induce clinical signs of cholinergic poisoning and concurrently reduce brain and plasma cholinesterase activities in the rat.

Keywords: insecticide, rat, cholinesterase activity, biochemical parameters,

INTRODUCTION

Pesticides, sometimes haphazardly applied in large amounts causing environmental pollution, are a cause for concern. Chlorpyrifos (O, O'-diethyl O-3, 5, 6-trichloro-2-pyridyl phosphorothionate) is an organophosphate insecticide with broad spectrum of action with many uses in urban and agriculture. Despite the restriction on its use, it continues to be one of the most commonly used insecticides (Costa et al., 2006; Mahaboob Khan et al., 2007; Rauh et al., 2011). The latter is responsible for acute cholinergic effects exerted by its active metabolite: chlorpyrifos-oxon inhibits acetylcholinesterase, thereby preventing the hydrolysis of acetylcholine, leading to a massive stimulation of cholinergic receptors (Jintana et al., 2009 Wang et al., 2014). These cholinergic systems are known to be involved in cognitive and behavioural functions that are widely disturbed in Alzheimer’s disease (AD). In particular, AD is characterized by a loss of forebrain cholinergic neurons that normally express high levels of acetylcholine (ACh) and acetylcholinesterase (AChE), resulting in the simultaneous depletion of both the neurotransmitter and its hydrolyzing enzyme (Morley et al., 2002; Darvesh et al., 2003; Giacobini, et al.,2004 ; Costa et al 2008 ; Quandt et al.,2010; Siva et al 2013).

Mammals have two main classes of cholinesterases: acetylcholinesterase (AChE, E.C.

3.1.1.7) and butyrylcholinesterase (BuChE, E.C. 3.1.1.8). These distinct enzymes possess differences in their tissue distribution, kinetic properties, specificity for synthetic and natural substrates and selective inhibitors although they are evolutionarily related. In humans, AChE is more abundant in the central nervous system, end plate of skeletal muscle and erythrocytes membranes while BuChE is more abundant in serum (Amitai et al., 1998). BuChE, also called nonspecific cholinesterase or pseudocholinesterase, is able to act on hydrophilic and hydrophobic choline esters. At this moment, the exact physiological function of BuChE is not yet clear (Thiermann et al., 2007) .Other systems that could be affected by pesticides intoxication are immune system pancreas, liver and biochemical changes (Neishabouri, et al., 2004; Kalender et al., 2005). The main objective of our study is based on the disruption of the functioning of the nervous system by inhibiting an enzyme essential for its proper functioning: cholinesterase and dysfunction of liver function following exposure to insecticides gavage male rats "Wistar". The parameters to be measured are: transaminases, alkaline phosphatase, and cholinesterase activity. This work is still very partial in the field of pesticide toxicology.

MATERIAL AND METHOD

Chemicals

Chlorpyrifos (O, O 9-diethyl–3, 5, 6-trichloro-2-pyridyl phosphorothioate) 98% pure; CAS Registry Number: 5598-13-0; was purchased from New England Nuclear, Boston Leading Manufacturer and supplier by (INRAA, ALGERIA). The structure of chlorpyrifos is shown in Figure 1.

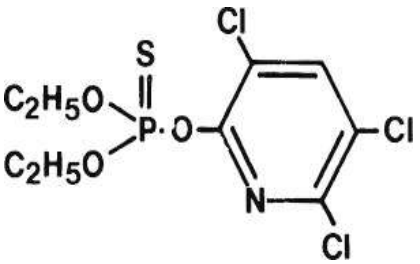

Figure 1. Structure of chlorpyrifos (O,0-Diethyl-0-(3,5,6-trichloro-2- pyridinyl)-phosphorothioate).

Animal and experimental design

All aspects of the experiment were conducted according to the guidelines provided by the ethical committee of the experimental animal care at Tlemcen University. Twenty male rats (Wistar Albino) were obtained from Animal Resource Centre (Algeria).10 weeks old, with an average weight of 250 ± 10 g. The rats were exposed to a daily 12 h photoperiod and an ambient temperature of 25 ◦C. They were fed a standard diet, the water being renewed daily. After the habituation period, the animals were divided into 2 groups of 10 rats each. Treatment of rats was performed by oral gavage (per os) administration in 1 mL at a dose of 13, 5 mg / kg body weight, corresponding to (1/10 DL50) of chlorpyrifos which was diluted in a solution corn oil according to the protocol (Goel et al., 2007). Control rats were treated orally with 1 ml of corn oil only. The feedings are made every other day for three months of experimentation. Body weight and food intake were recorded daily. At the end of the experiment (after three months), each batch of rats were sacrificed by decapitation under CO2 anesthesia. Blood is collected by puncture of the abdominal aorta. A quantity of blood is collected in heparinized tubes and centrifuged at 3000 rev / min for 15 minutes. Total blood and Plasma are immediately used for

the determination of the activity of butyrylcholinesterase. The plasma is also used for the determination of transaminases (AST, ALT) and alkaline phosphatase (ALP) which are markers of liver function. Brain was excised, cerebellum was separated and brain hemispheres were washed with an HEPES-saline buffer (HSB) and placed in a tray of ice (4 °C) used for the determination of protein and the activity of brain butyrylcholinesterase.

Brain extraction and assay of butyrylcholinesterase activity

Preparation of brain homogenates:

The day of the assay, the brain structures collected and stored in liquid nitrogen as homogenized at 4 ° C in 20 volumes of Tris / HCl (50mmol/L, pH 7.4) -sucrose (0,32mol /L) in a glass homogenizer (Kontes Glass , Duall). The homogenate is centrifuged at 1000g for 15 min at 4 ° C. After centrifugation, the surnageants are recovered for measurement of the activity of butyrylcholinesterase and the protein content.

Assay of butyrylcholinesterase activity

Butyrylcholinesterase was assayed in plasma and surnageant according to the method of (Ellman et al.,1961) using butyrylthiocholine as substrate (the normal range of BuChE is 1200-1600 U/L, from 23 normal sera analyzed). The reaction mixture consisted of 100mM potassium phosphate buffer pH 7.0, 3mM butyrylthiocholine iodide, 2mM 5, 5" dithio-bis (2-nitrobenzoic acid) reagent and 10µl serum in a volume of 500 µl. After incubation at 37oC for 10 minutes there action was stopped with 500µl 2 mM eserine sulphate and read at 412 nm. Units were calculated from the micromolar extinction co-efficient of 13.6 x 10-3 for 5-mercapto-2-nitrobenzoic acid.

Chemical analysis

Enzymatic assays (Kit Spinreact) were utilized for the purpose of measuring the plasma levels of glucose, albumin, total cholesterol, and triglycerides.

As for the renal function, it was assessed through the determination of plasma levels of creatinine, uric acid , and urea in blood (Kit Spinreact).

Regarding the liver function, it was evaluated via the enzymatic activity of transaminases, i.e. Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), bilirubin and Alkaline Phosphatase (ALP), and by measuring the total plasma protein level in blood (Kit Spinreact).

Statistical analysis

Results are expressed as means ± standard deviation (SD). After analyzing the comparison of the average variance is performed by the test ' t' Student between the two groups of rats (control , experimental) This analysis is performed using the STATISTICA software, version 4.1 (STATSOFT , TULSA , OK) . Differences are considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Chlorpyrifos is a widely used organophosphate insecticide in agricultural and urban pest control products throughout the world. Like most organophosphate pesticides, chlorpyrifos is a neurotoxin. Animals are killed or paralyzed by exposing to chlorpyrifos due to the irreversible

inhibition of cholinesterase (acetylcholinesterase and butyrylcholinesterase), resulting in the disruption of nervous system. Currently, the measurement of the inhibition of the cholinesterase has become one of the biomarkers most frequently used for detecting exposure to organophosphate compounds (Kwong, 2002; Woods et al., 2002; Siraj et al., 2009; Eamkamon et al., 2012; Dzul-Caamal et al., 2014).

Variations in body weight are frequently used as endpoints in acute toxicity studies in rats, as they can reflect the pathophysiological impact of exposure to a toxic substance. Indeed, notable changes in body weight, such as unusual weight loss or gain, are often early signs of deterioration in the state of health of the animals.In our study, variations in body weight were determined in control and experimental rats and recorded daily at the same time in the morning during the experimental period. Our results demonstrate that after four weeks of exposure to 13.5 mg/kg of chlorpyrifos (corresponding to 1/10 DL50), significant variations in physiological parameters were observed compared to the control group. Control rats showed significant weight gain (+30.87 g), indicating normal growth and metabolic adaptation. In contrast, the chlorpyrifos-treated rats showed minimal weight gain (+2.37 g), suggesting growth inhibition. This stagnation may be linked to disturbances in energy metabolism or reduced food intake induced by the pesticide. The treated group had significantly lower daily food intake (14.60 ± 1.12 g/day) than the control group (20.3 ± 1.20 g/day). This reduction may reflect pesticide-induced anorexia or alterations in neuroendocrine signaling pathways that regulate appetite. Water consumption was also markedly decreased in chlorpyrifos-exposed animals (16.73 ± 4.15 mL/day) compared to the control group (38.14 ± 5.4 mL/day). This decline could indicate disturbances in thirst mechanisms, digestive discomfort, or neurotoxic impairment of central fluid balance regulation (table 1).

In general, alterations in organ mass reflect toxicity following exposure to a harmful substance. The heart, liver, kidneys, spleen, brain, and lungs are among the first organs to be affected by the toxicant-induced metabolic response. In our study, the liver showed a marked increase in its absolute and relative weight (12.07 ± 2.42 g vs. 9.10 ± 0.82 g; 5.85% vs. 3.88%), suggesting early liver injury due to pesticide biotransformation. Similarly, the kidneys showed a moderate increase in their absolute weight (1.40 ± 0.10 g vs. 1.34 ± 0.12 g) and a more pronounced increase in their relative weight (0.78% vs. 0.57%), indicating renal stress related to increased excretory activity or nephrotoxic effects. In contrast, the brain showed a significant reduction in absolute (0.97 ± 0.03 g vs. 2.11 ± 0.12 g) and relative (0.47% vs. 0.90%) weight, reflecting neurotoxic lesions that may involve neuronal loss, delayed brain development, or cholinesterase inhibition-induced atrophy (table 2). Our results are consistent with those of (Araoud et al., 2013; Goel et al 2000; Noaishi et al 2013).

Table 1. Variation in Body Weight (g), Daily Food Intake, and Drinking Water Consumption in Control and Chlorpyrifos-Treated Rats After 4 Weeks of Treatment

	Control	Chlorpyrifos 13.5 mg / kg ($LD_{50/10}$)
Body weight (g) - Initial - Final - Weight gain	 203.84±12.58 234.71± 27.21 + 30.87	 203.84±12.58 206.21± 18.14 + 2.37
Food intake (g/day/rat)	20.3±1.20	14.60±1.12
Water intake (ml/day/rat)	38.14±5.4	16.73±4.15

Values are presented as means ± SD. Significant differences between control and experimental rats in each group are indicated by: *P < 0.05

Table 2. Variation in Absolute and Relative Weights (%) of Organs in Male Rats After 4 Weeks of Chlorpyrifos Exposure

Organ (%)	Control	Chlorpyrifos 13.5 mg / kg (1/10 LD50)
Liver		
Absolute Weights	9.10 ± 0.82	12.07 ± 2.42
Relative Weights	3.88± 0.15	5.85± 0.12
Kidneys		
Absolute Weights	1.34± 0.12	1.40± 0.10
Relative Weights	0.57± 0.08	0.78± 0.06
Brain		
Absolute Weights	2.11± 0.12	0.97 ± 0.03
Relative Weights	0.90± 0.02	0.47± 0.02

Values are presented as means ± SD. Significant differences between control and experimental rats in each group are indicated by: *P < 0.05

The biochemical profile of blood is one of the most sensitive parameters for assessing the toxicity of xenobiotics and pesticides in humans and animals; these results can provide important information about the internal environment of the organism. The evaluation of biochemical characteristics of different species has become an important means for understanding normal and pathological processes as well as toxicological impacts (Liu et al., 2013). Insecticides, even in very low concentrations, interfere with basal metabolism (Al-Shinnawy, 2008).

Insecticide exposure induced a decrease in albumin levels in rats. These results are consistent with those of El-Demerdash and Nasr (2014), who revealed a reduction in serum albumin levels in male rats treated with diazinon, another organophosphate insecticide (table 3).

The kidney is a primary target organ for xenobiotic compounds, particularly environmental pollutants, due to its high blood flow and its role in concentrating solutes. This susceptibility renders the kidney vulnerable to a wide range of toxic effects, often involving both tubular and glomerular cells (Mohamed et al., 2003). In our study, rats orally exposed to chlorpyrifos (1/10 LD_{50}) showed increased serum levels of urea, uric acid, and creatinine. Such elevations are considered biomarkers of renal dysfunction and tissue damage induced by this insecticide (table 3).

Regarding the lipid profile, our results showed a significant increase in total cholesterol and triglycerides in rats under the influence of chlorpyrifos. This increase can be attributed to the effect of the permeability of the liver cell membrane by pesticides (Yousef et al., 2006). In addition, hypercholesterolemia may be due to the obstruction of the bile ducts of the liver, which reduces or stops the secretion of cholesterol into the duodenum (Ogutcu et al., 2008) (Table 3).

Our results show that chlorpyrifos induces increased levels of transaminase activities and alkaline phosphatase reflecting a malfunction of the liver function (table 3). The transaminase and alkaline phosphatase enzymes are considered as indicator for tissue damage that is explained by the degree of exposure and the severity of toxic symptoms. The significant increase in transaminase and alkaline phosphatase activities in treated rats may be explained by destructive changes in hepatic cells induced by chlorpyrifos. Despite this specific mode of action, the toxic effects of chlorpyrifos are also manifested by severe liver dysfunction. It has been shown that acute or chronic exposure to this insecticide induced a significant increase in transaminases AST and ALT (Goel et al., 2000; Alavanja et al., 2004).

Table 3 . Plasma Biochemical Parameters in Control and Chlorpyrifos-Treated Rats

Parameter	Control	Chlorpyrifos13.5 mg / kg (1/10 LD50)
AST (UI)	56.62 ± 6.18	96.24 ± 2.02*
ALT (UI)	33.5 ± 1.64	68.89 ± 3.22**
ALP (UI)	93. 60 ± 6.9	114.10 ± 4.2**
Bilirubin (µmol/l)	6.78 ± 0.4	5.01 ± 0.2 *
Albumin (g/dl)	16.24± 0.73	14.24± 0.73*
CT (mmol/ml)	1.22± 0.03	1.98± 0.04*
TG (mmol/ml)	1.12± 0.11	1.76± 0.13*
Creatinine (mol/l)	27.42± 0.61	28.5± 0.51
Urea (mmol/l)	5.32± 0.11	7.05± 0.31*
Uric acid (mmol/l)	78.5± 3.14	80.03± 1.78*

TC: Total cholesterol; TG: Triglyceride; AST: Aspartate-Amino-Transferase; ALT: Alanine-Amino-Transferase; ALP: Alkaline phosphatase. Values are presented as means ± SD. Significant differences between control and experimental rats in each group are indicated by: *P < 0.05.

Our data showed an inhibition of blood and brain cholinesterases, with an increase of protein levels in brain reflecting neurotoxicity in rats following exposure to chlorpyrifos (figure 2). These data suggest that organophosphate insecticide administered at DL50/10 doses induce clinical signs of cholinergic poisoning and concurrently reduce brain and plasma cholinesterase activities in the rat. Chlorpyrifos, like other organophosphates, particularly exerts its action by inhibiting the activity of acetylcholinesterase (Amitai et al., 1998; Shenouda et al., 2009; Dzul-Caamal et al., 2014). Indeed, some pesticides such as lindane, endosulfan, dieldrin and Eldrine may result in inhibition of GABAergic receptors and activation of glutamatergic receptors in neuronal cells of mammals, inducing a hyperexcitability syndrome that can evolve up the occurrence of convulsions this is probably related to neuronal lysis sign of Alzheimer's disease in people exposed to pesticides (Sunol et al 2008; Muller et al., 2014).

These preliminary results confirm the presence of alterations in liver function and inhibition of cholinesterase activity reflecting induced neurotoxicity by the insecticide chlorpyrifos in Wistar rats. The measurement of target enzymes, cholinesterase in the case of organophosphates such as chlorpyrifos would be a valuable tool to determine the actual causes of the enhanced toxicity when organisms are exposed to pesticides in general.

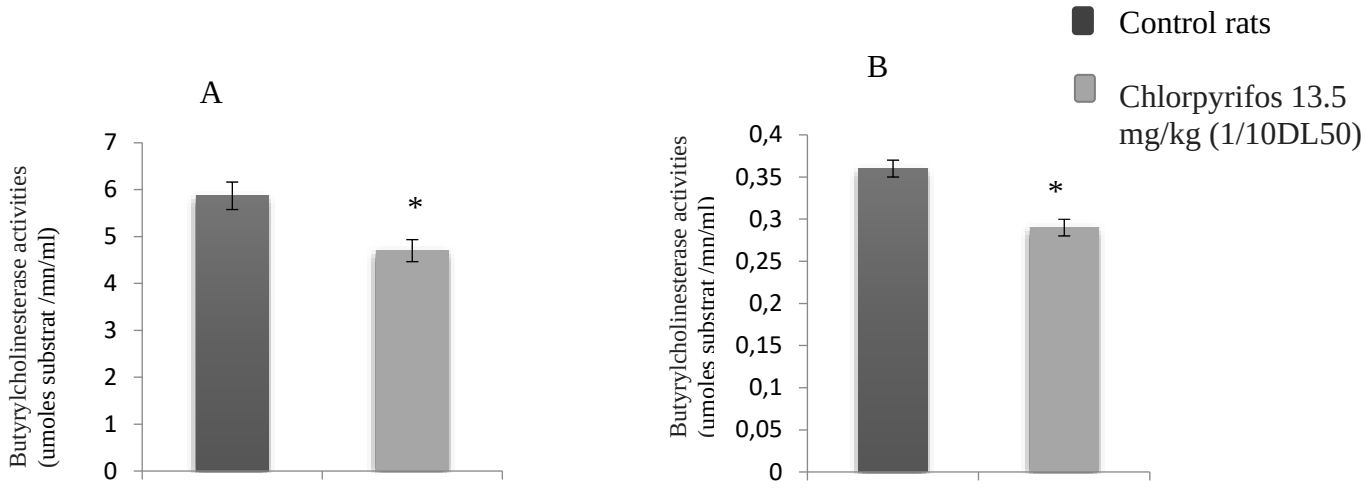

Figure 2. Butyrylcholinesterase activities in blood (A) and plasma (B) in control and experimental rats. Values are presented as means ± SD. Significant differences between control and experimental rats in each group are indicated by: *P < 0.05.

CONCLUSIONS

Chlorpyrifos exerts its action by inhibiting cholinesterase activity and impaired liver function. The commercial availability of this product is worrying, justifying the use of a wide prevention program to inform the public and the authorities of the danger organophosphates.

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BIOACTIVE SYNERGY OF CINNAMON –TURMERIC: IN VITRO EXPLORATION OF ANTIOXIDANT, ANTI-HEMOLYTIC AND ANTI-INFLAMMATORY EFFECTS AND THEIR IMPLICATIONS FOR HUMAN HEALTH

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ABSTRACT

Inflammatory and hemolytic disorders linked to oxidative stress represent an increasing public health challenge, thereby justifying the investigation of natural therapeutic alternatives that are effective, stable, and better tolerated. This study aims to evaluate, *in vitro*, the antioxidant, anti-hemolytic, and anti-inflammatory properties of a combination of *Cinnamomum verum* (cinnamon) and *Curcuma longa* (turmeric), prepared as aqueous (decoction) and methanolic (maceration) extracts, and compared with two reference standards: gallic acid and diclofenac sodium.

Phytochemical screening revealed a marked (+++) presence of tannins, flavonoids, terpenoids, and anthraquinones in the aqueous extract, whereas the methanolic extract exhibited moderate to high (++ to +++) levels of these compounds, except for terpenoids, which were present in lower concentrations. The decoction showed a high total polyphenol content (178.46 ± 21.66 mg GAE/g DW), while the maceration was richer in flavonoids (35.43 ± 1.25 mg QE/g DW).

Anti-hemolytic activity assessed on human erythrocytes demonstrated a stable and dose-dependent inhibition for the decoction (from 74.22% to 85.91% at 500 µg/ml), approaching the efficacy of diclofenac (89.35% at 500 µg/ml). The methanolic extract showed a peak effect at 150 µg/ml (86.17%), followed by a marked decrease at higher concentrations.

Regarding anti-inflammatory activity, measured by the inhibition of protein denaturation, a moderate dose-dependent response was observed: from 31.85% to 57.42% for the decoction, and from 28.73% to 52.38% for the maceration. Diclofenac demonstrated a stronger inhibition of 71.63% at 500 µg/ml. Based on the study results, the cinnamon–turmeric synergy, especially in decoction form, stands out for its dual protective bioactivity, highlighting its potential in the formulation of phytopharmaceuticals targeting oxidative and inflammatory conditions.

Keywords: Cinnamon–turmeric synergy**,** Antioxidant, Anti-Hemolytic , Anti-Inflammatory activity, in vitro study**,**

INTRODUCTION

Chronic inflammatory diseases and hemolytic disorders pose significant challenges to global public health. Although inflammation is essential for defense and tissue repair, it can become harmful when it escapes physiological regulatory control. This dysregulation can lead to persistent tissue damage driven by either the persistence of pathogens or functional abnormalities of immune cells (Chen et al., 2018).

The conventional treatment for inflammation relies primarily on steroidal (glucocorticoids) and non-steroidal (NSAIDs, such as aspirin) anti-inflammatory drugs. Despite their effectiveness, prolonged use is often limited due to significant side effects (Koshi et al., 2022). This has fueled growing interest in natural alternatives, particularly medicinal plants, which are a valuable source of bioactive compounds that could be used for safer and more effective therapies (Mukhtar et al., 2008).

In toxicological in vitro approaches, erythrocytes are widely used as an experimental model to assess the cytotoxicity of plant extracts. Their abundance, combined with the ease of monitoring hemolysis through hemoglobin release into the extracellular medium, makes erythrocytes a reliable and reproducible system (Ghlichloo, 2023).

Since antiquity, medicinal plants have played a central role in traditional healing practices worldwide. Their abundance of bioactive molecules gives them considerable therapeutic potential, a topic that modern research is increasingly exploring in relation to various pathological conditions, particularly inflammatory and metabolic disorders (Khatri et al., 2023; Kaur et al., 2024). Two spices are of particular interest: cinnamon, derived from the inner bark of trees in the genus Cinnamomum (Lauraceae), and turmeric (Curcuma longa), a rhizomatous herbaceous plant in the Zingiberaceae family.

These spices, originally from Sri Lanka and South Asia, respectively, are widely used in diet and phytotherapy. Rich in polyphenols and other secondary metabolites, they contain curcumin and cinnamaldehyde, which are well-documented for their antioxidant, anti-inflammatory, and antimicrobial properties (Kaur et al., 2024; Guo et al., 2024).

The present study aims to investigate the synergistic effect of the cinnamon–turmeric combination using an in vitro approach. Specifically, the study evaluates the combination's antioxidant, anti-hemolytic, and anti-inflammatory activities to highlight its potential relevance to human health. The experimental design uses assays such as protein denaturation inhibition and erythrocyte membrane stabilization to provide a framework for understanding the therapeutic potential of this plant-based synergy.

MATERIAL AND METHOD

This study used a mixture of cinnamon *(Cinnamomum verum*) inner bark and turmeric *(Curcuma longa)* rhizomes as plant materials. Both were obtained from a local herbalist in the Wilaya of Tlemcen in western Algeria. The materials were then dried and ground into a fine powder using an automatic grinder before undergoing extraction procedures (Figure 1).

Turmeric (Curcuma longa)

Cinnamon (Cinnamomum verum)

Figure 1: Turmeric rhizomes (Curcuma longa), turmeric powder and cinnamon bark (Cinnamomum verum) after grinding

Two types of extracts were prepared from the cinnamon-turmeric mixture. The methanolic extract was obtained by macerating 50 g of the powdered mixture in a 80:20 methanol-water solution for 24 hours. This process was repeated three times with fresh solvent. The combined filtrates were concentrated under reduced pressure at 40 °C, and the residue was dried and stored at 4 °C.

The aqueous extract was obtained by boiling 50 g of powder in 500 mL of distilled water for 30 minutes. After filtration, the filtrate was dried at 30 °C for 72 hours and stored at room temperature. The extraction yield was calculated as the ratio of the dry extract mass to the initial plant material mass, expressed as a percentage according to the following equation:

$$Re = (Me/Mv) \times 100$$

where Me is the mass of the dry extract obtained (g) and Mv is the initial mass of the plant material (g).

Phytochemical screening revealed the following results: Alkaloids were tested by treating 2 mL of extract with 2 mL of Wagner's reagent. The appearance of a yellow-white precipitate indicated a positive reaction (Maria et al., 2018). Tannins were identified by adding a few drops of 0.1% ferric chloride ($FeCl_3$) to 2 mL of the extract; a dark green color confirmed their presence (Warnasih et al., 2019). Sterols were detected using the Liebermann-Burchard test. The addition of 5 mL of acetic anhydride and 1 mL of concentrated sulfuric acid to 5 mL of the extract without shaking produced a red-brown ring and violet layer (Khan et al., 2012). Flavonoids were identified by treating 2 mL of the extract with a few drops of 37% HCl and 0.5 g of magnesium filings, which resulted in red or orange coloration (Saxena et al., 2013). Terpenoids were identified by adding 1 mL of chloroform and 1.5 mL of concentrated sulfuric acid to 2.5 mL of the extract. This produced two phases with a brown interface (Yadav & Agarwala, 2012). Cardiac glycosides were tested by dissolving 2 mL of the extract in chloroform and carefully adding 2 mL of concentrated sulfuric acid, yielding a reddish-brown

ring at the interface (Harborne, 1973). Anthocyanins were detected by adding 3 mL of 10% H_2SO_4 and 1 mL of 10% NH_4OH to 1 mL of the extract. This produced an intensified red color in an acidic medium and a blue shift in an alkaline medium (Diallo, 2000). Anthraquinones were tested by mixing 5 mL of the filtrate with an ammoniacal solution. A pink or violet color in the ammoniacal phase indicated a positive result (Odebeyi & Sofowora, 1978). Volatile oils were detected by adding 0.1 mL of 10% sodium hydroxide and a few drops of 10% diluted hydrochloric acid to 2 mL of the extract. This yielded a white precipitate (Mojab et al., 2003).

The quantitative determination of total polyphenols was carried out using the Folin–Ciocalteu reagent, as described by Wong et al. (2006). The reagent consists of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$) and is reduced during the oxidation of phenols. This reaction forms blue oxides of tungsten and molybdenum (Ribéreau, 1968). The resulting coloration, with maximum absorbance at 765 nm, is proportional to the polyphenol content (Ghazi & Sahraoui, 2005). Evaluation followed the protocol of Li et al. (2007). Briefly, 200 µL of each extract were added to 1 mL of Folin–Ciocalteu reagent diluted 10-fold in distilled water. After a 4-minute incubation period, 800 µL of a 75 g/L sodium carbonate solution were added. The mixture was then incubated for 30 minutes in the dark at room temperature, after which the absorbance was measured at 765 nm. Total polyphenol concentration was determined using a gallic acid calibration curve and expressed as milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g DE).

Total flavonoid content was determined using the aluminum chloride ($AlCl_3$) colorimetric method described by Lamaison and Carnet (1990). Briefly, 1 mL of the extract solution was mixed with 1 mL of a 2% $AlCl_3$ solution. The mixture was shaken vigorously and incubated for 10 minutes. Absorbance was then measured at 367 nm. Flavonoid content was calculated using a calibration curve prepared with rutin and expressed as micrograms of rutin equivalents per milligram of dry extract.

Condensed tannins were quantified using the vanillin-HCl method (Julkunen-Tiitto, 1985). This method is based on the formation of red complexes between vanillin and the terminal flavonoid groups of condensed tannins, which may convert further into anthocyanidols. In practice, 50 µL of the extract were mixed with 1.5 mL of a 4% vanillin/methanol solution, followed by the addition of 750 µL of HCl. The mixture was kept at room temperature for 20 minutes, after which the absorbance was measured at 550 nm. A calibration curve was established using catechin (100–700 µg/mL).

The antioxidant capacity was evaluated using the ferric reducing antioxidant power (FRAP) assay, as described by Yildirim et al. (2001). This method is based on the reduction of the ferricyanide complex (Fe^{3+}) to ferrous iron (Fe^{2+}) in the presence of antioxidants, which yields a green color proportional to the reducing power (Gülçin et al., 2003). For the assay, 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide were added to 1 mL of the extract. After incubating for 20 minutes at 50 °C, 2.5 mL of trichloroacetic acid (10%) was added, and the mixture was centrifuged at 3,000 rpm for 10 minutes. Then, 2.5 mL of the supernatant were mixed with 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ (0.1%). Absorbance was measured at 700 nm. The results were expressed as µg of ascorbic acid equivalents per mg of dry extract (µg AAE/mg DE).

The scavenging activity of the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was assessed using the technique described by Sánchez-Moreno et al. (1998), with modifications. In this assay, 1,950 µL of the DPPH-methanol mixture (0.025 g/L) were mixed with 50 µL of various extracts at different concentrations. After incubating for 30 minutes at ambient temperature, absorbance was measured at a wavelength of 515 nm against a blank. Then, the DPPH free radical scavenging activity, expressed as a percentage, was calculated using the following formula:

$$I\,(\%) = (A_c - A_s / A_c) \times 100$$

where *Ac* and *As* represent the absorbance values of the control and the sample, respectively.

Anti-hemolytic activity was evaluated using the human erythrocyte membrane stabilization method. First, a 10% erythrocyte suspension was incubated with the extracts in the presence of a hyposaline solution. Then, the mixture was centrifuged. Then, the absorbance of the supernatant was measured at 560 nm. The percentage of hemolysis inhibition was calculated by comparing it to the inhibition of hemolysis by gallic acid, which was used as a positive control.

$$\text{Inhibition of hemolysis (\%)}: = [\,(Ac - At) / Ac\,] \times 100$$

where Ac is the absorbance of the control sample and At is the absorbance of the test sample.

Anti-inflammatory activity was assessed by measuring the inhibition of the thermal denaturation of bovine serum albumin (BSA). A 0.2% BSA solution was incubated with various extract concentrations (100–500 µg/mL) before being heated to 72 °C. Absorbance was measured at 660 nm, and the percentage inhibition was calculated using diclofenac sodium as the reference standard according to the following equation:

$$\text{Inhibition (\%)}: 100 - [\{(At - Ac) / At\} \times 100]$$

Where At is the absorbance of the test solution (extract) and Ac is the absorbance of the control.

Statistical Analysis

All results are expressed as mean ± standard error. Statistical analysis was performed using Excel 2021.

RESULTS AND DISCUSSION

Extraction Yield

The extraction of the Cinnamomum verum–Curcuma longa mixture revealed a higher yield with water (16.36%) compared to methanol (12.96%) (Table 1). This difference can be explained by the high polarity of water, which favors the extraction of water-soluble metabolites such as polysaccharides, glycosides, and certain polyphenols. Methanol, although polar, exhibits lower affinity for some hydrophilic fractions, which accounts for its slightly reduced yield. These results corroborate the observations of Hegde et al. (2012), who highlighted the effectiveness of water as an extraction solvent for spices rich in phenolic compounds.

Table1: Extraction Yield (%) of Methanolic and Aqueous Extracts of the Cinnamon-Turmeric Mixture

Extraction Solvent	Methanolic extract	Aqueous extract
Yield (**(%)**	13.25	16.36

Qualitative Phytochemical Screening

Phytochemical screening, performed according to standardized methods (Nguyen et al., 2020), revealed the presence of major secondary metabolites — phenols, flavonoids, anthraquinones, and glycosides — while no sterols or Anthocyanins were detected (Table 2). This molecular diversity suggests that the cinnamon–turmeric combination concentrates bioactive compounds that account for its reported biological activities, particularly its antioxidants and anti-inflammatory effects (Sana et al 2019; Muazu et al. 2021; Fuloria et al., 2022; Pagliari et al., 2023).

Quantitative Phytochemical Analysis

Quantitative assessment (Figure 2) revealed a particularly high content of polyphenols in the aqueous extract, followed by flavonoids, terpenoids, and tannins. These results confirm the hypothesis that water is the most effective solvent for polyphenol extraction (Piba et al., 2021). The richness in polyphenols, well known for their strong antioxidant power, confers notable pharmacological potential to these extracts (Sana et al 2019; **Nakaee et al ., 2023**).

Biological Activities (Antioxidant, Anti-hemolytic, and Anti-inflammatory)

The radical scavenging activity, evaluated by DPPH and FRAP assays (Figure 3), showed that the methanolic extract exhibited the highest DPPH scavenging activity (23.28 ± 7.40) and 14.47 ± 4.43 mg Trolox equivalents/g DW), probably due to its higher alkaloid content (Dieng et al., 2017). However, the FRAP assay confirmed that both extracts possess significant reducing power, attributable to their richness in polyphenols (Sana et al 2019).

Table 2: Results of Phytochemical Tests of Different Extracts of the Cinnamon-Turmeric Mixture

Tests	Maceration	Decoction
Alkaloids Test	++	+
Tannins Test	++	+++
Steroids Test	–	–
Flavonoids Test	+++	+++
Terpenoids Test	+++	+++
Cardiac Glycosides Test	+++	+++
Anthocyanins Test	–	–
Anthraquinones Test	++	+++
Volatile Oils Test	+	+

+: Low presence; **++:** Moderate presence; **+++:** High presence; **–:** Absence

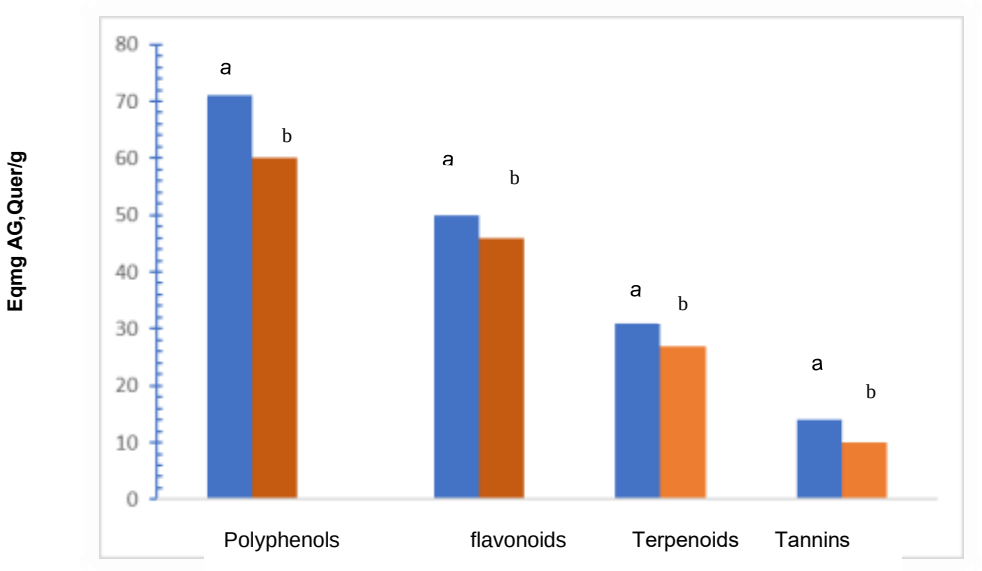


Figure 2: Total Phenolic Content of the Extracts

In parallel (figure 4) , the anti-hemolytic activity assessed on human erythrocytes revealed a stable and dose-dependent inhibition for the decoction (74.22–85.91% at 500 µg/ml), approaching the efficacy of diclofenac (89.35% at 500 µg/ml). The methanolic extract showed a peak activity at 150 µg/ml (86.17%), followed by a marked decrease at higher concentrations.In addition, the aqueous extract inhibited thermal hemolysis more effectively than diclofenac at different concentrations, the latter being a conventional non-steroidal anti-inflammatory drug (NSAID) that is well known and widely used worldwide in the treatment of inflammatory diseases (Procházková et al., 2011).

Anti-inflammatory activity, measured by the inhibition of protein denaturation, demonstrated a moderate, dose-dependent response: 31.85–57.42% for the decoction, 28.73–52.38% for the maceration, compared to 71.63% for diclofenac at 500 µg/ml. These findings suggest a protective effect linked to the synergistic action of flavonoids and terpenoids, capable of modulating pro-inflammatory signaling pathways (NF-κB, COX-2). The thermal process of decoction, by inactivating polyphenol oxidase (PPO), also helps to preserve thermostable compounds (Zawawi et al., 2022; Antony and Farid, 2022).

Overall, the cinnamon–turmeric mixture emerges as a rich source of bioactive secondary metabolites. The differences observed between aqueous and methanolic extracts reflect solvent selectivity and the importance of extraction conditions. The aqueous extract combines ethnopharmacological relevance, efficient extraction, and biological stability, whereas the methanolic extract, richer in alkaloids, exhibits strong antioxidant activity but less stability at higher concentrations. These findings support the rational valorization of the mixture in phytotherapy and highlight the need for further studies, particularly *in vivo*, to confirm its efficacy and clinical safety.

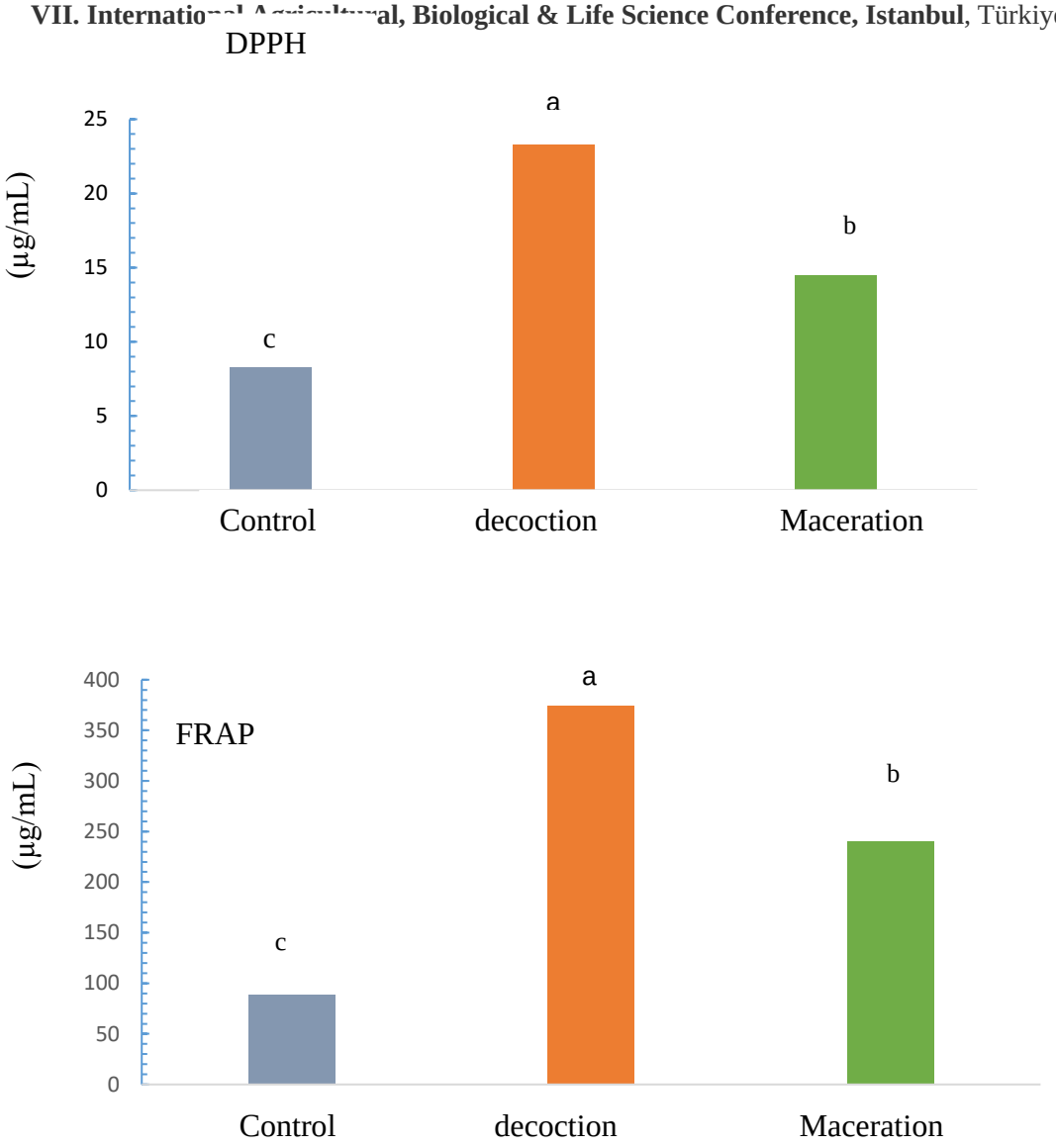
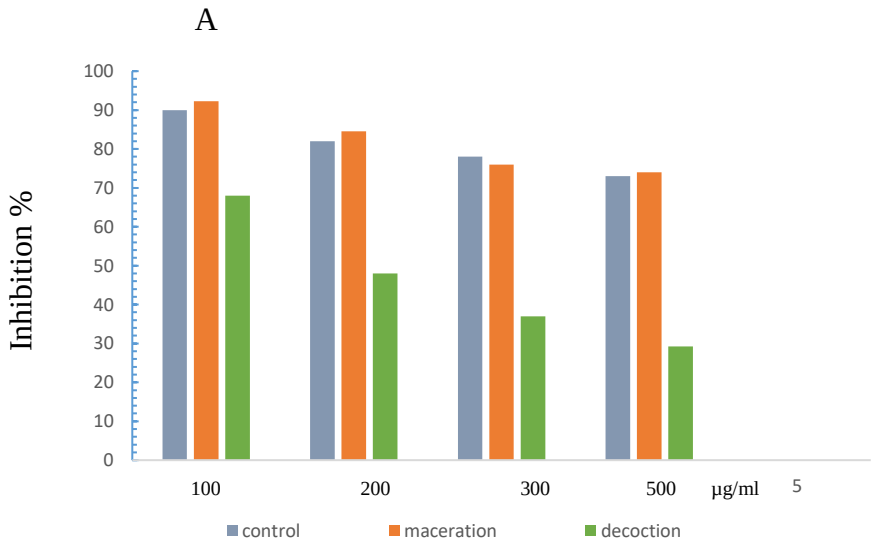


Figure 3: Antiradical Activity of the Extracts (DPPH and FRAP Assays)



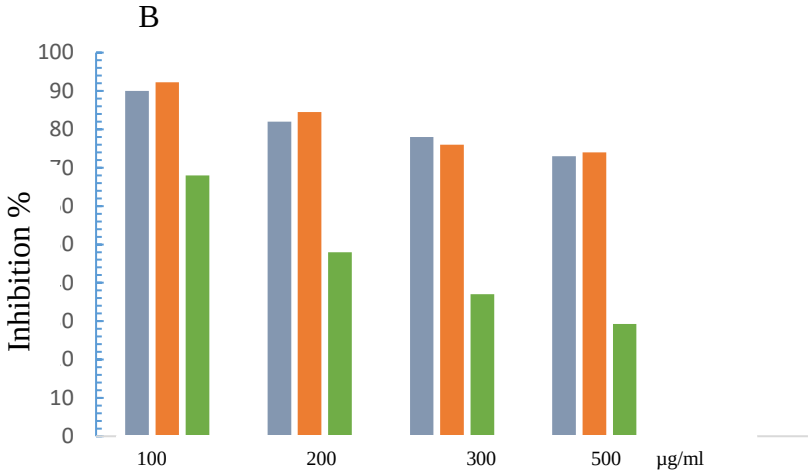


Figure 4: In vitro evaluation of the anti-hemolytic (A) and anti-inflammatory (B) activity

CONCLUSIONS

Based on the study ,the cinnamon–turmeric synergy, especially in decoction form, stands out for its dual protective bioactivity, highlighting its potential in the formulation of phytopharmaceuticals targeting oxidative and inflammatory conditions.

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NEW APPROACHES FOR ARTIFICIAL INTELLIGENCE-ASSISTED RETINOSCOPES

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ABSTRACT

Retinoscopy is a fundamental examination method that has been used for many years in the objective evaluation of refractive errors such as myopia, hyperopia, and astigmatism in the fields of ophthalmology and optometry. In recent years, with the digitalization of this technique, innovative tools such as smartphone-assisted systems and the Gimbalscope have gained increasing attention. The literature emphasizes the advantages of the Gimbalscope in educational settings, including real-time visualization, low cost, and high portability. Furthermore, AI-based systems have been shown to enhance effectiveness in both education and clinical diagnosis by automating the classification of retinoscopic reflexes. In this study, an AI model is planned to be developed to classify retinoscopic reflexes using publicly available datasets. This model aims to bridge theory and practice by proposing a sample system that can be applied particularly in healthcare technologies and optometry education. The proposed approach is expected to enable faster detection of refractive errors and increase access to eye examinations for a broader population.

Keywords: Retinoscopy, Gimbalscope, Refractive Error, Artificial Intelligence, Biomedical Diagnosis

INTRODUCTION

The idea of retinoscopy was first proposed by the ophthalmologist Cuignet in the 1800s (Enaholo et al., 2023; Pai-Dhungat et al., 2014; Norn, 2016; Hallak, 1976). Retinoscopy is a technique currently used to detect refractive errors, including myopia, hyperopia, and astigmatism (Musa et al., 2024). Retinoscopy helps determine the eyeglass prescription that should be used to correct a person's refractive errors. In recent years, retinoscopy has continued to develop, influenced by artificial intelligence (Mendez, A., 2016). Chan and colleagues combined a retinoscope and a smartphone camera to create a digital retinoscope (Chan et al., 2014). When we look at the studies on the subject in recent years, we see that interest in smartphone-supported systems has increased, and exciting innovations such as the Gimbalscope have emerged.

This new technology provides real-time visualization during training and also attracts attention due to its affordability and portability (Chandrakanth et al., 2022). Furthermore, AI-supported systems have demonstrated the ability to improve training and clinical outcomes through automation in the classification of the retinoscopic reflex (Krishnan et al., 2025). However, studies evaluating the performance of AI models based on publicly available datasets

for the classification of retinoscopic reflexes remain limited. This makes it difficult to standardize comparisons and evaluations.

In this study, Simple CNN and ResNet-18 were used as different deep learning models for the RetinaMNIST dataset, and their performances were examined. Thus, we evaluated the impact of model architecture, data preprocessing (grayscale and RGB), training time, and data augmentation on classification accuracy. The findings from this study provide new ideas for integrating AI-assisted retinoscopy into ophthalmology education and clinical practice.

MATERIAL AND METHOD

This study was conducted on the RetinaMNIST dataset, which consists of 2D retinal fundus images divided into five classes: myopia, hyperopia, astigmatism, normal, and other (Yang et al., 2023). The grayscale image database was initially resized into 224×224 pixels from the original size of 28×28 pixels, and grayscale and RGB transformations were the preprocesses performed over the database. To improve generalization, data augmentation techniques were applied in selected experiments. Two architectures were implemented: a Simple CNN and a ResNet-18 model for five-class classification, where layer freezing and dropout were additionally explored. Training was carried out for 10–30 epochs, and early stopping along with learning rate scheduling was employed to prevent overfitting. Model performance was evaluated in terms of training and test accuracy, which provided insights into the effectiveness of the models under different configurations.

RetinaMNIST was selected because it provides a benchmark setting for evaluating automated refractive error detection while reflecting the visual complexity of retinoscopy. Nevertheless, its limited size and clinical diversity impose constraints on generalization performance, motivating the use of preprocessing strategies, transfer learning, and data augmentation in subsequent experiments.

RESULTS AND DISCUSSION

The experiments on the RetinaMNIST dataset revealed that achieving high classification accuracy remains a challenge due to dataset complexity and class imbalance. The Simple CNN model reached a maximum test accuracy of **53.50%**, both with grayscale images trained for 30 epochs and when grayscale images were converted into RGB format. Despite its shallow architecture, the CNN provided competitive results when training duration or preprocessing was optimized. The ResNet-18 model slightly outperformed the CNN, achieving the **highest test accuracy of 53.75%** with RGB images at 10 epochs. However, increasing the number of epochs or applying pretrained weights did not consistently enhance performance, and in some cases even reduced accuracy (e.g., **46.75%** with pretrained weights at 20 epochs). Data augmentation and fine-tuning techniques such as layer freezing and dropout yielded moderate improvements but did not surpass the best-performing configurations. Overall, the accuracy values across both models ranged from **46% to 54%**, consistent with literature reporting relatively low performance on RetinaMNIST (Halder et al., 2024; Neghawi and Liu, 2024). These results highlight the difficulty of generalization in small, imbalanced biomedical datasets, where overfitting is a persistent issue. Future improvements may involve applying transfer learning from large-scale pretrained networks, exploring transformer-based architectures (e.g., ViT, Swin Transformer), and utilizing larger, more diverse retinal image datasets. From a practical standpoint, integrating AI-based classification with portable devices such as the Gimbalscope could provide scalable, cost-effective support in both clinical diagnostics and medical education.

Table 1. Comparison of model performance for the RetinaMNIST dataset

Model	Data Preprocessing	Epochs	Train Accuracy (%)	Test Accuracy (%)
Simple CNN	Grayscale	10	50.37	50.75
Simple CNN	Grayscale	30	51.11	53.50
Simple CNN	RGB	10	54.26	53.50
ResNet-18	RGB	10	68.24	53.75
ResNet-18	RGB	20	59.26	46.75
ResNet-18	Grayscale	20	54.54	48.00
ResNet-18	RGB + Augmentation	10	55.65	52.00
ResNet-18	RGB, Layer Freeze + Dropout	20	56.94	52.25

Figure 1. Example classification results on the RetinaMNIST dataset using ResNet-18.

Figure 1 shows sample predictions of the ResNet-18 model on the RetinaMNIST dataset, showing ground truth (GT) and predicted labels (Pred). Classes: Myopia, Hyperopia, Astigmatism, Normal, Other. In most cases, the ground truth and predicted classes are consistent, indicating that the model is capable of distinguishing major refractive errors. However, some misclassifications are observed between Myopia and Hyperopia, which are clinically similar categories. The results highlight both the potential and the limitations of deep learning based approaches in automated refractive error detection.

CONCLUSIONS

This study presented a comparative analysis of different deep learning models with experimental evaluation using the RetinaMNIST dataset. The CNN and ResNet-18 models achieved maximum test accuracies of 53.50% and 53.75%, respectively, which are consistent with results reported in the literature. While these accuracy levels highlight the challenges posed by the limited size and complexity of RetinaMNIST, the proposed framework underscores the potential of AI-assisted retinoscopy in both clinical and educational applications. Future work will focus on expanding dataset diversity, implementing advanced deep learning architectures such as Vision Transformers, and evaluating integration with real-world retinoscopy systems.

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OPTIMIZING GERMINATION OF NIGELLA SATIVA L. WITH GIBBERELLIC ACID AND SEED PRIMING TECHNIQUES

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ABSTRACT

The use of *Nigella sativa* L., commonly known as black cumin, in traditional and medicinal practices is well-documented, due to the wide range of its biological activities. In light of its significance, this study investigates the efficacy of various seed priming treatments, specifically Gibberellic Acid (GA3) and different priming durations, under laboratory conditions to enhance the germination stages of *Nigella sativa* L. The objective of these priming treatments is to improve germination rates, the germination index, and average germination time, with the potential to increase agricultural productivity.
The findings of this study demonstrate that priming durations and gibberellic acid doses have a significant impact on germination parameters under controlled conditions. These results provide valuable insights into the potential of these treatments to optimise crop growth. It can be concluded that appropriate seed priming can effectively enhance the early growth stages of *Nigella sativa* L., contributing to better crop establishment and yield.

Keywords: Nigella sativa L., Seed priming, Gibberellic Acid (GA_3), Germination rates, Germination index

INTRODUCTION

Medicinal plants, especially those with a broad spectrum of biological activities, have attracted considerable attention in both the scientific and medical fields. *Nigella sativa* L., commonly known as black cumin or black seed and belonging to the Ranunculaceae family, is notable for its widespread use in culinary and medicinal contexts (Mohamed et al., 2014; Kiralan et al., 2016). Its medicinal use has attracted worldwide interest, largely due to its efficacy in the treatment of numerous conditions. Historical accounts, including those attributed to the Prophet Muhammad, have enhanced the reputation of black seed as a remedy for various ailments, except death itself (Islam et al., 2019).
Traditionally, *Nigella sativa* seeds have been used to treat conditions such as fatigue, persistent headaches and melancholy. Roasted seeds mixed with honey or butter have been suggested to relieve colic and coughs, while the seeds are known for their lactogenic and antiseptic properties, particularly in the treatment of eye infections. The emergence of COVID-19 has

further sparked interest in potential antiviral treatments, with studies investigating Nigella sativa extracts for their ability to inhibit coronavirus replication and affect TRP gene expression during infection (Ulasli et al., 2014). The results suggest that *Nigella sativa* has a significant effect on viral load and gene expression associated with CoV infection, enhancing its therapeutic potential (Yimam et al., 2015).

The genus Nigella includes around 20 species, 14 of which are found in Türkiye (Nemtinov et al., 2022). It is cultivated globally, from Southern Europe to the Middle East and South Asia, and is particularly widespread in regions like Thrace, Northern Anatolia, and the Mediterranean in Türkiye, especially in provinces like Burdur, Afyon, Isparta, and Gaziantep (Inan et al., 2022).

Seed priming, a cost-effective method to promote early crop growth and uniform emergence, is a common agricultural practice. This involves treating seeds with osmotic solutions or plant growth promoters such as salicylic acid and gibberellic acid, or inorganic salts like KNO_3 and $CaCl_2$ (Farooq et al., 2008; Rehman et al., 2011). Priming typically accelerates germination, improves stand establishment, and enhances crop yield and quality. For example, KNO_3 priming not only speeds up germination and strengthens seedlings but also boosts yield and protein content when combined with other priming methods (Rehman, 2014).

A substantial body of research has demonstrated that a variety of priming techniques can positively influence seed germination and seedling growth (Paparella et al., 2015; Sharma et al., 2014; Khalaki et al., 2020). The primary objective of this study is to determine the optimal dose and duration of gibberellic acid (GA3) application for seed priming in Nigella sativa, with the aim of enhancing seed germination. While the study also aims to explore whether these optimal conditions observed in controlled laboratory settings can be effectively applied in field conditions, the central focus remains on identifying the most effective GA3 treatment parameters to improve seed germination.

MATERIAL AND METHOD

This study examines the impact of , Gibberellic Acid (GA_3), priming treatments on Nigella sativa L. seeds under controlled laboratory conditions. The seeds were subjected to germination tests, placed on four layers of moistened filter paper, and incubated in darkness at a constant temperature of 22°C. Germination was assessed by the emergence of a 1 mm radicle and monitored over a 10-day period. No signs of microbial contamination were observed during the experiment (Kamal et al., 2010).

For priming, there are four dose; Control dose, 100 ppm GA, 200 ppm GA and 300 ppm GA; three priming duration; 12 hour Priming, 24 hour Priming and 36 hour Priming applications. The experimental design was arranged in a completely randomized design with 4 replications of 25 seeds in the germination stage The seeds were placed in 10 × 10 cm plastic boxes lined with blotting paper.

Germination Rate (%): It is calculated as Scott et al., 1984.

$$GR = \frac{Number\ of\ germinated\ seeds}{Total\ number\ of\ seeds} * 100$$

Germination Index: The germination index is calculated by dividing the number of germinated seeds by the number of days to the first count, and adding that to the number of germinated seeds divided by the number of days to the final count (Benech Arnold et al., 1991).

Mean germination time (Day): It is calculated as Ellis &Roberts, 1981.

$$MGT = \frac{\Sigma(Dn)}{\Sigma n}$$

n : number of seeds germinated on day D,
D: number of days counted from the beginning of the germination the lightest seeds,
The obtained data were analyzed separately and differences were determined using the analysis of variance (one-way ANOVA). Variance analyses were performed using JMP Pro v.16 software. Statistically significant factors were grouped using the LSD (Least Significant Difference) test. Factors that were not significant in the LSD test were compared based on mean tables.

RESULTS AND DISCUSSION

The ANOVA analysis results highlight the significant effects of GA_3 (gibberellic acid) dosage and application time on the germination performance of Nigella sativa seeds. The results show that both factors have statistically significant impacts on parameters such as germination rate, germination index, and mean germination time. The GA_3 dosage has a highly significant effect on the germination rate at the 1% level ($p < 0.01$). Different GA_3 concentrations lead to noticeable differences in the seeds' germination success. Additionally, application time also significantly affects germination rate at the 1% level ($p < 0.01$), indicating that the duration of exposure to GA_3 plays an important role. However, the interaction between dosage and time was not statistically significant ($p > 0.05$), suggesting that the combined application of these two factors does not create a synergistic effect. GA_3 dosage significantly affects the germination index at the 5% level ($p < 0.05$), meaning that varying doses impact the rate of germination, though not as dramatically as for germination rate. On the other hand, application time did not significantly influence the germination index ($p > 0.05$), indicating that time does not largely affect germination speed. GA_3 dosage significantly affects the mean germination time at the 1% level ($p < 0.01$), showing that optimal dosages can shorten the time it takes for seeds to germinate. Similarly, application time also has a significant effect on the mean germination time at the 1% level ($p < 0.01$), indicating that longer exposure to GA3 can alter the germination process. However, the interaction between dosage and time did not significantly affect this parameter ($p > 0.05$) (Table 1).
These findings suggest that both GA_3 dosage and application time should be optimized independently to improve the germination performance of Nigella sativa seeds. The lack of a significant interaction between the two factors means that their combined application does not offer additional advantages, so it is important to focus on each factor separately for better results.

Table 1. Significance levels in variance analysis for the GA_3 priming dose applications and priming durations in *Nigella sativa* L. Plant.

Source of Variation	Df	Mean of Square		
		Germination Rate	Germination Index	Mean germination time (Day)
Rep.	**2**	21.33	0.127	0.048
Dose	**3**	564.74**	1.369*	0.173**
Time	**2**	112**	0.098	0.497**
Dose*Time	**6**	40.29	0.151	0.131
Error	**22**	21.33	0.9	0.053
Total	**35**			

The results presented in Table 2 show the effects of different GA3 doses and priming times on the germination parameters of mean value *Nigella sativa* seeds.
The 300 ppm GA_3 dose consistently yielded the highest germination rates across all priming times, with an average of 92.00%, significantly outperforming the control (72.88%) and lower GA_3 doses. The dose of 300 ppm GA_3 resulted in significantly higher germination compared to the other treatments ($p < 0.01$). When considering priming duration, 36-hour priming resulted in the highest average germination rate of 85.32%, followed by 12-hour (81.33%) and 24-hour (79.33%). The effect of the priming time was significant, indicating longer priming times positively influence germination rates. A similar trend is observed with the germination index. The highest index (4.05) was found in seeds primed with 300 ppm GA_3, indicating faster and more successful germination compared to the control (3.14) and other GA_3 concentrations. The effect of the dose on the germination index was statistically significant ($p < 0.05$). Among the priming durations, the 12-hour priming time slightly outperformed others with an average germination index of 3.57, though the differences between priming times were not as pronounced. Regarding the average germination day, seeds primed with 300 ppm GA_3 also exhibited the fastest germination, averaging 6.08 days. This was significantly faster compared to seeds primed with 200 ppm GA_3 (6.40 days) and the control (6.18 days). The effect of GA_3 dose on germination time was significant at $p < 0.01$. The longest germination time was recorded at the 36-hour priming duration (6.45 days), suggesting that longer priming may slow down the germination process, while shorter priming durations (12 and 24 hours) resulted in quicker germination.

Table 2. Effect of GA_3 priming dose applications and priming durations in *Nigella sativa* L. plant germination parameters.

GA Dose and Priming Time	Germination Rate				Germination Index				Avarage Germination Day			
	12 hour Priming	**24 hour Priming**	**36 hour Priming**	**Avg.**	**12 hour Priming**	**24 hour Priming**	**36 hour Priming**	**Avg.**	**12 hour Priming**	**24 hour Priming**	**36 hour Priming**	**Avg.**
Control dose	73.33	70.66	74.66	**72.88 C**	3.19	3.12	3.11	**3.14 C**	6.07	6.09	6.39	**6.18 AB**
100 ppm GA	81.33	72	86.66	**80.00 B**	3.61	2.94	3.5	**3.35 BC**	6.01	6.48	6.41	**6.30 AB**
200 ppm GA	78.66	84	86.66	**83.11 B**	3.33	3.64	3.35	**3.44 B**	6.24	6.09	6.87	**6.40 A**
300 ppm GA	92	90.66	93.33	**92.00 A**	4.17	3.89	4.08	**4.05 A**	5.89	6.2	6.15	**6.08 B**
Avg.	**81.33 B**	**79.33 b**	**85.32 A**		**3.57**	**3.39**	**3.51**		**6.05 B**	**6.21 B**	**6.45 A**	

CONCLUSION

The results of the analysis of GA_3 priming doses and durations on *Nigella sativa* seeds provide several key insights for optimising germination performance. The highest germination rates, germination index values and shortest average germination times were achieved with a GA_3 concentration of 300 ppm. This dose significantly improved germination performance compared to lower doses and the control, demonstrating its effectiveness in promoting faster and more successful seed germination. Of the different priming durations, 36 hours priming resulted in the highest average germination rate, but also resulted in a slightly longer average germination time. This suggests that while longer priming durations may improve the overall germination rate, they may also increase the time taken for seeds to germinate. Conversely, shorter priming durations (12 and 24 hours) tended to speed up the germination process, but with less pronounced improvements in germination index compared to the 36 hour priming.The results suggest that the GA_3 dose of 300 ppm combined with a 12- or 24-hour priming period may be the most balanced approach, providing high germination rates and relatively short germination times. The effect of the interaction between dose and priming time was not as significant as the individual effects of each factor.In conclusion, a GA_3 dose of 300 ppm is highly recommended to enhance the germination of *Nigella sativa* seeds, with 12 h or 24 h priming preferred for faster germination. Longer soaking times may be beneficial for overall germination rates but may slightly delay the germination process. Balancing these factors according to the specific needs of the seed and the desired results can lead to optimum germination performance.

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SPATIOTEMPORAL PATTERNS OF TRACE ELEMENTS (LI, MN, NI, BA) IN RIVERS FEEDING THE İSTANBUL STRAIT, TÜRKIYE

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ABSTRACT

Sediments in freshwater ecosystems are crucial reservoirs of both essential and toxic elements. While some elements are vital for life, prolonged or high-level exposure, much like with non-essential toxic elements, can be detrimental. The Istanbul Strait, a globally significant marine habitat in northwestern Türkiye, receives substantial input from numerous rivers and lakes. This study investigated the spatiotemporal variations and accumulation of lithium (Li), manganese (Mn), nickel (Ni), and barium (Ba) in the sediments of rivers flowing into the İstanbul Strait. It this research, sediment samples were collected from three key fluvial habitats (Göksu, Kağıthane and Alibeyköy streams) during the dry and wet seasons of 2022–2023. The findings of the study reveal that the average annual concentrations of these elements in the river sediments followed the order: Mn (542.585 ppm) > Li (33.905 ppm) > Ni (21.598 ppm) > Ba (0.425 ppm). Furthermore, significant seasonal differences were observed in trace element accumulations. Notably, average concentrations of Li, Mn, Ni, and Ba in the sediments increased approximately 11, 3, 1.5, and 2 times, respectively, during the wet season.

Keywords: İstanbul Strait, Riverine habitats, Sediment quality, Trace elements

INTRODUCTION

Manganese, an essential element, is crucial for the healthy growth and development of all living organisms. However, much like toxic elements such as lithium, nickel, and barium, an excess of even essential elements can negatively impact the environment. Therefore, maintaining trace elements at concentrations below harmful limits and ensuring a balanced presence of essential elements in aquatic habitats is paramount for environmental protection (Milne, 2000; Belitz et al., 2009; Köse et al., 2013; Çiçek et al., 2013; 2017; Tokatlı and Helvacıoğlu, 2020).

Freshwater sediments play a crucial and dual role in aquatic ecosystems. Not only do they act as transporters and reservoirs for numerous pollutants, but they also offer a rich archive of valuable environmental information (Tokatlı et al., 2014; Yüksel et al., 2024; Varol and Tokatlı, 2024). This makes their study indispensable for understanding the health and history of water bodies. Consequently, the analysis of elemental content in surface sediments is a vital practice for environmental monitoring and assessment (Varol et al., 2022; Ustaoğlu et al., 2022; Tokatlı and İslan, 2022). By examining these elemental signatures, researchers can gain insights into past and present anthropogenic impacts, natural geological processes, and the overall ecological status of freshwater habitats.

The Istanbul Strait stands as a critically important international marine habitat in northwestern Türkiye. Its extensive watershed, teeming with numerous rivers and lakes, unfortunately also subjects this vital aquatic environment to significant pollution stress (Tokatlı et al., 2023; 2024; Varol and Tokatlı, 2024). In this study, the accumulation and spatiotemporal distribution of lithium (Li), manganese (Mn), nickel (Ni), and barium (Ba) were investigated in

the sediments of the rivers that feed directly into the Istanbul Strait and the implications of these findings for ecosystem health in the region were evaluated.

MATERIALS AND METHODS

For this study, three fluvial ecosystems feeding the Istanbul Strait were selected (Figure 1). Surface sediment samples were then collected from the downstream locations of these rivers during both the wet season (end of winter) and dry season (end of summer) of 2022–2023. An Ekman Grab (Hydrobios) was used for sample collection, adhering to the standard protocol (EPA, 2001a).

To determine the concentrations of lithium (Li), manganese (Mn), nickel (Ni), and barium (Ba) in the sediment samples, each sample was first dried at 105 °C for 3 hours. For digestion, 0.25 g of each dried sample was placed into a CEM Mars Xpress microwave device. A mixture of acids, specifically perchloric acid ($HClO_4$) and nitric acid (HNO_3) in a 1:3 ratio, was added to the Pyrex reaction vessels. The samples then underwent mineralization by heating at 200 °C for 30 minutes. Following mineralization, the samples were filtered using a 0.45 µm cellulose nitrate filter. After filtration, ultrapure water was added to bring the final volume of each sample to 100 mL. The concentrations of these trace elements in the sediments were then precisely measured using an Agilent 7700 ICP-MS device. All measurements were performed in triplicate at the accredited (TS EN / ISO IEC 17025) central laboratory of Thrace University in Edirne, Türkiye (EPA, 1998; 2001b).

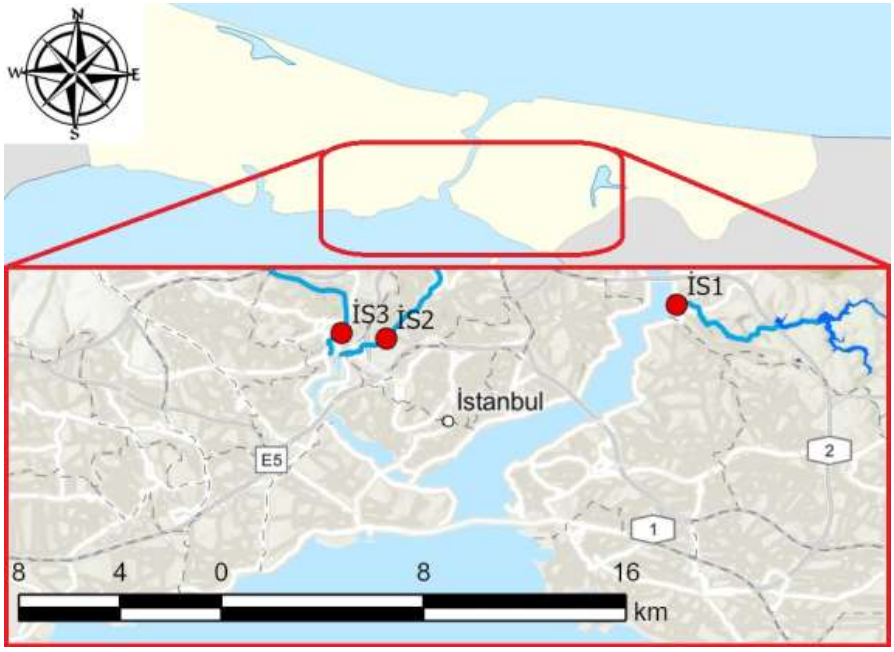

Figure 1. Study area and selected sampling locations

RESULTS AND DISCUSSION

Figure 2 presents the determined concentrations of lithium (Li), manganese (Mn), nickel (Ni), and barium (Ba) in the sediments collected from the designated sampling locations on the Göksu, Kağıthane, and Alibeyköy streams. These values illustrate the spatial distribution of the selected elements, offering valuable insights into the elemental composition of sediments within these fluvial water bodies during the sampling periods.

In the investigated streams, clear seasonal and spatial variations were observed in trace element concentrations. Lithium increased markedly during the wet season, reaching 112.159 ppm in Göksu Stream, compared to much lower values in the dry period (3.044–9.597 ppm). Manganese also exhibited a strong rise in the wet season, with the highest concentration measured in Göksu Stream (1248.808 ppm), whereas dry season levels remained between 234.523 and 359.089 ppm. Nickel showed site-dependent fluctuations, being higher in Kağıthane Stream during the dry season (25.156 ppm) and in Alibeyköy Stream during the wet season (35.766 ppm). Barium, on the other hand, remained at relatively low concentrations (0.183–0.411 ppm in the dry season), though it increased in the wet season, particularly in Alibeyköy Stream (0.764 ppm). These results indicate that wet season conditions, influenced by surface runoff, erosion, and anthropogenic inputs, considerably enhance the mobilization of certain elements, especially Li and Mn.

The pronounced seasonal increases in Li and Mn, particularly during the wet season, suggest that hydrological dynamics play a major role in the mobilization of these elements. Increased precipitation enhances surface runoff and leaching, resulting in the transport of metals from surrounding soils and anthropogenic sources into the streams. Elevated Ni and Ba concentrations in Alibeyköy and Kağıthane Streams further indicate the influence of urban and industrial discharges, which are well-documented contributors to trace metal pollution in densely populated catchments. Similar spatial and temporal patterns in trace metal accumulation have also been reported in previous studies conducted in Turkish aquatic ecosystems (Arslan et al., 2012; Tokatlı et al., 2020; Din et al., 2023; Ustaoğlu et al., 2025; Ali et al., 2025). Overall, the results highlight the combined effects of natural processes and anthropogenic pressures on metal dynamics in stream ecosystems.

CONCLUSIONS

This study demonstrated notable spatial and temporal variations in trace element concentrations in the sediments of Göksu, Kağıthane, and Alibeyköy Streams. Lithium and manganese showed remarkable increases during the wet season, suggesting that hydrological processes strongly influence their accumulation in sediments. Nickel and barium exhibited site-specific patterns, reflecting the impact of local anthropogenic pressures, particularly in urbanized catchments. Since these streams discharge directly into the Istanbul Strait, the observed sediment contamination patterns are of particular concern, as they may contribute to the transfer of trace metals into this ecologically and economically critical marine ecosystem. Therefore, continuous monitoring of these inflowing streams is essential to evaluate and mitigate their potential impacts on the Istanbul Strait.

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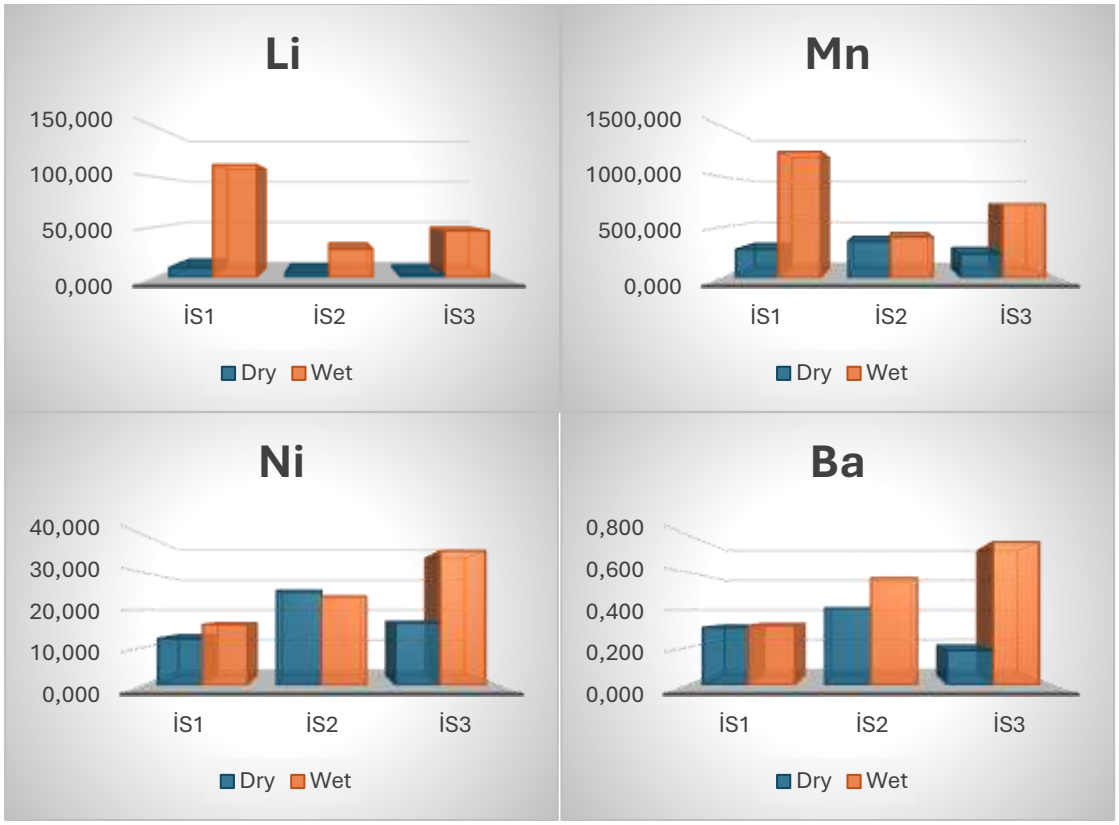

Figure 2. Spatiotemporal distributions of trace elements (ppm)

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THE EFFECTS OF CHITOSAN AND ESSENTIAL OIL APPLICATIONS ON FRUIT CRACKING PREVENTION AND QUALITY CRITERIA IN 0900 ZIRAAT CHERRY VARIETY (PRUNUS AVIUM. L)

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ABSTRACT

This study was conducted during the 2022-2023 period in the experimental plot located at the Çukurova University Pozantı Agricultural Research and Application Center, situated at an altitude of 1200 m. The study focused on the 0900 Ziraat cherry variety grafted onto MaxMa 60 rootstock, planted at a 5x5m spacing, and pruned in a Central Leader system. The trees were five years old. In the study, %0.5 $CaCl_2$, 300 ppm Green Stim, 100 ppm Chitosan, 400 ppm Thyme oil, combinations of these treatments, and water as a control were used. The study was conducted in a randomized complete block design with 3 replications, each containing 10 plants. As a result of this study, while the control application showed a 36% cracking rate, the lowest fruit cracking rate of 2% was obtained with the Thyme Oil (400 ppm) application. Particularly, the Thyme oil application had positive effects on fruit size. The fruit weight, which was 7.39 g in the control, was determined to be 8.42 g with the Thyme oil application, indicating a positive effect on fruit weight. The best result in fruit firmness was also achieved with the Thyme oil application. While the control had a fruit firmness of 4.5 kg, the Thyme oil application resulted in a fruit firmness of 5.42 kg.

Keywords: Cherry, quality, fruit cracking, essential oil

INTRODUCTION

Cherry (Prunus avium) is a type of stone fruit from the Rosaceae family, native to Asia (Özbek, 1978; Webster et al., 1996). Fruits, which constitute an important group among the species belonging to horticultural plants, are of great importance with their production values, growing areas, nutrition and share in exports. Fruits and vegetables are a source of vitamins, minerals, dietary fiber and antioxidants in terms of nutrition. The ratio of water-soluble dry matter, skin color, hardness of the fruit flesh, fruit diameter and weight are the most important criteria affecting consumer preferences in cherry, which is a low-calorie, good source of calcium, potassium and vitamins (vitamins A, B1, B2, C and malic acid) (Wani et al., 2014).

Cherries are produced in a wide area around the world. However, they are mostly produced in Turkey, the USA, Iran and Italy in commercial terms. Turkey, which ranks first in cherry production with the changes in climate conditions and years, is also one of the countries that have a say in cherry exports. Turkey's geographical structure and climate conditions allow for high-quality cherry cultivation suitable for export. The harvest time of cherries grown in Turkey can be earlier than in many European countries, and it extends until August as they rise above sea level (Küden, 2004). Cherries are grown almost everywhere in our country. Cherry production in our country is mainly carried out in the Kemalpaşa district of İzmir, Manisa, Konya-Akşehir, Afyonkarahisar-Sultandağı, Isparta-Uluborlu, Denizli-Honaz and recently in the Konya Hadim

and Taşkent regions. It is also produced in Çanakkale, Kütahya, Mersin, Antalya, Kahramanmaraş, Adana (Saimbeyli), Niğde (Ulukışla), Bursa, Amasya and Sakarya.

The area of cherry orchards in 2023 is 798,622 decares (Figure 1), export value and volume (Figure 2), the production amount is 736,792 tons (TÜİK, 2024).

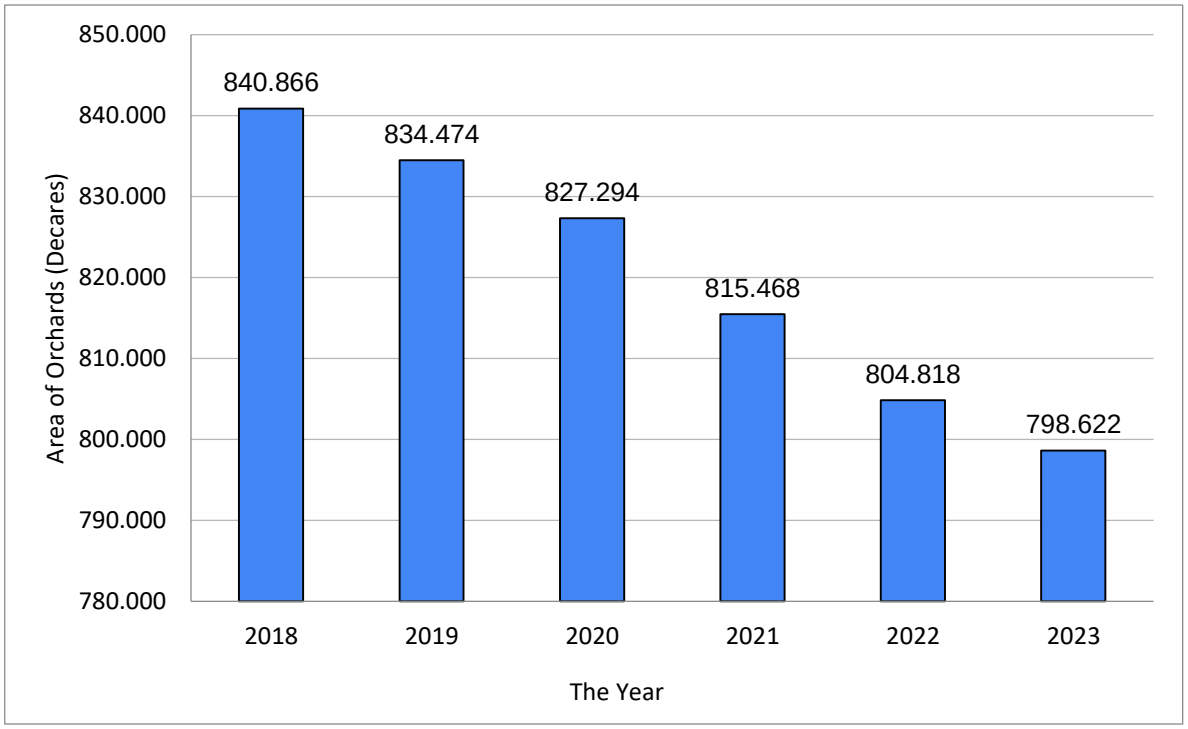

Figure 1. Turkey's Cherry production area by years

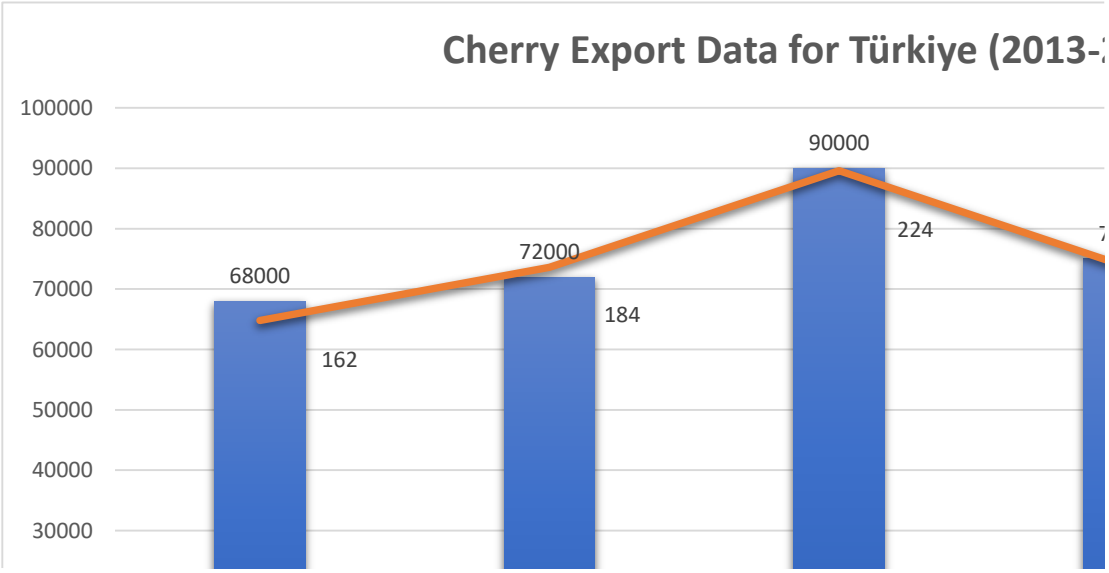

Figure 2. Turkey's Cherry export values and volume by years

One of the most important problems arising from climatic events in cherry cultivation is fruit cracking due to excessive rainfall occurring during harvest time and close to harvest, which causes great economic losses (İmrak et al., 2018; İmrak and Küden 2023). Cracking in cherries occurs in the cuticle of the fruit and generally occurs at 3 points (stalk pit, lateral face, and fruit tip) (Peschel and Knoche, 2005). The most common cause of cracking is accepted as rainwater entering the fruit

shell (Davenport, 1972). Although research on the causes of cracking began in the 1930s, this issue is still debated among scientists (Sekse, 1995). It has been accepted that many factors such as soil moisture, tree-water relations (Sekse, 1995), variety (Christensen, 1972a), fruit maturity stage (Christensen, 1973), soluble substance concentration (Christensen, 1972b), temperature (Christensen, 1972c), stomatal width and frequency (Christensen, 1972b), respiration rate (Pommier, 1989) play an important role in the cracking event.

In the study examining the susceptibility of Fermina, Regina, Lapins and 'Brooks varieties to cracking, it was stated that Regina and Fermina varieties were more resistant than Lapins and Brooks varieties (Moing et al., 2004). It is known that Regina variety is one of the most resistant varieties to fruit cracking (İmrak et al., 2018).

The main purpose taken into consideration in the selection of chemicals applied to reduce cracking is to reduce the amount of rainwater entering the fruit surface as much as possible during and after heavy rain. In many areas, calcium-containing solutions and the use of calcium together with substances such as copper, sulfate, nitrate are used against fruit cracking, as well as antitranspirants and various surface waxes (Sekse, 1995).

In cherry cultivation, calcium-containing solutions, which are used intensively to reduce cracking, are used effectively in many areas 2-3 weeks before harvest (Brown et al., 1995). In calcium applications, calcium blocks cell wall pectins, making the cell wall resistant to pressure and cracking (Alani, 1980).

In recent years, many applications have been made to prevent cracking, and one of them is the application of 0.5 mM methyl jasmonate applied in the pre-harvest period, which is stated to have a positive effect in preventing fruit cracking by increasing tolerance to abiotic stress (Ruiz-Aracil et al., 2023)

In the research, it was aimed to prevent cracking in the fruits of 0900 Ziraat cherry variety for export, caused by excessive rainfall during harvest time, by applying Calcium Chloride ($CaCl_2$), Green Stim, Chitosan and Thyme oil, thus increasing the quantity and quality of fruit for export.

MATERIAL AND METHOD

This study was carried out on 5-year-old 0900 Ziraat cherry variety grafted onto MaxMa 60 rootstock, planted at 5x5m planting distance and pruned in the Central Leader system in the experimental area located at Çukurova University Pozantı Agricultural Research and Application Center at an altitude of 1200m in the 2022-2023 period. In the study; 0.5% $CaCl_2$, 300ppm Green Stim, 100ppm Chitosan, 400ppm Thyme oil and water were used as control. The study was carried out according to the randomized block trial design with 3 replications and 10 plants in each replication.

Plant Material

0900 Ziraat cherry variety, which is mid resistant to cracking, is known as Turkish Cherry in Europe. Fruit characteristics; colour is bright dark red and large and heart-shaped fruits are resistant to transportation and storage. It has a thin, long and green stem, and its flesh is hard, juicy and delicious; with these features, it is among the best cherries in the world (Demirtaş and Sarısu, 2011).

It is in the Bigarreau group. It ripens late. Its fruit is very large (8.15 g), wide heart-shaped and bright dark red, fruit flesh is quite hard, crisp, juicy and very delicious; quality is excellent but needs pollinator, resistant to bacterial cancer and its flowering time is quite late. Its net mesocarp ratio is 14.31 and it is resistant to transport (Anonymous 2010).

Applications to Prevent Cracking in Fruits and Increase Quality

The plant growth regulators used in the study are given below. Water application was used as a control.

1- Calcium chloride ($CaCl_2$)
2- Calcium chloride ($CaCl_2$) + Green Stim
3- Calcium chloride ($CaCl_2$) + Chitosan
4- Calcium chloride ($CaCl_2$) + Thyme oil

Calcium chloride (CaCl2)

It is an inorganic compound, a salt with the chemical formula $CaCl_2$. It is a white crystalline solid at room temperature and is quite soluble in water. It can be formed by neutralizing hydrochloric acid with calcium hydroxide. Calcium chloride is commonly found as a hydrated solid with $CaCl_2 \cdot n\ H_2O$. These compounds are mainly used for deicing and dust control. Since anhydrous salt is hydroscopic and liquid, it is used as a desiccant (Kemp and Keegan, 2000). In calcium applications, calcium makes the cell wall resistant to pressure and cracking as a result of blocking cell wall pectin's (Alani, 1980).

The main purpose taken into consideration in the selection of chemicals applied to reduce cracking is to reduce the amount of rainwater entering the fruit surface as much as possible during and after heavy rain. In many areas, calcium-containing solutions and the use of calcium together with substances such as copper, sulfate, nitrate, as well as antitranspirants and various surface waxes are used against fruit cracking (Sekse, 1995).

Green Stim

It contains natural osmotic pressure balancers (glycine, betaine) (Imrak, 2019).

Chitosan

Chitosan is obtained as a result of deacetylation of chitin in an alkaline environment. It is insoluble in water and organic-based solvents (Abdou et al., 2008). Chitosan, the most widely used polysaccharide, is an economical natural biopolymer obtained from sea shells. (Sandford, 1989).

Thyme Oil

In our country, there are many fragrant plant species known as "Thyme" belonging to the Lamiaceae family, and their essential oils especially contain carvacrol and thymol. Studies have determined that Thyme oil suppresses many fungal diseases (Baydar, 2007; Bosquez et al., 2010; Camele et al., 2012).

General Characteristics of the Research Area

The research was carried out in the experimental area of Ç.Ü. Pozantı Agricultural Research Center in Pozantı, which is 1200 meters above sea level. Coordinate: 37.480050,34.903235.

The study was conducted between 2022-2023 and was set up in a randomized plot design with 3 replications and 10 trees in each replication. All applications were made approximately 30 days before harvest when the fruits turned from yellow to pink (vein fall) with a 15-liter backpack pump to wet the entire tree. The applications and their doses are given in detail in Table 1. All spray treatments were applied when the fruit color turns from green to yellow (22.06.2022)

Table 1. Applications and doses

Applications	Doses
Control	Water
$CaCl_2$	%0.5
$CaCl_2$+ Chitosan	%0.5 $CaCl_2$ + Chitosan 100 ppm
$CaCl_2$ + Thyme Oil	%0.5 $CaCl_2$ + Thyme Oil 400 ppm

$CaCl_2$ + Green Stim	%0.5 $CaCl_2$ + Green Stim 300 ppm

Phenological Observations

Budbreak, full bloom and harvesting dates were recorded (Westwood, 1987).

Pomological Analyses

Pomological analyses were performed with 3 repetitions and on 10 fruits at harvest maturity in each repetition (Emerce, 2004; İmrak, 2019).

Average Fruit Weight (g): The fruits obtained were weighed with a scale with a sensitivity of 0.01 g and calculated by dividing the total weight by the number of fruits. Here, 30 randomly taken fruit samples that could represent the analyzed variety were divided into 3, with 10 fruits in each repetition, and these fruits were weighed individually to determine the average fruit weight of each repetition.

The fruits obtained were measured with a caliper with a sensitivity of 0.01 mm and calculated by dividing the total value by the number of fruits. fruit length (mm), fruit width (mm), fruit height (mm)

Total Soluble Solids (TSS) %: The percentage value of the samples taken from the fruit juice was measured with a hand refractometer.

Total Acidity (%): After completing 5 ml of fruit juice with distilled pure water to 100 ml, titratable 0.1 Normality NaOH (Sodium Hydroxide) will be added to 5 ml of fruit juice completed with distilled water to 100 ml with the help of a pH meter until the digital display on the pH meter reaches 8.00-8.10 and then calculated in terms of citric acid according to the formula below.

Acidity Formula = Amount of NaOH consumed x 20 x Factor (0.963) x Acid value (Acid value for citric acid, 0.0067).

Fruit Flesh Firmness (kg): The hardness of the fruits of the varieties in the trial was measured using a hand penetrometer.

Color Measurements

Color measurements made according to the C.I.E. L*a*b* system were carried out with a Minolta color measuring device. In this system, the a* value indicates the color change from green to red, and the b* value indicates the color change from blue to yellow. Positive values of a* indicate red, and negative values indicate green. Positive values of b* indicate yellow, and negative values indicate blue (İmrak et al., 2018). The data obtained from the experiment were analyzed using the JMP v.8 statistical package program and the averages were compared using the Tukey test.

Cracking Index

The cracking index will be calculated in order to determine the cracking prevention or reduction effects of the applications (Bilginer et al., 1999). In this method, cherries that reached harvest maturity were collected in the morning hours (09:00-11:00) and brought to the laboratory for analysis in the cold chain.

In order to measure the cracking resistance of cherries, 50 fruits randomly selected from the fruits located 1.5 m above the ground in the outward facing area of the trees were used. In this method, cherries that reached harvest maturity were collected before noon and brought to the

laboratory for analysis in the cold chain. In order to measure the resistance of cherries to cracking, 50 randomly selected fruits were placed in 2-liter glass jars and kept in pure water at 20oC for 2-4-6 hours. At 2-hour intervals, the fruits were removed from the water and the cracked fruits were counted, and the healthy fruits were put back into the water. The same procedures were performed after 4 and 6 hours. The obtained data were calculated according to the cracking index formula used by Bilginer et al (1999) for cherries and explained below.

Cracking index = (5a + 3b + c) x 100/250

Statistical Analysis

The experiment was based on randomized blocking pattern as 3 replications and was assigned as one tree at each replication. The data were statistically analyzed using the Statistical Package for the. Means separation was determined by LSD test. Statistical analyses were carried out using JMP 5.0.1 version.

RESULTS AND DISCUSSION

It was observed that the applications made did not have any effect on the phenological stages. Phenological periods are presented in Table 2. It was determined that the dates specified in terms of phenological periods were parallel to previous studies and were found to be compatible with (İmrak and Küden, 2023).

Table 2. The effects of Applications on phenological stages in '0900 Ziraat' sweet cherry variety

Variety	Dormancy Breaking	Full Bloom	Harvesting time
0900 Ziraat	15.02.2023	19.04.2023	21.06.2023

Among the applications applied to the 0900 Ziraat cherry variety included in the experiment, the highest fruit flesh hardness was determined as 5.42 kg in the Thyme oil application, while the lowest fruit flesh hardness was determined as 4.5 kg in the control application. The fruit flesh hardness obtained as a result of other applications is between these values. Thyme oil application provided the highest fruit flesh hardness and clearly distinguished itself from all other applications. Thyme oil is known to have strong antimicrobial and antifungal properties among vegetable oils and can provide a protective effect on fruit tissue. This result supports the effectiveness of thyme oil in increasing fruit quality. There are findings in the literature that thyme oil applications provide similar positive effects in other fruit species (Sivakumar and Bautista-Baños, 2014). Chitosan application also showed a significant effect on fruit flesh hardness. With 4.83 kg fruit flesh hardness, chitosan application provided a significant increase compared to the control group. Chitosan is a natural biopolymer that can prevent water loss and microbial infections by forming a protective layer on the fruit surface. This shows that chitosan is an effective agent in improving fruit quality. Similarly, chitosan applications have been reported to increase fruit flesh firmness in other fruit species (El Ghaouth et al., 1992; Bautista-Baños et al., 2006). Calcium chloride application increased fruit flesh firmness to 4.65 kg. Calcium may increase fruit firmness by contributing to the strengthening of cell walls and the increase in the water retention capacity of cells. This finding is consistent with the literature showing that calcium applications provide similar effects in other fruit species (Conway et al., 1994; Saure, 2005) (Table 3).

The lowest L* value found in color measurement was determined to be 19.61 for Thyme oil application, while the highest L* value was determined to be 26.78 for Chitosan application (Table 3). The lowest a* value was determined to be 14.03 for Thyme oil application, while the highest a* value was determined to be 23.96 for control application. In addition, the lowest b* value was

determined to be 5.06 for Thyme oil application, while the highest b* value was determined to be 9.23 for control application.

Among the applications applied to the 0900 Ziraat cherry variety in the trial, the highest fruit weight was determined as 8.42 g in the Thyme Oil application, while the lowest fruit weight was determined as 7.39 g in the control. When we look at the average fruit weight data of all applications and control groups, it was determined that the average fruit weight of the Thyme Oil was higher (Table 4). The lowest SÇKM amount measurement value of the applications was measured as 14.63 in Green Stim, while the highest value was determined as 15.33 in Chitosan. In the pH value measurements of the applications in the trial, the lowest value was measured in the Calcium application as 2.88. The highest value was measured in Green Stim as 2.94. It was determined that the pH value of all applications and control groups was the highest and the lowest was in the Calcium application.

Table 3. The effects of Applications on fruit frimness and colour characteristics in '0900 Ziraat' sweet cherry variety

Applications	Fruit Frimness (kg)	L*	a*	b*
Control	4,5d	23,29ab	23,96a	7,01
$CaCl_2$	4,65c	20,44ab	17,21ab	6,06
$CaCl_2$+ Chitosan	4,83b	26,78a	17,38ab	6,47
$CaCl_2$ + Green Stim	4,53bc	24,8b	17,39ab	6,07
$CaCl_2$ + Thyme Oil	5,42a	19,61b	14,03b	5,06
$Lsd_{\%5}$	0,20	5,47	5,42	ns

The Calcium application provided the lowest pH value (2.88) and was significantly different from all other applications and the control group. The potential of calcium to reduce fruit pH may be due to the interaction of calcium ions with cell walls and intracellular matrices and increasing acidic properties. Similar findings have been reported in the literature that calcium applications improve product quality by reducing fruit pH (Conway et al., 1994; Saure, 2005).

The control group and chitosan application showed similar results with a pH value of 2.92. Chitosan can maintain pH balance by forming a protective layer on the fruit surface with its biopolymer structure. There are findings in the literature that chitosan improves fruit quality and provides pH balance (El Ghaouth et al., 1992; Bautista-Banos et al., 2006). Oregano oil application showed a result close to other applications with a pH value of 2.93. The antimicrobial and antifungal properties of oregano oil can help maintain pH balance by reducing microbial activity on the fruit surface. Similar findings on the positive effects of oregano oil applications on fruit quality are also available in the literature (Sivakumar and Bautista-Banos, 2014) (Table 4).

Table 4. The effects of Applications on pomological characteristics in '0900 Ziraat' sweet cherry variety

Applications	Average fruit weight (g)	Total soluble solid content TSS (%)	Titrable acidity (TA) (g citric acid/100 ml)

Control	7,39b	14,93	2,92ab
$CaCl_2$	7,97ab	15,23	2,88b
$CaCl_2$+ Chitosan	7,89ab	15,33	2,92ab
$CaCl_2$ + Green Stim	7,97ab	14,63	2,94a
$CaCl_2$ + Thyme Oil	8,42a	15,17	2,93ab
$Lsd_{\%5}$	0,77	ns	0,09

In the control group, the fruit cracking rate was determined to be the highest at 11.2%. This shows that a significant portion of the cherry fruits cracked when no application was made. 0.5% Calcium Chloride ($CaCl_2$) application reduced the fruit cracking rate to 2.4%. This shows that $CaCl_2$ significantly reduces fruit cracking. 300 ppm Green Stim application reduced the fruit cracking rate to 0.8%. This shows that Green Stim greatly reduces fruit cracking and is an effective application. 100 ppm Chitosan application also reduced the fruit cracking rate to 0.8%. This shows that chitosan is effective in reducing fruit cracking. 400 ppm Thyme Oil application achieved the lowest fruit cracking rate of 0.4%. This shows that thyme oil is the most effective application in preventing fruit cracking (Figure 3)

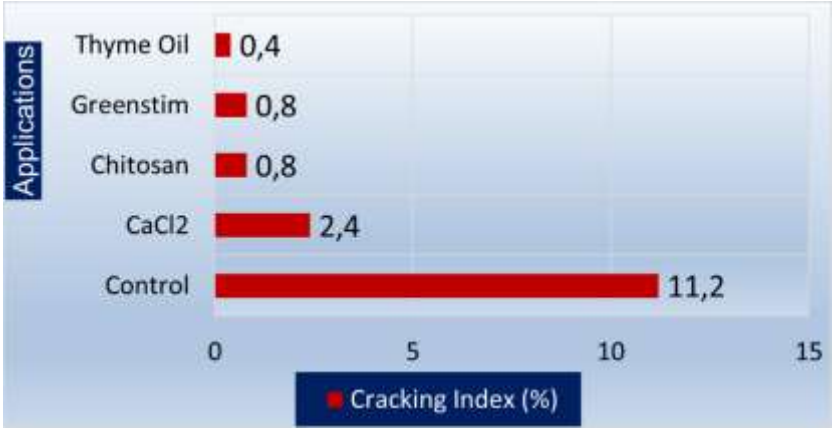

Figure 3. Effects of different treatments on 0900 Ziraat cherry fruit cracking Index

CONCLUSIONS

The results of this study have shown that all treatments significantly decreased the cracked fruit ratio compared to the control. But Thyme oil applications especially reduced the amount of 68.98 percent. In this study on 0900 Cherry, Thyme oil found to be the most effective Applications. It was followed by Green Stim, There were no detected any big differences Phenological and pomological (Except fruit weight) observations. Thus, Thyme oil gave the best result for reducing fruit cracking and Green Stim, Chitosan, $CaCl_2$ gave recommendable results compared to the control in 0900 Ziraat cherry cultivar in excessive rainfall years.

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INVESTIGATION OF PECTIN AND XANTHAN GUM DEGRADATION BY INTESTINAL BACTERIA

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ABSTRACT

The microbiota in the colon transforms polysaccharides such as pectin and xanthan gum and contributes to the maintenance of colon health by providing the formation of short-chain fatty acids. Various studies have shown that microbiota breaks down these components and form short-chain fatty acids, gas, and various metabolites, but in studies, the microbiota has generally been examined as a community, and the contribution of different bacterial species to biotransformation has not been sufficiently demonstrated. This study investigated whether Escherichia coli strains isolated from the human colon, which has not been studied before, produce short-chain fatty acids using pectin or xanthan gum in vitro. The results showed that when cultures containing different concentrations of polysaccharides were compared with control cultures (without polysaccharides), the lowest pH values were observed in cultures containing 5 mg/ml xanthan gum and 10 mg/ml pectin. However, gel formation was observed in the media prepared at these concentrations, and problems were experienced during the pipetting stages. For these reasons, 2 mg/ml xanthan gum and 5 mg/ml pectin concentrations were selected to test the production of short-chain fatty acids. The results of this study showed that these strains could not produce short-chain fatty acids under the conditions examined, but it aims to examine different bacterial strains in further studies.
Keywords: pectin, xanthan gum, microbiota, E. coli, short-chain fatty acids

INTRODUCTION

The human digestive system harbors a complex and dynamic population of microorganisms called the gut microbiota. This population of microorganisms includes bacteria, archaea, eukaryotes, and viruses (Koppel and Balskus, 2016). This population is larger than the number of human body cells, and it is estimated that there are at least 100 trillion (1014) microorganism cells in the human gut (Clemente et al., 2012). The gut microbiota has a significant effect on the health and disease status of the host. The microbiota benefits the host by performing physiological functions such as strengthening intestinal integrity, shaping intestinal epithelial cells, obtaining energy, protecting against pathogens, and strengthening the host's immunity. Disturbance of the balance of the gut microbiota (dysbiosis) has been associated with the pathogenicity of many inflammatory diseases and infections (Thursby and Judge, 2017). The composition of the gut microbiota varies significantly among individuals. It is affected by many factors such as genetics, host physiology (age, disease, stress, etc.), medications used, diet, and living environment (Holscher, 2017). Although studies on the presence of microorganisms in placenta have been published in recent years (Aagard et al., 2014; Rodriguez et al., 2015), the common belief is that the intestinal microbiota begins to form at birth (Thursby and Judge, 2017). It is also known that antibiotic use in adulthood causes changes in the composition of the intestinal microbiota and that these changes can be long-term (Modi et al., 2014).

Diet is one of the important factors affecting the intestinal microbiota. There are many published studies, which indicates the influence of diet on microbiota. For instance, when the

microbiota of people in a hunter-gatherer society in Tanzania was compared with those of people on a Western-style diet, it was found that the microbiota richness and diversity were higher in hunter-gatherers. This was associated with the fact that the diet of hunter-gatherers, unlike Western societies, included more carbohydrates that can be degraded by the microbiota (Schnorr et al., 2014). It is known that each of the macronutrients (fat, protein, carbohydrates) and many micronutrients can change the intestinal microbiota. The most prominent macronutrient among these is carbohydrates. For example, in a study examining fructose alone, it was found that it changed the composition of the intestinal microbiota (Mastrocola et al., 2018). An animal model study examining sucrose as part of a Western-style high-fat, high-sugar diet, it was observed that the microbiota was rapidly restructured (Collins et al., 2016). Furthermore, nutrients that humans cannot digest are used by bacteria in the large intestine in their basic biological processes. Therefore, changes in the human diet also affect the metabolism of these bacteria.

According to the Codex Alimentarius Commission (CAC) definition, dietary fiber is defined as carbohydrate polymers that are composed of 10 or more monomers and cannot be digested and absorbed in the human small intestine and is examined in 3 categories. These are classified as; edible carbohydrate polymers naturally found in foods; edible carbohydrate polymers obtained from raw food materials by physical, enzymatic or chemical means and having beneficial physiological effects based on scientific evidence; and finally, edible synthetic carbohydrate polymers having beneficial physiological effects based on scientific evidence. However, the definition of how many monomers dietary fiber will consist of may vary from country to country (Holsher, 2017). Dietary fibers can be obtained from plant and microbial sources. The main dietary fibers can be listed as: Cellulose, hemicellulose, guar gum, xanthan gum, alginate, carrageenan pectin, etc. (Guillon et al., 2011). These dietary fibers are broken down by intestinal bacteria when they reach the large intestine. As a result, they affect the stool mass, stool frequency, colon pH, and the energy obtained from these indigestible dietary fibers in individuals. However, the type of dietary fiber is broken down is important in terms of the metabolites formed (Guillon et al., 2011).

Epidemiological studies have shown that there is an inverse relationship between dietary fiber intake and cardiovascular diseases. The main important products formed as a result of the fermentation of these dietary fibers in the large intestine are short-chain fatty acids. The main short-chain fatty acids are acetate, propionate, and butyrate. Short-chain fatty acids play an important role in the health of the host. Butyrate contributes to the integrity of the intestinal barrier in the large and small intestines and provides energy to the epithelial cells in the large intestine (Chambers et al., 2018). In addition, studies have shown that butyrate has an inhibitory effect on colon cancer and the development of tumors. Different dietary fibers are formed with a wide variety of monosaccharide bonds. Although the human digestive system is inadequate in breaking down these bonds, the intestinal microbiota, with its diversity and gene pool, contains enzymes that can break down these bonds and can use unbreakable complex carbohydrates as an energy source (Gentile and Weir, 2018). Nutrition is one of the most important factors that shape and change the intestinal microbiota throughout human life. In addition, it is also a fact that the intestinal microbiota changes or transforms the foods consumed and the components in these foods. In this sense, the intestinal microbiota is one of the most important links in this bioavailability chain.

Pectin is one of the structural polysaccharides of plants and has a structure consisting of a polygalacturonic acid chain (Vandamme et al., 2002). It has a wide usage rate in the food industry due to its gelling, thickening and stabilizing properties. Since pectin can also be considered in the oligosaccharide class, it can also be fermented by intestinal bacteria (Willats et al., 2006). However, the rate of use by bacteria varies depending on the chemical structure and methylation degree of pectin (Hamaker et al., 2014). In some studies, it has been reported that pectic fractions support the development of bacteria such as Bifidobacterium and Lactobacillus, although it depends on the composition and prebiotic index of pectin. Thus, its benefits in protecting intestinal health and increasing digestion have been observed (Wicker et al., 2014). Gulfi and his colleagues

(2007) determined that total short-chain fatty acid production was high as a result of fermenting pectin, which was extracted from apples and has a branched structure, with bacteria in human feces. In a study conducted with mice, Fåk and his colleagues (2015) observed that pectin increased the level of short-chain fatty acids in the cecum and blood, and stated that acetic acid production was low in low-methylation pectins.

Xanthan gum is a microbial polysaccharide produced by Xanthomanas campestris, which is widely used as a thickener and stabilizer in food, pharmaceutical and agricultural applications (Rosalam and England, 2006). Since it is soluble in hot and cold water, it is used in many products in the food industry, and in the pharmaceutical industry, it is also used as a coating material with other polysaccharides such as chitosan (Giavasis, 2013). Xanthan gum, which is considered among the digestible carbohydrates, is also called dietary fiber and its use by intestinal bacteria was also investigated. In a study conducted by Bourquin et al., in vitro fermentation of various dietary fibers with bacteria isolated from feces was investigated, and they found that intestinal bacteria fermented xanthan and could produce high levels of acetic acid (Bourquin et al., 1996). Additionally, Schnizlein et al. (2020) found that in mice fed a diet supplemented with xanthan gum, the production of short-chain fatty acids increased and the efficacy of an antibiotic used to prevent colonization by Colostroides difficile bacteria was enhanced.

Studies showed that dysbiosis has been linked with many diseases. Various approaches, such as probiotics, prebiotic supplements, or fecal microbiota transplantation, have been intensively studied to cure the gut microbiota imbalance. Restoration of the levels of short-chain fatty acids is another therapeutic approach to cure dysbiosis (Fusco et al., 2023). Studies where the human microbiota was examined cumulatively showed that microbiota can degrade pectin and xanthan gum and can form acetate, propionate, and butyrate in different proportions. What has not been fully revealed in these studies and is worth examining is the contribution of individual members of the microbiota to this transformation. Escherichia coli is a bacterium commonly found in the human intestine. Previous studies have shown that E. coli strains can degrade different glucosides thanks to their different Glucosyl Hydrolase (GH) enzymes. In this context, this study investigated whether 2 different E. coli strains isolated from the human intestine can degrade pectin and xanthan gum and form short-chain fatty acids in vitro.

MATERIAL AND METHOD

E. coli strains used in the study were obtained from Karadeniz Technical University, Faculty of Medicine, Department of Microbiology. Nutrient Broth (Oxoid) was used to develop E. coli strains. After the liquid medium was prepared, the pH was adjusted to 6.8± 0.2. Pectin (pectin from Citrus peel, Galacturonic acid≥74.0) and xanthan gum (Xanthan from Xanthomonas campestris) used in the analyses were obtained from Sigma Aldrich.

The effect of different pectin and xanthan gum concentrations on the growth of E. coli strains were tested in the medium, 50 ml containing different concentrations of pectin (1, 2.5, 5, 10 mg/ml) and xanthan gum (0.5, 1, 2, 5 mg/ml). The media were inoculated with fresh E. coli F or E. coli C cultures and incubated for 8 hours in a shaking incubator (Heidolph, Germany) at 37°C. At the end of the incubation period, microbial growth in the cultures was determined by OD600 measurements made with a spectrophotometer (Thermo Scientific, Multiskan GO, US). The controls without pectin or xanthan gum were also prepared.

After determining the appropriate pectin and xanthan gum concentration, 50 ml media were prepared for each E. coli strain. These media were inoculated with fresh E. coli F or E. coli C cultures and incubated for 24 hours in a shaking incubator (Heidolph, Germany) at 37°C. At the end of the incubation period, pH measurements of the cultures were also made. In addition, fresh cultures of E. coli F and E. coli C were inoculated together on media containing pectin or xanthan gum (3 parallels, 50 ml medium). These media were incubated for 24 hours in a shaking incubator (Heidolph, Germany) at 37°C, and pH measurements were made at the end of the incubation

period. After the prepared cultures were transferred to clean falcon tubes, they were stored at -20°C until analyzed.

GC-MS analysis was performed using the method Schneider et al. used (2006) with modifications. 0.4 ml of 50% sulfuric acid and 2 ml of diethyl ether were added to a sufficient amount (2 ml) of culture; the sample was mixed in an orbital shaker for 45 minutes and then centrifuged at 3000 rpm for 5 minutes. Potassium persulfate was added to remove the remaining water and injected into the device. Shimadzu GC-MS QP2010 Ultra and DB-FastFAME 30 m, 0.25 mm, 0.25 µm (Agilent, US) columns were used in the analyses. The temperature program was programmed to increase from 100°C to 240°C and lasted for a total of 20 minutes. The split ratio was 10:1, the injection volume was 1 µl, the solvent delay time was 0, He was selected as the carrier gas, and the flow was adjusted to 1 ml/min. He was set to 30 ml/min, H2 flow to 40 ml/min, and dry air flow to 400 ml/min. The external standard method was used to quantify the samples; acetic acid, butyric acid, and propionic acid standards were run in the device at different concentrations (5, 10, 12.5, 25, 50 mol/l), and their retention times in the column were determined.

RESULTS AND DISCUSSION

Initially, each medium's pH values varied between 6.70-6.98. After the incubation, no pH below these values was observed in any culture. The samples were compared with control cultures to determine the pH decrease due to short-chain fatty acids that may occur due to E. coli activity during the incubation process. When the control cultures (without polysaccharides) were compared with the cultures containing different concentrations of polysaccharides at the end of the incubation, it was observed that the lowest pH values were generally in cultures containing 5 mg/ml xanthan gum and 10 mg/ml pectin (except 10 mg/ml for E. coli F). Gel formation was observed in the media prepared at these concentrations, and problems were experienced during the pipetting stages. For these reasons, 2 mg/ml xanthan gum and 5 mg/ml pectin concentrations were selected to test the production of short-chain fatty acids.

The retention times determined for these standards are as follows: Acetic acid; 3.2 min, propionic acid; 4.1 min, butyric acid; 5.5 min. These short-chain fatty acids were not detected in any of the examined samples. Noise peaks were observed in the GC-MS chromatograms of the samples; a sample chromatogram is shown in Figure 1.

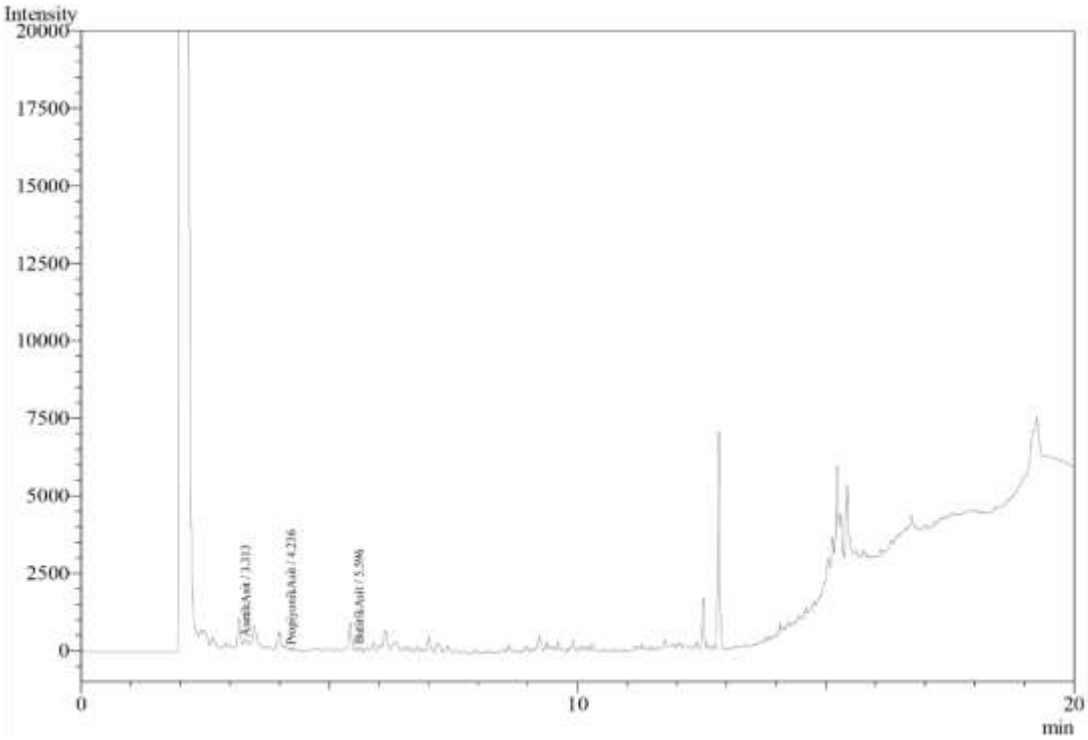

Figure 1. GC-MS chromatogram

CONCLUSIONS

Although there is no definitive information in the literature that E. coli can break down pectin or xanthan gum, it has been found that D-galacturonic acid units, which are monomers of pectic components, are broken down by E. coli in a unique way (Richard and Hilditch, 2009), and some previous studies have shown that some bacteria members of the Enterobacteriaceae family can break down pectin with their own enzymes (Abbott and Boraston, 2008). The fact that E. coli is an important member of the human intestinal microbiota and has strains that can be both pathogenic and probiotic makes this bacterium significant. Therefore, although the existing E. coli strains were tested for short-chain fatty acid production in the first step, our aim is to identify new bacterial strains that can produce short-chain fatty acids in the human intestine. In future studies, it is aimed to isolate bacteria that can use these polysaccharides and test the production of short-chain fatty acids in these strains by culturing them in media where pectin or xanthan gum is used as the sole carbon source from human feces solution.

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ASSESSMENT OF ETHYLENE EFFECTS ON POST-HARVEST CHARACTERISTICS OF CUT SNAPDRAGON FLOWERS

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ABSTRACT

Snapdragon (*Antirrhinum majus* L.) flower has unique inflorescence, attractive colors, and shapes, and is known as a cut flower in the world flower market, however, it is considered a sensitive flower to ethylene. Ethylene hormone plays an important role in the senescence of ethylene-sensitive flowers, especially snapdragon flowers, and causes the stem bending of cut flowers. This complication greatly reduces the ornamental value of snapdragon flowers and causes economic damage to producers and consumers. This study investigated different levels of ethylene hormone (0, 1, 10, and 100 µl. L^{-1}) on some growth characteristics and postharvest vase life of snapdragon flowers. The first visible response of snapdragon cut flowers to ethylene treatment was florets dropping whereas the flowers were turgid. The florets dropping was observed in all three levels of ethylene and indicated a significant difference with the control treatment. Ethylene also caused a disturbance in water absorption. Consequently, the relative water content, fresh weight, protein content, and vase life decreased. The curvature angle was also maximum at 100 µl. L^{-1} ethylene.

Keywords: *Antirrhinum majus* L, Curvature angle, Fresh weight, Post-harvest, Senescence

INTRODUCTION

The Snapdragon plant, scientifically known as Antirrhinum majus L., is a well-liked decorative plant appreciated for its attractive and aromatic blooms. In the United States, the sales revenue from freshly-cut snapdragons amounted to $12.18 million in 2015, positioning it among the top ten fresh-cut flowers. Challenges arise in the postharvest stage due to the plant's vulnerability to ethylene, which triggers florets' abscission and wilting. This sensitivity contributes to issues like limited vase life, incomplete flower opening, suppression of pigmentation, and stem curvature, impacting snapdragons' overall production and market performance (Kato et al., 2022; Xiang et al., 2020).

Snapdragon petals undergo abscission upon ethylene exposure, suggesting the sensitivity of snapdragon flowers to ethylene. Ethylene is recognized for its pivotal regulatory functions across diverse plant species. The biological activity of ethylene is evident even at very low concentrations ranging from 0.01 to 1.0 parts per million (ppm) (Heffron and Korban, 2022; Kato et al., 2022).

The longevity of cut snapdragon flowers is typically dictated by stem bending, as flowers with bent stems are unsuitable for commercial use. Regrettably, stem bending frequently manifests in snapdragon cut flowers within a brief period of vase life, making it a significant postharvest challenge (Naing et al., 2021). Research using ethylene and ethylene-action inhibitors like silver thiosulfate (STS) and 1-methylcyclopropene (1-MCP) has aimed to determine the involvement of ethylene as a positive regulator in snapdragon stem-bending. However, results varied, with some studies showing no significant impact on bending with STS or 1-MCP, while others reported a

blocking effect, suggesting further research is needed to clarify ethylene's role in this mechanism (Çelikel et al., 2010; Naing et al., 2021; Woltering, 2016).

In the present study, we treated snapdragon cut flowers with ethylene and investigated the influence of ethylene on various vase-life characteristics of snapdragon, such as curvature angle and florets abscission.

MATERIAL AND METHOD

Snapdragon F1 (Antirrhinum majus 'Legend White') seeds were germinated in Petri dishes under controlled conditions and placed in a greenhouse with regulated temperature and natural light. The plants were watered every other day until they reached the flowering stage, at which point they were moved to a growth chamber for ethylene treatment. Snapdragon flowers were trimmed to 40 cm and then treated with ethylene at 1, 10, and 100 µl l-1 for 48 h at 22 °C (each treatment with 10 repetitions). The treatment was performed in 38.8 × 39.0 × 38.8 cm glass chambers. The KOH solution (1 M) was placed inside the chamber to reduce CO2 concentration (Chamani et al., 2005).

The cut stems were weighed on day 0 (before treatment), day 1 (1 day after treatment), day 3 (3 days after treatment), day 5 (5 days after treatment), day 7 (7 days after treatment), day 9 (9 days after treatment), and day 11 (11 days after treatment). The water absorption was also recorded at these time points. The floret's abscission was counted on day 1.

Vase life was determined by recording the number of days from treatment initiation (day 0) until 50% of the flowers in the spike began wilting or drying, indicating the end of their commercial viability. Soluble protein content in the petals was measured using the Bradford assay method to quantify the total protein content (Bradford, 1976) on day 1. Stem curvature in snapdragon cut flowers was measured using a protractor and stated in degrees on day 11. A straight stem was defined 0°.

Statistical analysis of the data was conducted using SPSS V16 software, with significance levels determined through Duncan's multiple range test at a 5% probability.

RESULTS AND DISCUSSION

Gradually, the weight of the shoot in the control increased with a gentle slope and decreased after 5 days. On the other hand, ethylene treatment (1, 10, and 100 µl. L-1) caused a significant decrease in shoot weight 1 day after the treatment. With increasing ethylene concentration, weight loss occurred at a faster rate (Figure 2A). According to the changes in shoot weight, water absorption also revealed a significant increase in the control. Concentrations of 10 µl. L-1 and 100 µl. L-1 prevented water absorption in flower stems and as a result, the weight of the shoots decreased (Figure 2B). The treatment of cut snapdragon flowers with ethylene (10>100>1 µl. L-1) caused the florets to drop severely. The lowest drop of florets was observed in the control (Figure 2C). As a result, the vase life of untreated flowers was recorded up to 8 days (Figure 2D). Ethylene also caused a significant decrease in total soluble protein content, which indicated the rapid degradation of proteins under ethylene treatment (Figure 2E). Furthermore, the degree of stem curvature observed in E10 and E100 treatments was significantly higher than those of the E1 and control treatments at 11 days after treatment (Figure 2F).

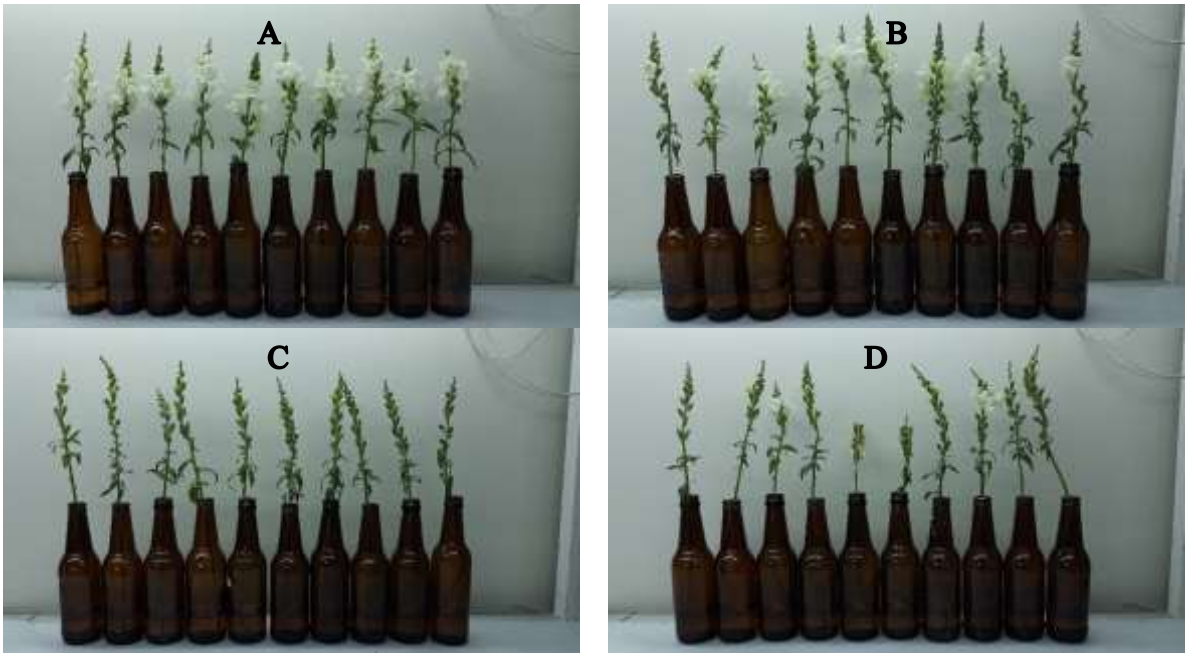
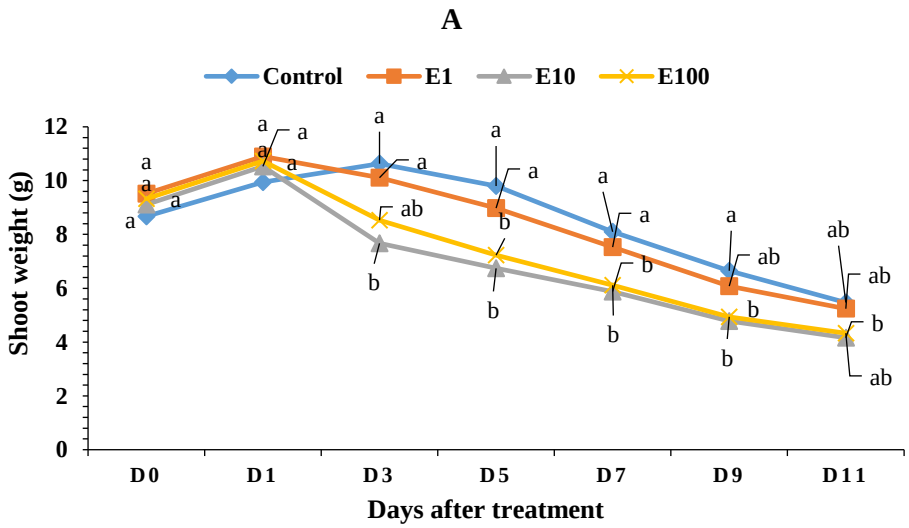


Figure 1. Comparison of the effects of different concentrations of ethylene on postharvest quality of snapdragon cut flowers. (A): control (0 µl. L^{-1}), (B): E1 (1 µl. L^{-1}), (C): E10 (10 µl. L^{-1}), (D): E100 (100 µl. L^{-1}). The photos were taken at day 5.



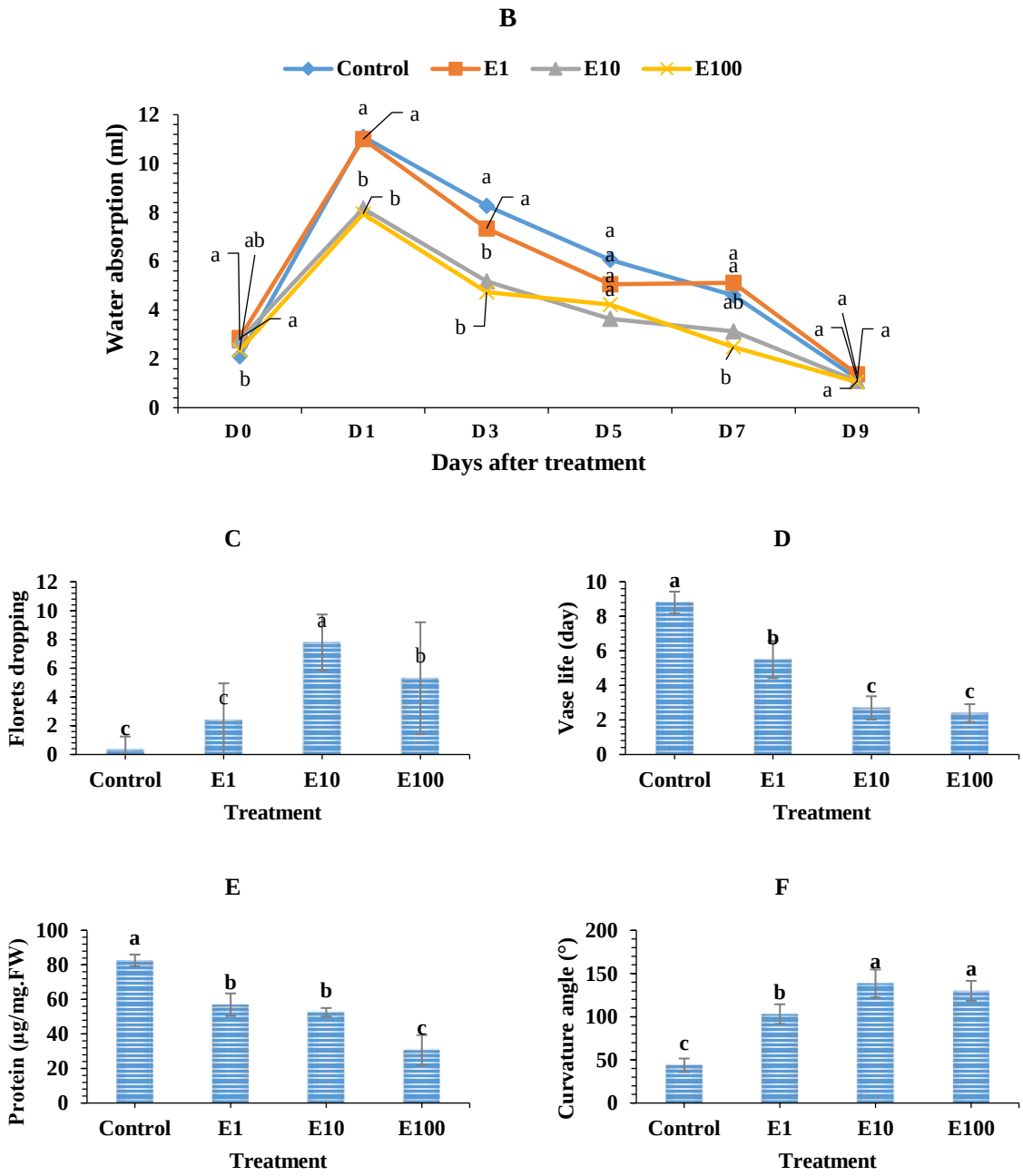


Figure 2. Some post-harvest characteristics of cut snapdragon flowers under ethylene treatment (0 µl. L^{-1}, Control; 1 µl. L^{-1}, E1; 10 µl. L^{-1}, E10; 100 µl. L^{-1}, E100). (A): shoot weight, (B): water absorption, (C): florets dropping, (D): vase life, (E): total soluble protein, (F): curvature angle. Different letters indicate significant differences at $p \leq 0.05$ (Duncan's test). Error bars represent ±SD, n=10.

In this study, we investigated the effect of different concentrations of ethylene on some characteristics of snapdragon flower post-harvest. This study indicated that increasing the concentration of ethylene can strongly increase the angle of curvature and bending of the stem. This incident was accompanied by a decrease in the weight of the stem, a decrease in water absorption, severe dropping of florets, and a lack of soluble protein, which ultimately led to the loss of the useful vase life of the cut flower.

Ethylene can stimulate many degenerative processes in cut flowers (Lukaszewska, 1985). In the present study, different concentrations of ethylene decreased total protein content. Proteins, as vital cellular components, are severely sensitive to adverse environmental conditions. The decrease in total protein is attributed to the involvement of ethylene in the expression of genes related to

aging, activation of plant proteases, and proteolytic degradation (Chamani et al., 2007; Eason et al., 2002; Jones et al., 2005).

Many studies have been done on the effect of ethylene regulation on stem bending. However, the results are contradictory and it is not yet clear whether ethylene stimulates stem bending or not (Naing et al., 2021). In a study, the treatment of cut tulip stems with ethephon (ethylene-releasing compound) caused a delay in stem bending (Van Doorn et al., 2011). On the other hand, in some species such as Calendula officinalis and gerbera, ethylene inhibiting compounds have reduced the rate of stem bending, which depends on the structural nature of the stems of different species (Hemati et al., 2019; Lone et al., 2021; Naing et al., 2021).

CONCLUSIONS

This study revealed that the initial observable reaction of snapdragon cut flowers to ethylene exposure was the dropping of florets (in the turgid condition). This phenomenon was evident across all levels of ethylene, highlighting a notable contrast with the control group. Furthermore, ethylene led to a disruption in water uptake, resulting in reductions in relative water content, fresh weight, protein levels, and vase longevity. The highest curvature angle was recorded at an ethylene concentration of 100 µl. L^{-1} and had a significant difference with control.

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ASSESSING THE IMPACT OF GLOBAL WARMING ON SOIL HEALTH AND AGRICULTURAL PRODUCTIVITY

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ABSTRACT

Global climate change imposes increasing and multifaceted pressures on soil health and fertility. Rising temperatures, irregular precipitation patterns, and the accumulation of greenhouse gases in the atmosphere significantly affect the physical, chemical, and biological properties of soils. Elevated temperatures accelerate the decomposition of soil organic matter, reduce soil organic carbon, and increase the risk of salinization and erosion. Intense and erratic rainfall events disrupt soil aggregate stability, lower cation exchange capacity, and enhance nutrient leaching. Conversely, reduced rainfall and drought limit soil moisture availability and nutrient uptake, while suppressing biological nitrogen fixation. Although increased atmospheric CO_2 levels may stimulate microbial activity and nutrient cycling, they can also lead to net carbon losses from the soil. This review explores the complex effects of climate change on soil health, focusing on structural degradation, shifts in pH, salinization, nutrient cycling disruptions, and microbial imbalance. Furthermore, the study discusses mitigation and adaptation strategies (such as conservation agriculture, organic matter management, and integrated nutrient management) that can help reduce climate-related soil degradation. Implementing climate-resilient soil management practices is essential to sustain long-term soil health and agricultural productivity.

Keywords: Climate change, Greenhouse gases, Carbon cycle, Sustainable agriculture

INTRODUCTION

Soils underpin terrestrial life and global food systems by regulating water and nutrient cycles, storing vast amounts of carbon, and hosting extraordinarily diverse biota that drive decomposition and fertility. Soils contain more carbon than the atmosphere and terrestrial vegetation combined, positioning them as a critical yet vulnerable component of both the climate system and agricultural productivity. Recent studies and guidance from agricultural and climate agencies emphasize that the size and persistence of this soil carbon pool confer both opportunity and risk: management can enhance sequestration, but warming and land-use pressures can accelerate losses (Keller and Handler 2024). Anthropogenic climate change, which has resulted in approximately 1.1 °C of global warming relative to the 1850–1900 baseline, has already led to widespread and multifaceted impacts on both natural and managed ecosystems. There is high confidence that continued warming will exacerbate risks to soil health and food security. The IPCC's Sixth Assessment Synthesis Report (2023) highlights that increasing temperatures, shifting precipitation regimes, and more frequent extremes are altering hydrologic and biogeochemical processes that determine soil health, with cascading effects on crop yields and rural livelihoods.

Warming directly influences the soil carbon balance by accelerating microbial metabolism and decomposition, potentially shifting soils from net sinks to net sources of CO_2 in sensitive

regions. Recent whole soil warming experiments and model data analyses published since 2023 highlight the significant vulnerability of soil organic carbon, including deeper soil layers that were previously considered relatively stable. These studies consistently report increased carbon emissions under sustained warming and underscore considerable uncertainty in both the magnitude and timing of these losses (Ren et al. 2024). Hydroclimatic change compounds these thermal effects. Intensifying heavy-precipitation events increase erosion, destabilize aggregates, and enhance nutrient leaching, while prolonged drought reduces soil moisture, suppresses biological nitrogen fixation, and impairs microbial functioning. Emerging assessments and regional projections show rising erosion risk under warmer, more variable climates, with implications for water quality and long-term soil fertility (Yin et al. 2025; Lua et al. 2024; IPCC 2023). At the farm and landscape scales, these physical and biogeochemical stresses translate into measurable risks for crop production. A 2023 article in *Nature Reviews Earth & Environment* notes that warmer temperatures, elevated CO_2, and altered water availability jointly modulate cereal yields, often exposing heat and water-stress thresholds that management must address. Complementary experimental evidence suggests that climate change and intensified cropland use can reduce soil multifunctionality, defined as the concurrent provision of nutrient cycling, carbon storage, and water regulation. This reduction in multifunctionality may, in turn, constrain agronomic resilience (Sünnemann et al. 2023)

Given soils' centrality to both mitigation (through carbon storage) and adaptation (through water retention, nutrient buffering, and biological resilience), safeguarding soil health is a prerequisite for maintaining agricultural productivity under climate change. This review synthesizes current understanding of how warming, altered precipitation, and rising atmospheric CO_2 interact to affect soil physical structure, chemistry (pH, salinity, cation exchange capacity), and biology (microbial communities and nutrient cycling). It also evaluates evidence based strategies such as conservation agriculture, organic matter management, and integrated nutrient management, which have the potential to slow or reverse climate related soil degradation while maintaining agricultural productivity.

CLIMATE CHANGE DRIVERS AFFECTING SOIL HEALTH

Rising temperatures

Global warming has led to significant increases in both air and soil temperatures, with profound implications for ecosystem processes and climate feedbacks. In China, analyses of five decades of temperature records (1962–2011) revealed that surface soil temperatures rose 31% more than air temperatures and that warming penetrated as deep as 3.2 meters, amplifying soil respiration rates by up to 28% and extending the growing season by approximately 20 days (Zhang et al. 2016). Similar long-term trends have been documented in other regions, such as North Rhine-Westphalia, Germany, where soil warming ranged from 0.36 °C to 0.47 °C per decade depending on depth (Dorau et al. 2022). Moreover, projections for the coming decades imply continued soil warming, especially in deeper layers, potentially altering global temperature regimes and cascading into ecological disruptions (Bradford 2019; Lembrechts 2022). Taken together, these findings highlight the increasingly urgent need to incorporate soil temperature dynamics, alongside atmospheric warming, into climate models and ecosystem assessments, as soil warming acts both as a driver and an amplifier of climate change.

Altered precipitation regimes

Global warming is profoundly altering precipitation regimes worldwide, intensifying both the wet and dry extremes and restructuring seasonal patterns. Rising global temperatures increase the atmosphere's capacity to hold moisture, by approximately 2 to 3 percent per degree Celsius on a global scale, with extreme precipitation events intensifying at an even faster rate. This results in more severe rainfall events and prolonged dry periods, even as average precipitation shows an overall increasing trend (Douville, 2021). Climate models reveal that precipitation becomes less frequent yet more intense in the tropics, with heavy precipitation events increasing by up to 35 % per 1°C warming, despite an overall decline in light rainfall (Guo *et al* 2022). Furthermore, the annual range of precipitation widens significantly; maximum precipitation increases by roughly 3.6 % $°C^{-1}$, while minimum precipitation rises by only 0.2 % $°C^{-1}$ (Guo et al. 2012). Changes in wet and dry season patterns are also evident: across the tropics, many regions are transitioning from annual to biannual precipitation regimes, with wet seasons shortening by an average of about 7 days and beginning 6 days later, while dry seasons grow both longer and drier (Guo et al. 2012). Moreover, projections suggest that large precipitation accumulations, which have been rare in historical records, will become exponentially more likely under continued warming, with some events projected to increase by several hundred to over one thousand percent (Neelin et al. 2017). Collectively, these alterations not only pose escalating risks for flooding, drought, and agricultural disruption but also challenge water-resource management and infrastructure resilience in both regional and global contexts.

Drought

Anthropogenic global warming is exacerbating drought globally through a combination of elevated atmospheric evaporative demand (AED), altered precipitation dynamics, and soil moisture depletion. Recent high-resolution global drought data (1901–2022) demonstrate that increased AED (driven by rising temperatures) has amplified drought severity by an average of 40 %, and has helped expand drought-affected land by approximately 74 % in the period 2018–2022 compared to 1981–2017, with nearly 30 % of the terrestrial surface experiencing moderate to extreme drought in 2022 alone, 42 % of which is attributable to AED (Gebrechorkos et al. 2025). This pattern is echoed regionally; in the Iberian Peninsula, intensified evaporative demand has led to more frequent and severe hydrological droughts, significantly reducing streamflow in both natural and regulated river basins (Lorenzo, 2014). Projections indicate that under moderate emissions scenarios (SSP2-4.5), by mid-century nearly 70 % of global land could face declining soil moisture, increasing agricultural drought risk particularly in regions like India, China, Europe, and the United States (OECD, 2025). Moreover, observational and modeling evidence indicates that drought durations, particularly the longest annual dry spells, may lengthen by the end of the century, substantially exceeding midrange estimates when models are calibrated with observational data. This convergence of rising temperatures, increased evaporation, and declining moisture storage highlights the urgent need for resilient water resource management to address more frequent, prolonged, and severe drought conditions.

Elevated atmospheric CO_2 (Eco_2)

Elevated atmospheric carbon dioxide, a central driver of anthropogenic climate change, exerts a fundamental influence on plant physiology, hydrology, and ecosystem carbon cycling. However, the magnitude and consistency of these effects remain highly dependent on environmental context. The dramatic rise in CO_2 from preindustrial ~280 ppm to over 427 ppm by 2024 underscores its critical role in enhancing the greenhouse effect and global warming (NOAA, 2024). On the positive side, Eco_2 enhances photosynthetic carbon uptake, thereby increasing terrestrial gross primary productivity (GPP) and improving leaf-level water-use efficiency by reducing stomatal conductance and transpiration (Lucas et al. 2019). Free Air Carbon Dioxide Enrichment (FACE) studies report biomass increases of approximately 20 to 30 percent, although these gains are often limited by soil nutrient availability, particularly nitrogen and phosphorus. These nutrient constraints account for a substantial portion of the variability observed in carbon fertilization responses (Fu et al. 2024). Additionally, elevated CO_2 can partially buffer plants against drought and heat stress by improving water status and mitigating oxidative damage, as seen in meta-analyses across diverse species and controlled experiments such as those involving Arabidopsis (Wang et al. 2022). That said, Eco_2 can also yield counterintuitive outcomes: enhanced leaf area may increase canopy transpiration, and changes in plant hydraulics (e.g., altered stomatal behavior or hydraulic conductance) may reduce drought resilience in certain contexts (Becklin et al. 2017). Moreover, the CO_2 fertilization effect may be waning globally due to nutrient constraints and ecosystem saturation, potentially weakening the land carbon sink and increasing reliance on mitigation strategies. Thus, although elevated carbon dioxide plays a critical role in modulating plant responses under climate change, its overall influence is determined by species-specific traits, environmental interactions, and resource availability. This underscores the need for nuanced, multifactor assessments in ecosystem modeling.

SOIL PROPERTY ALTERATIONS UNDER CLIMATE STRESS

The climate stress factors mentioned above reconfigure soils' physical, chemical, and biological properties in ways that directly govern fertility and yield stability.

Physical properties: structure, aggregation, and erosion susceptibility

Under climate stressors such as intensified precipitation, prolonged droughts, and fluctuating temperatures, soil physical properties undergo marked degradation. Elevated temperatures and extreme heat events can reduce cohesion among clay particles, shifting textures toward more silt-sized fractions and compromising structural integrity through induced cracking and dehydration of 2:1 clay minerals (Mangi et al. 2017). Drought exacerbates these effects by suppressing the production of root mucilage and microbial exudates that normally stabilize aggregates, leading to a decline in mean weight diameter (MWD) and aggregate stability; the removal of vegetation during drought further accelerates macroaggregate breakdown and diminishes aggregate-associated organic carbon (Reinsch et al. 2024). When heavy rainfall follows such weakened soil states, erosion susceptibility increases markedly: less aggregated soils, particularly coarser-textured ones, show higher erodibility and decreased stability across successive rainfall events, with significant reductions in soil K-factor observed (Yao 2019). Moreover, intensifying land use changes under warming climates influence aggregation through altered thermal amplitudes and

moisture regimes, which in turn affect porosity, bulk density, and infiltration potential (Li et al. 2023). Collectively, these climate-induced disruptions in soil structure, aggregation, and susceptibility to erosion threaten soil health, water retention, and ecosystem resilience.

Chemical properties: Ph, salinity, cation exchange capacity (CEC), and nutrient leaching

Under climate stressors such as shifting precipitation patterns, rising temperatures, and sea level rise, soil chemical properties are significantly disrupted. Soil Ph is highly sensitive to climate-driven changes in temperature, precipitation, and biogeochemical cycling. Increased precipitation and extreme rainfall events accelerate the leaching of base cations (Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}), which normally buffer acidity, leading to progressive soil acidification (Yaseen 2024). At the same time, enhanced mineral weathering and the oxidation of soil organic matter under warming release hydrogen ions (H^{+}) and mobilize aluminum (Al^{3+}), further lowering Ph and impairing nutrient availability (Brinkman, 1990). In drought-prone regions, reduced leaching and the accumulation of soluble salts can, conversely, raise soil Ph (alkalinization), particularly in calcareous soils where carbonates become concentrated in the profile (Bui, 2017). Moreover, rising atmospheric CO_2 and associated shifts in soil respiration influence carbonic acid equilibria, which can modify Ph dynamics seasonally. Overall, climate stress exerts a bidirectional influence: acidification dominates in humid and high-rainfall systems, while alkalinization often occurs in arid and semi-arid landscapes both with profound consequences for nutrient cycling, microbial activity, and long-term soil fertility.

In coastal and semi-arid zones, sea-level rise and saltwater intrusion have increased soil salinity by between 1 % and 33 % over recent decades, while elevated evapotranspiration in arid regions inhibits salt leaching, promoting accumulation in the root zone (Rahman et al. 2018). Climate-induced warming accelerates the decomposition of soil organic matter (SOM), which critically underpins soil Cation Exchange Capacity (CEC) a determinant of the soil's ability to retain key nutrient cations. As SOM declines, especially in coarse-textured or low-clay soils, CEC diminishes, reducing nutrient retention and amplifying leaching losses when intense precipitation occurs (Mandal and Sathyaseelan, 2012). Together, these shifts diminish nutrient retention, elevate the risk of nutrient leaching, and impair soil fertility and ecosystem function under ongoing and future climate stress.

Biological properties: microbial diversity, processes, and symbioses

Climate-induced stressors such as warming, drought, and altered precipitation patterns have pronounced effects on soil microbial diversity, functional processes, and symbiotic relationships. Meta-analyses reveal that on average, global warming reduces soil bacterial and fungal diversity by approximately 16% and 20%, respectively, with projected declines reaching 56% (bacteria) and 81% (fungi) under high-emission scenarios by mid-century trends that threaten soil organic carbon (SOC) sequestration and ecosystem resilience (Sun et al. 2025). Long-term experimental observations in grasslands indicate that warming restructures microbial successional trajectories, making fungal communities increasingly divergent over time and emphasizing deterministic environmental filtering over stochastic assembly (Guo et al. 2018). Drought compounds these effects: prolonged moisture deficits and elevated temperatures significantly alter the composition of bacterial and fungal communities, reduce their richness, and disrupt established symbioses, including mycorrhizal colonization patterns (Seaton et al. 2012). At the functional level, changes

in temperature and precipitation influence the complexity of microbial networks. In temperate steppes, warming has been shown to increase fungal network complexity and stability, particularly during cooler seasons, indicating a shift toward more interconnected, fungi-driven processing networks (Yu et al. 2024). Moreover, symbiotic relationships are altered: drought promotes arbuscular mycorrhizal fungi (AMF) colonization and structural adaptations like increased hyphal networks and vesicle formation, enhancing plant drought tolerance and nutrient uptake via osmolyte production and water relations adjustments (Silva et al. 2025). Collectively, climate stress reduces microbial diversity, reshapes community assembly, alters biogeochemical functioning, and reshapes symbiotic partnerships, with profound implications for nutrient cycling, plant resilience, and ecosystem stability.

CONSEQUENCES FOR AGRICULTURAL PRODUCTIVITY

Climate-induced soil degradation exerts cascading effects on agricultural systems, ultimately eroding crop yields, resource efficiency, and resilience.

Declines in Crop Yields and Soil Multifunctionality

Climate driven soil degradation, reflected in declines in soil organic carbon, increased erosion, loss of structural integrity, and reduced microbial function, significantly undermines both crop yields and soil multifunctionality. Intense warming and drought drive accelerated SOC decomposition and erosion, reducing moisture retention, nutrient availability, and key ecosystem services that support plant growth (e.g., carbon and nutrient cycling) (Cheng et al. 2017). Empirical modeling shows that warming reduces global yields of maize by 3.4%, wheat by 2.4%, rice by 0.3%, and soybean by 5%, while improving SOC can dramatically mitigate these losses bringing yield reductions down to near-neutral levels in many cases (Deng et al. 2023). Furthermore, precipitation reduction in semi-arid grasslands disrupts microbial biodiversity and network complexity, resulting in diminished soil multifunctionality such as nutrient provisioning, microbial growth efficiency, and organic matter decomposition (Wang et al. 2023). Conservation agriculture, which enhances soil health through practices like reduced tillage and increased SOC and microbial biomass, has been shown to sustain or even boost crop yields (e.g., 9.3% wheat yield increase) under long-term warming, highlighting how degraded soils may lose resilience in the absence of such practices (Teng et al. 2024). Together, these findings illustrate a clear mechanistic pathway: climate-induced soil degradation erodes the suite of soil functions that underpin crop productivity and ecosystem resilience, unless countered by proactive soil management strategies.

Nutrient Inefficiencies and Increased Fertilizer Demand

Climate driven soil degradation, including the loss of organic matter, erosion, altered moisture regimes, and disruption of nutrient cycling, exacerbates nutrient inefficiencies in agroecosystems and consequently increases fertilizer demand. Declining soil organic matter (SOM) and fertility force farmers to rely more heavily on synthetic fertilizers, yet as SOM diminishes, soils' physical and biological ability to retain nutrients also deteriorates, causing a substantial portion of applied nutrients to be lost to leaching or volatilization (Mosier et al. 2021). For example, degraded dryland agroecosystems often exhibit both nutrient depletion and reduced fertilizer-use efficiency, prompting escalating fertilizer applications that further increase greenhouse gas emissions (Iqbal et al. 2023). Furthermore, rising temperatures above critical thresholds (e.g., 28 °C) have been empirically linked to sharp increases in fertilizer use particularly

nitrogen in irrigated regions, as farmers attempt to offset heat- and moisture-induced yield declines, even as nutrient uptake efficiency falls (Quan et al. 2024). Together, these dynamics reveal a vicious cycle: climate stress degrades soil, impairs nutrient retention, and forces greater fertilizer inputs, often with diminishing returns and increased environmental and economic costs.

Water Stress and Drought Vulnerabilities

Climate-driven soil degradation such as erosion, loss of organic matter, structural decline, and vegetation removal, significantly heightens water stress and drought vulnerability in agroecosystems. The degradation of soil structure and aggregation due to intensified erosion and compaction diminishes infiltration rates and reduces soil water–holding capacity, leading to faster runoff and reduced water availability during dry periods (Radulov and Berbecea, 2023). Degraded soils lacking organic matter retain less moisture and are more prone to moisture deficits under warming and erratic precipitation, which amplifies drought impacts on plant growth and ecosystem resilience (IUCN, 2024). Land-use changes such as deforestation and the conversion of forests and rangelands to bare or cultivated land further impair the land's capability to buffer drought by disrupting vegetation cover and hydrological buffering (Liu et al. 2025). Empirical evidence from drought studies also shows that soil drying reduces microbial function and alters soil carbon and nitrogen processes, degrading the soil habitat and making ecosystems less resistant to prolonged water deficits (De Silva, 2025). Together, these processes create a feedback loop whereby degraded soils lose both physical and biological resilience, leaving landscapes more susceptible to water stress and prolonged drought under changing climate conditions.

Enhanced Greenhouse Gas Emissions and Feedbacks

Climate-induced soil degradation through warming, drying, erosion, and land-use change leads to an increase in greenhouse gas emissions from soils, thus reinforcing a positive feedback loop that accelerates climate warming. Experimental warming studies demonstrate that a ~1.5 °C rise in temperature significantly elevates soil CO_2 emissions (~13%), nitrous oxide (N_2O) emissions (~35%), and methane (CH_4) emissions (~23–38%) in various ecosystems, collectively adding 1.8 gigatonnes CO_2-equivalent per year and undermining the mitigation potential of terrestrial carbon sinks (Liu et al. 2020). Decomposition of previously stable soil organic carbon including deep SOM and permafrost carbon is accelerated by microbial activity under warming conditions, potentially releasing hundreds of petagrams of carbon over time and substantially reducing overall SOC storage (Cheng et al. 2017). Soil erosion and topsoil loss also enhance CO_2 emissions: an experimental study found that removal of the top 20 cm of soil increased seasonal CO_2 efflux from cropland, whereas adding soil reduced emissions, indicating that degradation itself can amplify GHG flux (Liang, 2018). In peat-rich ecosystems, draining and degradation of peatlands commonly undertaken for agriculture, transforms these systems from net carbon sinks into potent sources of CO_2 (and additionally CH_4 upon rewetting), making them a major driver of GHG emissions (Leifeld and Menichetti, 2018). Together, these processes illustrate that soil degradation triggers heightened emissions of CO_2, N_2O, and CH_4, creating self-reinforcing feedbacks jeopardizing both soil carbon stocks and broader climate stability.

Table 1. Climate Change Impacts on Agricultural Productivity

Process	Impact Mechanism	Productivity Consequence
Yield reduction	Stress from heat, drought, and fragmentation of multifunctionality	Lower output and food security risks
Nutrient losses	Leaching, runoff, low CEC	Reduced fertilizer efficiency, increased inputs
Water stress	Poor infiltration, structure loss, reduced water retention	Greater drought sensitivity, yield instability
Greenhouse feedbacks	SOC loss and N_2O emissions	Climate feedback loop and inefficiency

MITIGATION AND ADAPTATION STRATEGIES

Soil resilience to climate change depends on integrated, site-specific combinations of practices that protect soil structure, rebuild organic matter, improve nutrient and water use efficiency, and enhance biological function. Below we synthesize the evidence for key strategies and note important caveats.

Conservation agriculture (reduced/no-till, residue retention, cover crops)

Conservation agriculture (CA), anchored by the principles of reduced or no-tillage, residue retention, and diversified cropping (including cover crops), serves as a pivotal approach for both climate change mitigation and adaptation in agroecosystems. Reduced soil disturbance preserves structure and surface organic matter, enhancing water infiltration, reducing erosion, and promoting soil carbon accumulation (Zomer, 2017). Residue retention and cover cropping further bolster these benefits by maintaining soil moisture, regulating temperature extremes, and incrementally increasing soil organic carbon (SOC), soil aggregation, and nutrient cycling (Page, 2020). Meta-analyses reveal that CA often enhances yields modestly with cumulative gains in maize of ~4 % while improving drought resilience and yield stability under variable rainfall (Corbeels et. Al. 2020). Significantly, CA practices substantially reduce nitrous oxide (N_2O) emissions by about 7 % with no-till alone, and up to 30 % when combined with residue retention and rotation and increase crop yield by over 9 %, offering synergistic mitigation and productivity gains (Zhu et al. 2025). In drought settings, implementing cover crops and residue retention has shown striking results for example, soybean yields up to 29 % higher, along with improved water-use and photosynthetic performance (Whippo, 2024). Altogether, conservation agriculture offers a multifaceted strategy: it mitigates greenhouse gas emissions, enhances soil health and resilience, and stabilizes crop productivity under climate stress though site-specific outcomes may vary.

Organic matter management: composts, manures, green manures, and biochar

Organic matter management, including the application of composts, manures, green manures, and biochar, is increasingly recognized as an effective strategy for mitigating and adapting to climate change impacts on agricultural productivity. The incorporation of composts and animal manures improves soil fertility by supplying essential macro- and micronutrients,

enhancing cation exchange capacity (CEC), and stimulating beneficial microbial activity, which together bolster plant growth and resilience under climate stress (Goldan et al. 2023). Green manures, particularly legumes, contribute biological nitrogen fixation and enhance soil organic carbon (SOC) stocks, thereby improving soil aggregation, water-holding capacity, and nutrient cycling, which are critical under conditions of drought and variable rainfall (Mandal et al., 2004). Biochar, due to its stability and high carbon content, provides long-term carbon sequestration potential while also enhancing soil structure, nutrient retention, and water-use efficiency, and has been shown to reduce nitrous oxide (N_2O) and methane (CH_4) emissions from soils (Lehmann et al., 2011). Empirical studies demonstrate that co-application of biochar with organic manures or compost further improves soil physicochemical properties, crop productivity, and microbial community dynamics, highlighting the synergistic potential of integrated organic matter management strategies (Wang et al. 2019). Collectively, these practices not only enhance soil health and crop yields but also provide critical climate mitigation benefits by sequestering carbon and reducing greenhouse gas emissions, positioning them as vital tools for sustainable agriculture in a warming world.

Integrated nutrient management (INM) and precision fertilization

Integrated Nutrient Management (INM), which combines organic sources (e.g., manures, composts, crop residues) with judicious use of inorganic fertilizers, enhances soil fertility, synchronizes nutrient release with crop demand, and improves nitrogen use efficiency thereby reducing greenhouse gas emissions and enhancing resilience under climate variability Long-term field studies in rice–wheat systems demonstrate that INM significantly boosts soil organic carbon (SOC), aggregate stability, and soil health compared to sole inorganic treatments, while supporting sustained crop productivity (Paramesh et al. 2023). Empirical trials in dryland maize production reveal that INM using crop residue plus fertilizer substantially increases SOC (by up to ~52%) and enhances water retention, thereby bolstering drought resilience and productivity (Wang et al. 2023). Precision fertilization through spatial diagnostics, remote sensing, and targeted nutrient placement further refines INM by aligning application timing and dosage with site-specific crop needs, minimizing nutrient losses (e.g., nitrate leaching, N_2O emissions), and enhancing fertilizer use efficiency (Bayu, 2020). Overall, the integrated application of INM and precision fertilisation offers a robust pathway for climate-smart agriculture, achieving dual goals of improved productivity and reduced environmental impact under changing climatic conditions.

Water management: mulching, micro-irrigation, and landscape retention

Effective water management strategies encompassing mulching, micro-irrigation, and landscape-level retention, play a critical role in enhancing agricultural resilience amid climate change. Mulching, whether organic (e.g., straw, gravel) or synthetic (e.g., plastic film), significantly reduces soil evaporation, moderates soil temperature, enhances moisture retention, suppresses weeds, and improves soil structure and fertility, thereby reducing irrigation demand and promoting water-use efficiency under arid and semi-arid conditions (El-Beltagi et al. 2022). Micro-irrigation systems, such as drip or trickle irrigation, deliver water precisely to the root zone, minimizing losses via evaporation or runoff; they enhance water-use efficiency, support deeper root development, and can be integrated with fertigation for optimized nutrient delivery (Bwire et al. 2024). The synergy between mulching and micro-irigation yields even greater benefits: combining mulching with drip systems can reduce water consumption by as much as 40–50%,

decrease irrigation needs by 30%, and substantially boost crop yield and water productivity in vegetable production. At the landscape or catchment scale, soil mulching and micro-irrigation can lower the blue water footprint, delivering water savings of millions of cubic meters per year in some basins (Nouri et al. 2019). Furthermore, strategic landscape water retention such as enhancing soil organic carbon can markedly buffer yield losses under drought, as demonstrated in modeling studies showing that a 1% increase in soil organic carbon reduced potato yield loss from 16.4% to 7% during a severe drought (Heinz et al. 2025). Collectively, these practices form an integrated adaptation and mitigation framework, reducing vulnerability to shifting precipitation and water stress, stabilizing crop outputs, and enhancing efficiency and sustainability of agricultural systems in a warming climate.

Agroforestry, diversification, and perennial integration

Agroforestry, diversification, and perennial integration constitute complementary mitigation–adaptation strategies that increase the resilience and sustainability of agricultural landscapes by strengthening ecological function, buffering climatic shocks, and enhancing long-term productivity. Agroforestry by integrating trees and shrubs with crops and/or livestock modulates microclimate, reduces erosion and surface runoff, improves water and nutrient retention, and stores carbon in woody biomass and soil, thereby delivering both adaptation and mitigation co-benefits at field and landscape scales (Ghanbari et al. 2025). Crop and system diversification (spatial, temporal and genetic diversification, intercropping and mixed farming) reduces yield and income volatility, spreads climate and pest/disease risk across species and time, and improves soil health and biological pest regulation, making farm livelihoods less sensitive to extreme weather and market shocks (Mihrete and Mihretu, 2025). Integrating perennial species whether as tree crops, forages, silvopasture, or newly domesticated perennial grains reduces the need for annual tillage, increases below-ground carbon allocation through deeper, longer-lived root systems, curbs nutrient leaching and erosion, and can improve drought resilience through more extensive water capture and use (Chapman et al. 2022). When combined in diversified, perennial-rich agroecosystems (for example agroforestry mosaics, perennial–annual polycultures, and mixed silvopasture), these approaches produce synergistic outcomes higher and more stable ecosystem services (carbon sequestration, water regulation, soil conservation), improved on-farm biodiversity, and reduced vulnerability of production systems to climate variability while also opening policy and market pathways for scaling adoption.

Biological and engineered soil amendments (microbial inoculants, engineered bio-products)

Biological and engineered soil amendments including microbial inoculants and cutting-edge bioengineered products offer promising pathways to bolster agricultural resilience amid climate change. Microbial inoculants, or plant growth–promoting microorganisms (PGPMs), improve soil health by enhancing nutrient cycling, increasing plant resilience to drought and salinity, and reducing reliance on synthetic fertilizers and pesticides a source of considerable greenhouse gas emissions (Díaz-Rodríguez et al. 2025). These inoculants help restore soil microbial communities degraded by intensive agriculture, enriching ecosystem functions crucial for climate mitigation and sustainable productivity (Campos-Avelar et al 2023). Furthermore, targeted applications of PGPMs in wheat under drought stress have demonstrated significant improvements in plant drought tolerance, biomass, and soil enzymatic activity highlighting their

practical effectiveness under abiotic stress (Li et al. 2023). In parallel, engineered bio-products such as biochar produced via pyrolysis of organic biomass provide multifunctional benefits: sequestering carbon over centuries, enhancing soil water-holding capacity and nutrient retention, increasing microbial activity, and reducing emissions of nitrous oxide and methane (Shyam et al. 2025). Emerging biochar–microbe synergistic systems have shown spectacular results; for example, in calcareous soils, combining biochar with auxin-producing microbial inoculants (and L-tryptophan) improved canola yield by over 200%, while amplifying nutrient uptake and soil microbial activity (Mustafa et al. 2025). These methodologies underscore a powerful suite of mitigation and adaptation strategies: they enhance soil function, improve plant stress tolerance, and help sequester carbon all critical in transitioning toward climate-smart agriculture.

Policy, incentives, and carbon programs

Policy frameworks, targeted incentives, and carbon programs form a crucial triad for catalyzing climate-resilient agricultural transformations. Effective policy instruments ranging from subsidies, taxes, rural finance, land tenure reforms, extension services, to certification schemes, can significantly enhance the adoption of climate-smart agricultural (CSA) practices among smallholder and large-scale farmers, by reducing financial risk and aligning actions with public goods objectives (Van Asseldonk et al. 2023). Carbon programs, including credits for soil carbon sequestration or regenerative practices, offer a direct financial reward mechanism for climate-positive farming. Yet program design matters greatly: recent reporting highlights that in the U.S., while programs like the USDA's EQIP and related climate-smart initiatives channel billions in incentives, over half the funding may not effectively reduce greenhouse gas emissions; implementation gaps likewise limit program efficacy (The Guardian, 2024). Similarly, corporate-led carbon credit initiatives, such as those by Indigo Ag, have supported farmers transitioning to regenerative agriculture by paying premiums for verified carbon credits, yet critics warn that delays in payments and inequitable cost-sharing can undermine farmer engagement (Reuters, 2024). Internationally, the European Union's Common Agricultural Policy (CAP) and eco-schemes offer adaptation and mitigation support via agri-environmental commitments and climate-related rural development funds, although recent critiques argue that policy alignment with climate goals remains inconsistent due to imprecise metrics and diluted incentives (European Commission, 2025). Collectively, these insights underscore that a blend of well-calibrated policies, robust incentive structures, and transparently governed carbon programs is essential for driving agricultural systems toward sustainability, resilience, and equitable climate mitigation.

FUTURE DIRECTIONS AND RESEARCH GAPS

Safeguarding soil health under climate change requires closing several scientific and implementation gaps. Below I synthesize the most important open questions highlighted in recent literature, then propose concrete research priorities to guide the next decade of work. Key thematic gaps are

6. Measurement, monitoring, reporting and verification (MRV) for soil carbon and other soil health indicators;
7. Mechanistic understanding of carbon stabilization and losses across depths and land-uses;
8. The soil microbiome as an actionable lever for resilience;
9. Scalable, location-specific practice packages and socio-economic pathways to adoption; and

10. Modelling and remote sensing tools for up-to-date, trustworthy decision support.

RECOMMENDATIONS

Safeguarding soil health in the era of climate change requires integrated approaches that bridge scientific research, land management, and socio-economic policy. Healthy soils underpin climate mitigation and adaptation by sequestering carbon, regulating water, and supporting biodiversity, yet they remain vulnerable to degradation from unsustainable practices, land-use change, and governance gaps. Embedding soil health explicitly into climate policies at national and international levels is therefore essential to meet long-term environmental and food security goals, as exemplified by initiatives such as the European Union's Green Deal.

At the same time, economic incentives play a pivotal role in aligning farmer and landowner decision-making with environmental objectives. Payment for ecosystem services, subsidies for agroecological practices, and programs that reward carbon sequestration can make sustainable land stewardship financially viable. Secure land tenure and robust governance frameworks further underpin these efforts, empowering communities to prioritize long-term soil health investments over short-term exploitation.

Moving forward, several priorities emerge for policymakers and practitioners. Developing standardized soil health indicators encompassing physical, chemical, and biological dimensions, will enable consistent monitoring and adaptive management across regions. Greater investment in research and farmer education is equally vital, fostering innovation and knowledge transfer that accelerate the adoption of resilient practices. Moreover, the promotion of sustainable agricultural systems, including crop rotation, cover cropping, reduced tillage, and organic farming, offers proven pathways to strengthen soil functions and buffer climate extremes. Finally, broad-based public awareness and inclusive stakeholder engagement are critical to ensure that soil health remains a shared societal priority.

In sum, advancing soil health requires more than agronomic interventions; it demands coherent policies, supportive institutions, and active engagement across scales. By aligning science, practice, and governance, soil can be secured as both a natural capital asset and a cornerstone of climate resilience in the decades ahead.

CONCLUSION

Soil health is a cornerstone of sustainable development and climate resilience. Effective policies that integrate soil conservation into broader environmental and economic frameworks are essential for safeguarding this vital resource. By implementing the recommendations outlined above, governments can foster an environment where soil health is prioritized, leading to enhanced agricultural productivity, environmental sustainability, and climate change mitigation.

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SHORT-TERM EFFECTS OF DIFFERENT ORGANIC FERTILIZERS ON SOIL CHEMICAL PROPERTIES AND PLANT GROWTH IN MELON AND WATERMELON CULTIVATION

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ABSTRACT

The integration of organic fertilizers into horticultural systems has gained increasing importance as a sustainable strategy to enhance soil fertility and crop productivity under challenging soil conditions. This study aimed to evaluate the effects of various organic fertilizers including manures from small ruminants, cattle, poultry, and vermicompost on the chemical properties of a sandy-calcareous soil and the growth performance of melon (*Cucumis melo L.*) and watermelon (*Citrullus lanatus Thunb.*). The study focused on how recommended fertilizer doses influence soil chemical quality and plant growth. The results indicated that organic fertilization altered soil electrical conductivity (EC) and available phosphorus (P) levels. Poultry and cattle manures led to notable improvements in soil nutrient content. Additionally, plant growth parameters such as stem diameter and fruit weight were positively affected by organic fertilizers, particularly poultry manure. Crop-specific responses were observed: melon plots exhibited higher soil EC and available P, while watermelon plants showed superior performance in stem diameter, fruit flesh thickness, and soluble solid content. In contrast, melon plants recorded higher chlorophyll levels. Overall, poultry manure emerged as the most effective input for enhancing plant growth, while vermicompost and cattle manure also contributed depending on the crop type. These results suggest that organic fertilizers, especially poultry manure, are valuable tools for improving soil fertility and crop productivity in calcareous soils.

Keywords: Calcareous soils, Fruit quality, Mediterranean climate, Organic amendments

INTRODUCTION

Sustainable agricultural systems are an emerging model that promotes the integrated use of organic materials as alternatives to chemical inputs are being developed to ensure more environmentally friendly and efficient food production. In this context, organic fertilizers that enhance soil fertility have come to the forefront. The core component of organic fertilizers, organic matter, serves as a primary source of energy and nutrients for soil-dwelling microorganisms. However, in Turkey and many other agricultural countries, inadequately matured animal manures characterized by high C/N ratios and the presence of root rot pathogens or weed seeds have long been used in crop production. These manures often fail to provide the expected agronomic benefits for both soil and plants (Karaman 2012).

In recent years, there has been a growing demand particularly in intensively cultivated horticultural and greenhouse production areas for thermally treated or fermented organic fertilizers. This trend reflects efforts to promote sustainable agriculture and to optimize the

combined use of organic and chemical fertilizers. Nevertheless, these processed fertilizers are significantly more expensive than traditionally matured manures, due to additional processing, packaging, registration, and storage costs. Furthermore, their claimed benefits for practical use often rely more on manufacturer assertions than on robust scientific evidence. Therefore, it is essential to evaluate their effects on soil fertility and plant growth in order to better understand their potential contribution to sustainable agricultural systems and to promote their effective and economical use.

Melon (*Cucumis melo* L.) and watermelon (*Citrullus lanatus* Thunb.), among the most widely produced and consumed vegetables globally and in Turkey, are annual crops adapted to warm and temperate climates. While both crops are cultivated in open fields and greenhouses, only about 3% of melon and 2% of watermelon production is conducted under protected cultivation (Anonymous 2001; Taşkaya and Keskin 2004). These crops are valued not only for their edible parts but also for their seeds and rinds, which are utilized across the food, pharmaceutical, and cosmetic industries, making them strategic export commodities. Deep, well-drained, sandy-loam or loamy-sand soils with high water-holding capacity are ideal for watermelon cultivation, while poorly drained soils with shallow groundwater levels (below 1 meter) are unsuitable (Tuna and Özer 2005). Like many other cultivated plants, melons and watermelons respond positively to organic fertilization. Accordingly, the application of solid and/or organic fertilizers in the cultivation of these crops is considered important for improving both yield and quality, as well as enhancing soil health (Bayram et al. 2020).

In this study, the effects of processed organic fertilizers including sheep, cattle, and poultry manures, as well as vermicompost on the chemical properties of calcareous sandy soils and on plant growth in melon and watermelon cultivation were investigated. Additionally, the study aimed to assess how the optimum application rates recommended by organic fertilizer manufacturers for plant growth influence soil fertility. By comparing the effects of these commonly used organic fertilizers on both soil chemical parameters and plant growth indicators, this study identified the strengths and weaknesses of each fertilizer type. The findings offer valuable insights into the practical use of organic fertilizers in sustainable farming systems.

MATERIAL AND METHODS

Site Description

This study was conducted as a field trial at the Finike campus of Akdeniz University (36°18′31″N, 30°08′48″E; altitude: 40 m). According to meteorological data collected during the trial period, the site had an average daily temperature of 19.3 °C, an average daily sunshine duration of 9.10 hours, an average daily open surface evaporation of 125.58 mm, an average daily rainfall of 1.4 mm, an average daily wind speed of 1.6 m/sec, and an average relative humidity of 66.03%.

Soil samples collected to represent the trial area were analyzed prior to the experiment. The soil was classified as sandy loam in texture. It had a neutral pH and was classified as non-saline. The soil also contained a high level of lime and was rated as sufficient in terms of humus content. Total nitrogen (N) and available phosphorus (P) levels were determined to be adequate, while the cation exchange capacity (CEC) was classified as low.

Organic Fertilizers and Fertilization Practices

In this trial, various heat-treated organic fertilizers (processed at 65–70 °C) were applied, including small ruminant manure (goat and sheep manure; T1), cattle manure (T5), poultry manure (T4), and vermicompost (T2) (Table 1). Additionally, control (T3) plots were established, which received only chemical fertilizers (detailed below) without any organic fertilizer input. The application rates of the organic fertilizers were determined based on the manufacturers' recommendations and supported by findings from previous studies (Maltaş 2023; Maltaş et al. 2024). This approach aims to develop practical organic fertilization strategies suitable for field conditions, specifically for melon and watermelon cultivation, while improving soil chemical properties and supporting plant growth.

Table 1. Some properties of organic fertilizers used in the experiment

Parameter	Organic fertilizer			
	Vermicompost	Chicken manure	Cow manure	Goat-sheep manure
Organic matter, %	45	62	35	70
EC (dS/m)	6.0	2.6	11.2	7.5
Organic C, %	26.1	35.96	20.3	40.6
Humic/fulvic acid, %	41.5	36	15	28
Total N, %	3.0	2.2	1	1.5
Total P, %	1.5	1.76	1.5	1.5
Total K, %	1.0	1.62	-	2
Humidity max., %	20	20	20	20
pH	6-8	6.2	7-9	8-10
C/N	9/1	16/1	20/1	27/1
C/P	17/1	20/1	13/1	27/1

The application rates were as follows: 4 Mg ha^{-1} for vermicompost, 8 Mg ha^{-1} for cattle manure, 6 Mg ha^{-1} for goat manure, and 4 Mg ha^{-1} for poultry manure. In addition to these inputs, chemical fertilizers certified for use in organic farming were applied through a drip irrigation system to support plant nutrition throughout the experimental period. These chemical inputs were selected based on the initial soil analysis and estimates of nutrient removal by the crops (Zengin 2023).

Previous studies have demonstrated that the use of organic mulching materials (such as straw, sawdust, and compost) can improve microclimatic conditions and enhance the effectiveness of organic fertilizers (Gao et al. 2019; Teasdale and Mohler 2000). Mulching has been shown to promote soil biological activity, thereby improving nutrient uptake efficiency by plants and contributing to sustainable farming practices (Li et al. 2019). Therefore, in this study, a mulch was applied to the experimental plots to maximize the effectiveness of organic fertilizers.

Implementation of the Experiment

In this study, melon and watermelon cultivation was carried out using four types of heat-treated organic fertilizers. The experimental setup was established according to a randomized block design with three replications. The organic fertilizers were applied uniformly to the plots based on their respective application rates (in dry weight).

Melon (*Cucumis melo L.*) and watermelon (*Citrullus lanatus Thunb.*) seedlings were procured from a commercial nursery. Melon seedlings (Anka F1) were transplanted at a spacing of 2.0 m between rows and 0.3 m within rows, while watermelon seedlings (Peykan F1) were

planted at 2.0 m between rows and 0.6 m within rows. Both crops were cultivated over a vegetation period of approximately 90–100 days.

Throughout the growing period, all essential cultural practices such as weed control, hoeing, and plant protection treatments were carried out. In addition, irrigation and fertigation were performed using a drip irrigation system. The daily irrigation requirements of the plants were calculated based on climatic parameters (including rainfall, temperature, and relative humidity), soil texture and moisture, and plant needs (e.g., signs of wilting). These water requirements were integrated with the optimized fertilization strategy to ensure proper crop management.

Sampling and Analysis

Soil samples were air-dried and sieved through a 2 mm mesh prior to analysis. To characterize the initial soil conditions, samples were collected from the 0–30 cm soil depth using an auger before the experiment began. These samples were analyzed for texture, pH, electrical conductivity (EC), lime content, organic matter, total nitrogen (N), available phosphorus (P), and cation exchange capacity (CEC). Additionally, to evaluate the effects of organic fertilizer applications on soil chemical properties, samples collected at harvest were analyzed again for organic matter, total nitrogen, available phosphorus, and CEC.

The methods used for analyzing the above-mentioned soil parameters were as follows: soil texture was determined using the hydrometer method (Bouyoucos 1955); pH and EC were measured in a 1:2.5 soil-to-water suspension (Jackson 1967; Anonymous 1982); lime content was determined using the calcimeter method (Çağlar 1949); organic matter was measured using the modified Walkley-Black method (Black 1965); total nitrogen was determined using the Kjeldahl method (Kacar 1995); available phosphorus was analyzed according to the Olsen method (Olsen and Sommers 1982); and CEC was determined using the ammonium acetate method (Kacar 1995).

At the end of the experiment, pomological and physiological traits were assessed in plant and fruit samples (Figure 1 and Figure 2). For each treatment replication, measurements and evaluations were performed on three randomly selected plants per plot. The results were expressed as the average of these three plants.

Figure 1. Views of selected physiological and pomological traits in watermelon plants (A: Soluble solids content, B: Chlorophyll content, C: Fruit flesh thickness, D: Stem base diameter, E: Fruit juice pH, F: Fruit weight)

Figure 2. Views of selected physiological and pomological traits in watermelon plants (A, C: Harvested fruits; B: Fruit juice pH)

The evaluated pomological characteristics included: stem base diameter (cm; measured with a caliper), fruit fresh weight (g; measured with a precision balance), and yield (kg da^{-1}; calculated). The physiological parameters included: soluble solids content (SSC), fruit juice pH, total chlorophyll content (measured with a chlorophyll meter), and rind thickness (mm; measured with a caliper).

Statistical Analyses

The numerical data obtained in this study were statistically analyzed using the SPSS 23.0 software package. The significance levels of soil chemical properties and plant pomological and physiological parameters were determined through analysis of variance (ANOVA) at the 5% significance level. Parameters showing statistically significant differences were further evaluated using Duncan's multiple range test for mean separation. In addition, Pearson correlation analysis was performed to determine the relationships among the measured parameters (SPSS 2008).

RESULTS AND DISCUSSION

Soil Chemical Properties

At the end of the growing season, the effects of different organic fertilizer treatments on soil cation exchange capacity (CEC), organic matter (OM) content, total nitrogen (N%), and available phosphorus (P, mg/kg) were evaluated for watermelon cultivation (Table 2). According to the statistical analysis, changes in CEC, total N, OM (%), and available P values in watermelon plots were found to be statistically non-significant.

Table 2. Effects of applications on CEC, OM, total N and available P contents in watermelon soil

Treatments	CEC (meq/100)	% OM	% N	P (mg/kg)
T1	26.54	2.26	0.16	22.17
T2	29.33	2	0.15	19.47
T3	31.04	2.1	0.16	13.36
T4	28.11	2.17	0.18	18.52
T5	30.58	1.92	0.18	38.54
ANOVA (%5)	NS[1]	NS	NS	NS

[1] NS; not significant.

In contrast, in melon cultivation plots, although changes in CEC, total N, and OM (%) were also statistically non-significant, the variation in available phosphorus content was statistically significant at the $P < 0.05$ level (Table 3). Among the treatments, T4 (48.60 mg/kg) resulted in the highest increase in available phosphorus content compared to the other applications.

Table 3. Effects of applications on CEC, OM, total N and available P contents in melon soil

Treatments	CEC (meq/100)	% OM	% N	P (mg/kg)
T1	32.82	2.29	0.15	13.31 b[1]
T2	30.99	2.26	0.16	23.72 ab
T3	30.10	1.85	0.14	9.36 b
T4	29.32	1.97	0.13	48.60 a
T5	29.68	2.00	0.17	40.04 ab
ANOVA (%5)	NS	NS	NS[3]	2.966 **[2]

[1] Differences between values not indicated by the same letter are significant at the 5% level. [2] **; significant at the 1% level. [3] NS; not significant.

Previous studies have indicated that increasing doses of poultry manure significantly contribute to the enhancement of soil nitrogen content (Okur et al. 2008), while farmyard manure has been found to have a more pronounced effect on available phosphorus levels in soils (Agbenin and Goladi 1997). In the present study, the available phosphorus level in soils under melon cultivation (T4: 48.60 mg/kg) was approximately 20% higher than that in watermelon soils.

Göksu and Kuzucu (2017), in their study investigating the effects of different vermicompost doses on yield and quality parameters in watermelon, reported that vermicompost and chemical fertilizer applications increased the soil ion exchange capacity. However, in the current study, organic fertilizer treatments did not result in statistically significant changes in CEC for either melon or watermelon soils.

Similarly, Kılıç and Sönmez (2019), in their study on the effects of increasing doses of various organic materials (vermicompost, poultry manure, farmyard manure, leonardite) on soil fertility parameters in lettuce cultivation, found that the effects on soil organic matter content were statistically significant. In contrast, the findings of the current study showed that different organic matter applications had no statistically significant effect on soil organic matter levels in either melon or watermelon cultivation.

Plant Growth Properties

At the end of the cultivation period, among the yield and quality parameters obtained from the watermelon plant, leaf chlorophyll content (SPAD), fruit flesh thickness, fruit juice pH, and soluble solid content were found to be statistically non-significant (Table 4). However, collar

diameter was found to be statistically significant at the $P < 0.01$ level, with higher values observed in T1 and T4 (23.41 mm and 23.23 mm, respectively) compared to other treatments. Fruit weight was also found to be statistically significant at the $P < 0.05$ level, with higher fruit weights recorded in T2, T4, and T5 (7.38 kg, 8.13 kg, and 7.52 kg, respectively).

Table 4. Effects of treatments on SPAD, root collar diameter (RCD), fruit weight (FW), fruit flesh thickness (FFT), fruit juice pH (FJpH) and soluble solid content (SSC) in watermelon

Treatments	SPAD	RCA (mm)	FW (kg)	FFT (cm)	FJpH	SSC
T1	37.13	23.41 a[1]	6.99 ab	16.13	5.34	8.38
T2	38.99	17.74 c	7.38 a	15.53	5.60	9.26
T3	34.48	15.95 c	5.50 b	17.55	5.70	8.43
T4	34.71	23.23 a	8.13 a	14.29	5.49	8.70
T5	31.01	20.08 b	7.52 a	10.31	5.46	8.71
ANOVA (%5)	NS[4]	21.282 ***[3]	3.575**[2]	NS	NS	NS

[1] Differences between values not indicated by the same letter are significant at the 5% level. [2] **; significant at the 1% level. [3] ***; significant at the 0.1% level. [4] NS; not significant.

Regarding the melon plant, leaf chlorophyll content (SPAD), fruit flesh thickness, fruit juice pH, and soluble solid content were also found to be statistically non-significant (Table 5). However, root collar diameter was found to be statistically significant at the $P < 0.05$ level, with higher values observed in T1, T3, and T4 (14.67 mm, 14.83 mm, and 14.85 mm, respectively). Fruit weight was found to be statistically significant at the $P < 0.01$ level, with the highest fruit weight observed in T1 (2.02 kg).

Table 5. Effects of treatments on SPAD, root collar diameter (RCD), fruit weight (FW), fruit flesh thickness (FFT), fruit juice pH (FJpH) and soluble solid content (SSC) in melon

Treatments	SPAD	RCA (mm)	FW (kg)	FFT (cm)	FJpH	SSC
T1	42.53	14.67 ab[1]	2.02 a	9.78	5.93	4.80
T2	36.74	13.22 b	1.20 b	9.12	5.79	4.13
T3	48.32	14.83 ab	1.42 b	11.65	5.60	4.13
T4	44.78	16.85 ab	0.95 b	9.98	5.85	4.37
T5	40.28	16.15 a	1.45 b	10.97	5.86	3.97
ANOVA (%5)	NS	1.701 **[2]	5.448 ***[3]	NS[4]	NS	NS

[1] Differences between values not indicated by the same letter are significant at the 5% level. [2] **; significant at the 1% level. [3] ***; significant at the 0.1% level. [4] NS; not significant.

Üçok et al. (2019), in their study investigating the effects of different organic fertilizer applications on yield, quality, and plant nutrient contents in lettuce (*Lactuca sativa* L. var. crispa), reported no significant differences in chlorophyll content among the treatments. Similarly, in our study, although the differences between applications were not statistically significant for both watermelon and melon plants, the chlorophyll value in melon was found to be approximately 20% higher than in watermelon.

Özen (2018) investigated the effects of different doses of mushroom compost, leonardite, and vermicompost on lettuce yield and quality, and reported that increasing doses of organic material applications resulted in increased collar diameter. In a study conducted on watermelon (*Citrullus lanatus* (Thunb.) Matsum & Nakai), it was reported that different doses of vermicompost (0, 300, and 600 kg/da) did not have statistically significant effects on fruit flesh thickness, fruit juice pH, and soluble solid content (Göksu and Kuzucu 2017). Similarly, in our study, although the differences between treatments were not statistically significant in both watermelon and melon, the collar diameter, fruit flesh thickness, and soluble solid content in watermelon were found to be 40%, 40%, and 100% higher, respectively, compared to melon. In addition, the fruit weight of watermelon was found to be six times higher than that of melon. One of the main reasons for this large difference is the physiologically larger fruit size of watermelon compared to melon.

Correlations Between Soil Properties and Plant Growth

Based on the numerical data obtained from the trial, relationships between soil chemical properties and plant growth parameters were examined. Accordingly, in melon soils, a negative correlation was observed between fruit flesh thickness and organic matter content (r = -0.539*), while a positive correlation was found between root collar diameter and organic matter content (r = 0.728**). These correlations suggest that soil organic matter may play a dual role in influencing plant morphological traits. While the positive correlation between root collar diameter and organic matter content implies a supportive role of soil organic matter in promoting vegetative growth and stem development, the negative correlation between fruit flesh thickness and organic matter content might indicate a potential trade-off between vegetative and reproductive growth processes under certain soil conditions. This could be due to nutrient partitioning or changes in water-holding capacity and aeration associated with increased organic matter. However, these relationships are likely to be influenced by plant species, growth stage, and environmental factors.

CONCLUSION

This study aimed to evaluate the effects of various organic fertilizers including manures from small ruminants, cattle, poultry, and vermicompost on the chemical properties of a sandy-calcareous soil and the growth performance of melon (*Cucumis melo* L.) and watermelon (*Citrullus lanatus* Thunb.). The study focused on how recommended fertilizer doses influence soil chemical quality and plant growth. Poultry and cattle manures stood out in improving soil chemical properties in watermelon cultivation areas, whereas vermicompost and poultry manure significantly enhanced plant growth parameters. For melon cultivation, cattle manure was most effective for soil chemical improvements, while small ruminant, poultry, and cattle manures showed prominent positive effects on plant development. Overall, poultry manure emerged as the most effective input for enhancing plant growth, while vermicompost and cattle manure also contributed depending on the crop type. These results suggest that organic fertilizers, especially poultry manure, are valuable tools for improving soil fertility and crop productivity in calcareous soils. Nonetheless, expanding these findings through comprehensive studies involving different crops, soil types, cultivation locations, and production systems (e.g., greenhouse or organic farming) will be essential to deepen understanding and broaden the applicability of these organic fertilization strategies. These results underscore the critical role of optimized organic fertilization in promoting sustainable agricultural productivity, supporting both soil health and crop performance in Mediterranean agroecosystems.

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CYTOTOXIC ACTIVITIES OF DIFFERENT EXTRACTS OF *INONOTUS TAMARICIS* (Pat.) *MAIRE* FROM SOUTHWEST ANATOLIA, TÜRKİYE

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ABSTRACT

Cancer is a disease caused by uncontrolled cell proliferation and abnormal cells that gain the ability to spread to surrounding tissues. According to the 2025 Cancer Statistics, prostate, lung and colorectal cancers are the most common cancers in men, while breast, lung and colorectal cancers are the most common in women, with prostate and breast cancers alone having the highest rates.

In recent years, the increase in the incidence of cancer and its tendency to be seen at young ages has attracted attention, while healthy nutrition and natural bioactive compounds, especially medicinal mushrooms (such as *Inonotus obliquus*, *Schizophyllum commune*, *Trametes versicolor*, *Grifola frondosa*, *Ganoderma lucidum*, *Lentinula edodes*, *Laetiporus sulphureus*, *Boletus edulis*) have significant potential in the field of cancer prevention and supportive therapy.

In this study, it was aimed to investigate the cytotoxic activity of different extracts of *Inonotus tamaricis* (Pat.) Maire from Southwest Anatolia, Türkiye, against lung and colon cancer cell lines (A549 and HT-29) and healthy fibroblast cell line (CCd18Co). This mushroom is naturally distributed in south west anatolia, non-toxic, consumed as tea and has commercial potential. Extractions of the fungus (hexane, ethyl acetate, methanol and water) were carried out according to increasing polarity respectively, then the cytotoxic activities of the extracts were investigated using MTT method. As a result, ethyl acetate extract of *I. tamaricis* showed highly selective cytotoxic activity against HT-29 colon cancer cells (IC_{50}: 25.02 µg/mL). In A549 lung cancer cells, IC_{50} was determined as 150.81 µg/mL. Hexane and water extracts were moderately effective in HT-29 (IC_{50} ~100 µg/mL), while methanol extract showed weak activity. All extracts showed low toxicity in healthy fibroblast cells (IC_{50} >100 µg/mL). *I. tamaricis* shows a promise as a source of bioactive compounds and detailed phytochemical investigations are needed. Future studies on this species may reveal new bioactive metabolites with pharmaceutical potential, especially in colon cancer.

Keywords: *Inonotus tamaricis*, mushroom, cytotoxic activities, MTT, lung cancer, colon cancer

INTRODUCTION

In recent years, there has been a significant increase in the incidence of cancer worldwide and the average age of patients is getting younger. This trend poses serious risks to the general health status and treatment processes of cancer patients (Górska-Jakubowska et al., 2025). In 2025, the most commonly diagnosed cancers in men are prostate cancer (30%), lung cancer (11%) and colon & rectal cancer (8%), accounting for around 48% of all new cases, with prostate cancer alone accounting for 30% of cases. In women, breast cancer (32%), lung cancer (12%) and colon &

rectum cancer (8%) accounted for 51% of all new diagnoses, with breast cancer alone accounting for 32% of cases (Siegel et al., 2025).

Fortunately mushrooms contain polysaccharides (especially *β*-glucans), triterpenoids and phenolic compounds and wood mushrooms show protective effects against cancer by mechanisms such as strengthening the immune system, reducing oxidative stress and regulating the cell cycle (Deveci et al., 2019; Zhao et al., 2021; Meng et al., 2022; Korkmaz et al., 2024;). The anticancer, antioxidant, antitumor, antiviral, antibacterial, antibacterial, antifungal, anti-inflammatory, immunomodulatory activities and cholesterol-lowering effects of lectins, polysaccharides, polysaccharide-peptides, polysaccharide-protein complexes, lanostene-type triterpenoids, phenolic and flavanoid-structured compounds obtained from edible mushroom species have been revealed (Duru and Çayan, 2015; Maity et al., 2021; Lowenthal et al., 2023; Şavkıncı et al., 2024). Recent studies support the anticancer potential of species such as *Ganoderma lucidum* (Reishi), *Trametes versicolor* (Turkey tail) and *Inonotus obliquus* (Chaga) (Tel-Çayan et al., 2015; Camilleri et al., 2024; Górska-Jakubowska et al., 2025; Ikram et al., 2025).

Türkiye, has a very important geographical position in terms of species richness of macromycetes. *I. tamaricis* belongs to the Hymenochaetaceae family (Sesli et al., 2020). In this present study, *I. tamaricis*, which is naturally distributed in our country, consumed as tea and has commercial potential, was extracted with increasing polarity (hexane, ethyl acetate, methanol and water) and cytotoxic activities of the extracts on lung (A549) and colon cancer (HT-29) cell lines as well as normal colon cell line (CCd18Co) were investigated using MTT ((3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method.

MATERIAL AND METHOD

1. Mushroom Material

I. tamaricis was collected from *Tamarix parviflora* DC species in this natural habitat in Datça, Türkiye. The collected samples were taxonomically identified using the identification keys provided by Piątek (2001), Ghobad-Nejhad and Kotiranta (2008), Sharma et al. (2013), and the updated List of Fungi of Turkey (Sesli et al., 2020). A voucher specimen was deposited in the Natural Products Laboratory of Muğla Sıtkı Koçman University under the code ME2305. Macroscopic images of *I. tamaricis* is shown in Figure 1.

Figure 1. Macroscopic views of *I. tamaricis*

2. Mushroom Extract

A total of 1000 grams of dried *I. tamaricis* material was subjected to solvent extraction at room temperature using a sequential method with increasing solvent polarity: hexane, ethyl acetate, methanol, and water. Each extraction was performed three times (24 h × 3). After filtration, solvents were evaporated under reduced pressure using a rotary evaporator. The masses and yields of each extract are listed in Table 1.

Table 1. Quantities and yields of *I. tamaricis* extracts.

Mushroom	Extract	Code	Quantities (g)	% Yields
I. tamaricis	Hexane	ICH	1.95	0.20
	Ethyl acetate	ICE	20.33	2.03
	Methanol	ICM	12.95	1.30
	Water	ICS	9.94	0.99

3. Cytotoxic Activity

The cytotoxic effects of *I. tamaricis* extracts (hexane, ethyl acetate, methanol and water) were evaluated against two human cancer cell lines A549 (lung cancer cell line) and HT-29 (colon cancer cell line) as well as a non-tumorigenic normal human colon fibroblast cell line (CCd18-Co). Cytotoxicity was assessed using the MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), according to the method described by Plumb (2003) and Korkmaz et al (2024). The IC_{50} values (± standard deviation) for each extract were calculated for the 72-hour treatment period to assess the cytotoxic potential against cancer cell lines and normal cell lines.

4. Statistical Analysis

For cytotoxic activity, statistical analysis was performed using GraphPad Prism version 8, including one-way ANOVA followed by Tukey's multiple comparison tests. p value < 0.05 was considered statistically significant. The IC_{50} values were determined from regression curves generated from the percentages of inhibition or absorbance at different concentrations, as previously described.

RESULTS AND DISCUSSION

The cytotoxic effects of *I. tamaricis* extracts (hexane, ethyl acetate, methanol and water) on A549 and HT-29 cancer cell lines and healthy human cell line CCD18Co were determined after 72 hours treatment based on IC_{50} values. The cytotoxic activity results of the extracts are given in Table 2.

Table 2. Antioxidant Activity Results of *I. cuticularis* Extracts ($p<0.05$).

Extracts/ Referance	Cancer Cell Lines		Human Healthy Cell Line
	A549	HT-29	CCD18-Co
		IC_{50}(µg/mL)	
Hexane	>200	100.02± 0.03*	>100
Ethyl acetate	150.81± 0.03	25.02± 0.02*	180.85±0.05
Methanol	>200	147.25± 0.04	>200
Water	172.53± 0.02	100.03± 0.07*	>100
Referance			

Cisplatin	4.28±0.05	4.05± 0.04	NT

*Activity results expressed as mean ± standard deviation (SD) of 3 parallel tests ($p<0.05$)

As shown in Table 2, at the end of the 72-hour test period, *I. tamaricis* ethyl acetate extract showed the highest cytotoxic activity in HT-29 (IC_{50}: 25.02± 0.02 µg/mL) and A549 cell lines (IC_{50}: 150.81± 0.03 µg/mL) compared to other extracts (hexane, methanol and water). Because IC_{50} value (180.85µg/ml) of ethyl acetate for normal cell line CCD18-Co is higher than those of A549 and HT-29, it can be concluded that ethy acetate exyracts of *I tamaricis* is selective for cancer cell lines.

The hexane and water extracts of *I. tamaricis* showed moderate cytotoxic effect on HT-29 cancer cell line (IC_{50}: 100.02± 0.03 and IC_{50}: 100.03± 0.07µg/mL, respectively) compared to methanol extract (IC_{50}: 147.25± 0.04 µg/mL). In contrast, the toxicity of all *I. tamaricis* extracts on healthy human fibroblast cell line was IC_{50}>100.

The main active components of polysaccharide (anticancer, anti-inflammatory, antiviral, antioxidant, immunomodulatory, hypoglycemic, hypolipidemic, hepatoprotective), polyphenol (antioxidant), triterpenoid (anticancer, anti-inflammatory, antiviral) and melanin (antioxidant, anti-inflammatory, antiviral) isolated from the tree fungus *Inonotus obliquus* (Hymenochaetaceae) have been reported to be the source of increasing pharmacological research over the last two decades (Lu et al. , 2021; Camilleri et al., 2024; Ikram et al., 2025). In the study by Lee et al. (2021), substance found in Chaga (*I. obliquus*) called ergosterol peroxide works as an antiproliferative agent and prevents colony formation in colon cancer cell lines. By inhibiting the -catenin pathway in colorectal cancer, it raises the possibility that it can be used to treat the disease (Camilleri et al., 2024).

We believe that in the future, the extraction, purification and identification techniques of bioactive compounds from edible and medicinal fungi will be improved; the mechanism of anticancer effects (especially targeted studies in colon cancer) of these species will be more comprehensively understood and will find wider use in pharmaceutical applications.

CONCLUSIONS

In conclusion, ethyl acetate extract of *I. tamaricis* exhibited higher cytotoxic activity against cancer cell lines compared to the other extracts examined in this study. Therefore, ethyl acetate extract of this macrofungi requires extensive research on the characterization and purification of their bioactive metabolites, potentially opening new windows in mycochemistry, natural discovery and medicine.

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CURRENT STATUS AND POTENTIAL APPLICATION OF MICROALGAE PRODUCTION

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ABSTRACT

Microalgae production has garnered significant attention across various industries due to its diverse applications and potential benefits. This study explores the current status and potential applications of microalgae production, focusing on its role in biodiesel production, cosmetics, thalassotherapy, bioremediation, and feed materials. The high lipid content of certain microalgae species makes them a promising source for sustainable biofuels, while their utilization in cosmetics and thalassotherapy underscores the value of microalgae-derived compounds in high-value products. Microalgae are also being explored for bioremediation purposes, particularly in treating textile wastewater, showcasing their versatility in environmental applications. Genetic engineering strategies are being developed to enhance biofuel production by increasing lipid accumulation in microalgae strains.

Keywords: Microalgae, feed, food, biofuel, treatment, waste

INTRODUCTION

Microalgae production has gained significant attention due to its diverse applications and potential benefits across various industries, including renewable energy, biopharmaceuticals, and nutraceuticals. The potential of microalgae production extends to biofuels, feed, food products, and other valuable compounds. Researchers have highlighted the rich energy content, rapid growth rate, cost-effective cultivation methods, and environmental benefits of microalgae, positioning them as a promising feedstock for biofuel generation. The current status of microalgae production is characterized by ongoing research and technological advancements aimed at maximizing the efficiency and scalability of microalgae cultivation. One prominent application of microalgae lies in biodiesel production, where the high lipid content of certain microalgae species presents a promising source for sustainable biofuels (Wu et al., 2012). Beyond biofuels, microalgae are being explored for bioremediation purposes, particularly in treating textile wastewater, showcasing the versatility of microalgae in environmental applications (Andrade & Andrade, 2018). The potential of microalgae extends to genetic engineering for enhanced biofuel production, with metabolic engineering strategies being developed to increase lipid accumulation in microalgae strains (Banerjee et al., 2016). Moreover, the biotechnological applications of microalgae have expanded to include the production of cosmetics, health products, fertilizers, feeds, and food, emphasizing the versatility of microalgae biomass in various industries (Ugya & Meguellati, 2022). Additionally, the utilization of microalgae in cosmetics and thalassotherapy has gained traction, highlighting the value of microalgae-derived compounds in high-value products (Mourelle et al., 2017).

The biotechnological potential of native microalgae from diverse ecosystems, such as the Peruvian Amazon, underscores the growing relevance of microalgae biotechnology in harnessing

unique strains for specific applications (Cobos et al., 2020). Furthermore, the incorporation of microalgae into innovative food products and the extraction of valuable compounds like phytosterols for functional food and pharmaceutical applications highlight the growing interest in utilizing microalgae for health and nutrition purposes (Caporgno & Mathys, 2018; Luo et al., 2015). The development of low-cost and high-volume open microalgae biofuel production technologies signifies progress towards commercial-scale microalgae biofuel production, addressing the need for sustainable and renewable energy sources (Moheimani et al., 2013). Additionally, the genetic manipulation of microalgae for bioproducts and enhancing microalgae traits demonstrate the continuous advancements in biotechnology for optimizing microalgae-based applications (Vázquez-Villegas et al., 2018; Shin et al., 2016).

In conclusion, the current status and potential applications of microalgae production encompass a wide range of industries, from biofuels and cosmetics to bioremediation and feed products. The ongoing research and technological innovations in microalgae cultivation and genetic engineering are paving the way for a sustainable and versatile bio-based economy with microalgae at its forefront.

Curent status of Microalgae Production

Potential Applications

Food and feed material

Microalgae production is recognized for its potential as a sustainable and versatile source of food and feed materials, offering a wide array of nutritional and functional benefits. Microalgae have historically been consumed by humans for their nutritional value and medicinal properties (Sathasivam et al., 2019).

Studies have demonstrated the feasibility of large-scale cultivation of microalgae for feed purposes, particularly in livestock and poultry production (Saadaoui et al., 2021). In the realm of food production, microalgae present a sustainable alternative as feed material for monogastric animals, providing a viable option to supplement or replace traditional feedstuffs like corn and soybean (Chaves et al., 2021). The nutritional composition of microalgae, which includes proteins, polyunsaturated fatty acids, antioxidants, and phytosterols, positions them as promising sources for food and feed applications (Sui & Vlaeminck, 2020). Additionally, the bioactive lipids found in microalgae have applications not only in aquaculture feed but also in the food and pharmaceutical industries, showcasing the versatility of microalgae-derived compounds (Luo et al., 2015).

Microalgae play a crucial role in aquaculture by serving as essential sources of polyunsaturated fatty acids for aquatic species (Soto-Sánchez et al., 2023). Their high nutritional value makes them suitable food sources for larval bivalves, shrimps, and fish, emphasizing their significance in aquaculture feed formulations (Ma, 2023). Furthermore, the industrial utilization of microalgae biomass includes the production of feeds, underscoring their importance in the food chain (Ugya & Meguellati, 2022). The nutritional composition and functional properties of microalgae proteins have been well-established, positioning them as a simple and effective protein source for food and feed applications (Hashim et al., 2023). Commercially, microalgae are already integrated into various food products, demonstrating their potential as versatile ingredients with nutritional benefits (Igual et al., 2021). The expanding industrial applications of microalgae encompass food, nutraceuticals, pharmaceuticals, animal feed, cosmetics, and biofertilizers, showcasing the diverse opportunities presented by these microorganisms (Udayan et al., 2021).

Microalgae production holds vast promise as food and feed material, providing sustainable and nutrient-rich alternatives for various industries. The nutritional value, bioactive compounds, and functional properties of microalgae make them valuable resources for addressing food scarcity, ensuring adequate nutrition, and promoting sustainable living practices.

Pharmaceutical and cosmetic industries

The pharmaceutical sector is increasingly exploring the therapeutic potential of microalgae-derived compounds, such as antioxidants, anti-inflammatory agents, antimicrobials, and anti-cancer agents, highlighting their pivotal role in biomedical applications (Khavari et al. 2021). These bioactive metabolites from microalgae hold significant promise for developing novel pharmaceutical products due to their diverse pharmacological activities (Kaur et al., 2023). In the cosmetic industry, microalgae have gained attention for their cosmetic and cosmeceutical applications, with compounds like astaxanthin being utilized as pigments in cosmetics and as active ingredients in skincare products (Villaró et al., 2021). The bioactive compounds present in microalgae, including pigments, vitamins, and minerals, offer a natural and sustainable source for formulating cosmetic products, catering to the increasing consumer demand for natural and eco-friendly alternatives (Dussably et al., 2022). Moreover, marine microalgae and cyanobacteria have been identified for their potential use in cosmetics and thalassotherapy, underscoring their significance in the cosmetic industry (Mourelle et al., 2017). The pharmaceutical and cosmetic industries are increasingly turning to microalgae as a valuable resource for developing high-value products. Microalgae's ability to produce a wide range of bioactive compounds, such as carotenoids, phytosterols, and fatty acids, positions them as versatile sources for pharmaceutical and cosmetic applications (Balasubramaniam et al., 2021). The bioactive metabolites synthesized by microalgae have demonstrated antioxidant, anti-inflammatory, and anti-microbial properties, making them attractive candidates for pharmaceutical formulations and cosmetic products (Morais et al., 2015). Furthermore, the sustainable production of microalgae biomass offers a cost-effective and environmentally friendly approach to sourcing bioactive compounds for pharmaceuticals and cosmetics (Jeevanandam et al., 2020). The potential of microalgae in producing value-added lipids, proteins, and other bioactive molecules presents opportunities for developing innovative pharmaceutical and cosmetic formulations (Conde et al., 2021). The bioprospecting of microalgae for biopharmaceutical applications underscores the growing interest in harnessing the therapeutic potentials of microalgae in the pharmaceutical industry (Saeed et al., 2021). In conclusion, microalgae production holds immense promise for the pharmaceutical and cosmetic industries, offering a sustainable and bioactive-rich source for developing a wide range of products. The diverse bioactive compounds synthesized by microalgae present opportunities for formulating novel pharmaceutical drugs, nutraceuticals, and cosmetic products, catering to the evolving needs of these industries.

Biodiesel production

Microalgae production has attracted significant interest as a sustainable and efficient feedstock for biodiesel production, offering a promising solution to the global energy demand and environmental concerns. The potential applications of microalgae in biodiesel production have been extensively studied, highlighting their viability as a renewable energy source (Ajala et al. 2022). Microalgae possess characteristics that make them ideal for sustainable biodiesel production, including high lipid content and the ability to be genetically optimized for enhanced lipid accumulation (Mohamed et al., 2022).

The utilization of microalgae for biodiesel production involves various advanced technologies and influencing parameters that contribute to maximizing the efficiency of lipid extraction and conversion processes (Cao et al., 2013). The unique properties of microalgae, such as their ability to effectively remove nutrients and heavy metals from wastewater, further enhance their appeal as a biodiesel feedstock (Cobos et al., 2020). Additionally, the direct conversion of lipids within microalgae cells into biodiesel through in situ transesterification has emerged as an effective and simplified approach in biodiesel production (Hidalgo et al., 2015).

The potential of microalgae for biodiesel production extends to their high lipid content, which allows for the generation of saturated and monounsaturated fatty acids ideal for biodiesel

synthesis (Mohmmed et al., 2022). The sustainable production of biodiesel from microalgae offers advantages over traditional plant crops, as microalgae can accumulate significant quantities of triglycerides and free fatty acids, making them a valuable resource for biofuel production (Andrade & Andrade, 2018). Furthermore, the joint production of biodiesel and bioethanol from microalgae biomass presents a cost-effective and efficient approach to biofuel production (Sendzikiene & Makareviciene, 2022). The biodiesel potential of microalgae is further underscored by their ability to grow in the presence of sunlight and carbon dioxide, making them excellent sources of biofuels after processing (Ma et al., 2014). The differential fatty acid profiles of microalgae grown with organic materials highlight the versatility of microalgae in biodiesel production and wastewater treatment applications. The integration of oleaginous microalgae cultivation with wastewater treatment is considered a low-cost and sustainable approach for producing algae-based biodiesel.

Biomethane and biogas production

Microalgae production has emerged as a promising avenue for biomethane and biogas production, offering sustainable solutions for renewable energy generation and waste management. The potential applications of microalgae in biomethane and biogas production have been extensively explored, showcasing their versatility as a renewable energy feedstock (Poggio et al. 2023). Microalgae possess unique characteristics that make them ideal for biomethane and biogas production, including their ability to capture CO_2 and convert it into valuable methane gas (Prandini et al., 2016). The integration of microalgae-based wastewater treatment with biogas purification accelerates nutrient removal while producing valuable biomass and biomethane, highlighting the dual benefits of microalgae in waste management and energy production (Ahmad et al., 2014). Furthermore, co-digestion of microalgae with organic waste streams, such as palm oil mill effluent, has been shown to enhance biogas and biomethane production rates, demonstrating the potential of microalgae in optimizing anaerobic digestion processes (Córdova et al., 2018).

The advantages of using microalgae as a substrate for biogas and biomethane production stem from their high productivity, ability to capture CO_2, and convert organic matter into biofuels efficiently (Jensen & Skovsgaard, 2017). The upgrading of raw biogas to biomethane involves removing excess CO2 to produce a higher quality fuel suitable for various applications, such as transportation and heat generation (Machado, 2023). The scalability of anaerobic digestion of microalgae biomass within water resource recovery facilities further emphasizes the potential of microalgae in large-scale biomethane production (Milbrandt et al., 2016). Photosynthetic biogas upgrading has enabled the production of biomethane while concurrently treating digestate in constructed wetlands to obtain biofertilizers, showcasing the integrated approach of utilizing microalgae for energy and environmental benefits (Díez-Montero et al., 2020). The techno-economic analysis of biogas production from microalgae through anaerobic digestion highlights the feasibility and sustainability of biomethane production from this renewable feedstock (Murano et al., 2021). Moreover, modeling studies on hydrogen sulfide removal under biomethane production underscore the importance of optimizing biogas purification processes for efficient biomethane generation (Chernysh et al., 2021).

Bioremediation of air and wastewater

Microalgae have been recognized as a versatile and sustainable solution for bioremediation of air and wastewater, offering a promising approach to environmental cleanup and resource recovery. Extensive research has demonstrated the ability of microalgae to efficiently remove pollutants and contaminants from various environmental matrices (Jalilian et al. 2020; Uguz et al., 2022). Microalgae are well-known for their capacity to thrive in wastewater environments and effectively remediate pollutants, making them valuable assets in the pursuit of cleaner ecosystems and sustainable resource management.

The utilization of microalgae for bioremediation of wastewater presents a win-win paradigm, where the bioremediation capacity of microalgae is harnessed to treat wastewaters, thereby promoting the development of the microalgae industry and offering alternatives to conventional wastewater treatment processes (Delrue et al., 2016). Microalgae-based bioremediation processes have been shown to be cost-effective, energy-efficient, and highly effective in reducing pollutant levels compared to traditional wastewater treatment methods (Singh et al., 2022). The ability of microalgae to assimilate organic nutrients from wastewater for growth while simultaneously reducing pollutant content underscores their dual role in bioremediation and biomass generation (Chew & Show, 2022). Studies have demonstrated that microalgae can effectively remove contaminants from wastewater, leading to a reduction in biological oxygen demand and nutrient levels, thereby contributing to cleaner water sources and sustainable environmental practices (Rawat et al., 2011). The bioremediation potential of microalgae extends to the treatment of various types of wastewaters, including industrial effluents and urban waste, highlighting their versatility in addressing pollution challenges (Mutanda et al., 2020). The integration of microalgae-based bioremediation processes with biofuel production further enhances the sustainability and efficiency of wastewater treatment, offering a holistic approach to environmental stewardship (Renuka et al., 2014). Moreover, the application of microalgae in bioremediation of air and wastewater is not only effective in pollutant reduction but also contributes to biomass production, which can be utilized for various purposes, including biofuel production and nutrient recycling (Miazek et al., 2015). The ability of microalgae to thrive in contaminated environments and remove pollutants, such as heavy metals and organic compounds, underscores their potential as eco-friendly bioremediation agents (Li et al., 2018). The integration of microalgae with other remediation technologies, such as sulfate-reducing bacteria, in continuous treatment systems further enhances the efficiency and effectiveness of bioremediation processes. In conclusion, microalgae offer a sustainable and effective solution for bioremediation of air and wastewater, providing a dual benefit of environmental cleanup and biomass generation. The diverse applications and promising results of microalgae-based bioremediation processes highlight the potential of microalgae in promoting environmental sustainability and resource recovery.

CONSLUSION

Microalgae production is at the forefront of a sustainable bio-based economy, with applications in various industries. High lipid content in some microalgae species makes them a promising source for sustainable biofuels, addressing energy demands and environmental concerns. Their bioactive metabolites are increasingly used in pharmaceuticals and cosmetics. Microalgae biomass production provides a cost-effective and eco-friendly source of bioactive compounds for these industries. On the other hand, microalgae offer nutrient-rich alternatives for livestock, poultry, aquaculture feed, and food products, addressing food scarcity and promoting sustainability. Additionally, microalgae serve as feedstock for biomethane and biogas, offering renewable energy solutions and waste management. Integrating microalgae-based wastewater treatment with biogas purification accelerates nutrient removal and biomass production, showcasing dual benefits. Their application in bioremediation of air and wastewater offers sustainable solutions for environmental cleanup, contributing to cleaner ecosystems. In summary, microalgae-based technologies hold significant potential for environmental sustainability, resource recovery, and developing a bio-based economy that addresses energy demand, pollution, and resource management challenges.

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THE ROLE OF THE WOMEN ON ACHIEVING THE LDN

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ABSTRACT

Land degradation is one of the most significant problems affecting global habitats, leading to a decrease in productivity and food production. Considering these negative and destructive effects, urgent and decisive actions need to be taken on a global scale. Despite there being individual country efforts on this issue, it is also on the agenda of the sustainable development. In this regard, the concept of Land Degradation Neutrality (LDN) is seen as an important tool, aiming to prevent land degradation where possible and to restore degraded land in other cases. There are numerous links between the LDN and the Sustainable Development Goals (SDGs). One of the main ones is the role of women farmers in providing the LDN target. The women farmers are predominantly employed in low-paid and precarious jobs in the agriculture and are therefore disproportionately affected by the socio-economic challenges posed by the land degradation. In order to achieve the LDN objectives, a gender equality perspective needs to be integrated into the process and women's knowledge, labour and leadership must be brought to bear in the agriculture sector. Based on this information, the results of the survey in Türkiye conducted within the scope of the study in order to show the impact of women farmers on achieving the LDN goals are shared.

Keywords: Women, Survey, Sustainability, Degradation, Land Management

INTRODUCTION

Population growth continues with a rising momentum, and ongoing expectation is to reach approximately 9.8 billion by 2050, which significantly result in increases in food demand (UN, 2025). 2025 Global Report on Food Crises points out that 53 countries and regions experience acute food insecurity and need urgent food, nutrition, and livelihood assistance in 2024 (GRFC, 2025). In this case, Türkiye ranks 49th among 113 countries in terms of the 2022 Global Food Safety Index (GFSI, 2022). In addition to this indicator, Türkiye differs from the other countries in terms of some economic aspects, e.g. food price stability.

Besides this situation, degradation processes become a dominant pressure on arid and semi-arid fragile parts of Türkiye (Karagöz et al., 2017). Recently, it is reported that 12.7% of total land is categorized as "weak", 53.2% is "moderate", and 25.5% is located in the "high-risk" class in terms of desertification scale (CEM, 2017; Türkeş et al., 2019). Due to these compelling effects on limited natural resources, the Ankara Initiative has been prepared (UNCCD, 2019) to achieve Land Degradation Neutrality (the LDN) as a priority step to be taken by Türkiye. In addition to reaching the LDN targets, this document also aimed to implement the decisions taken at the 12th meeting of the Conference of the Parties to the Convention on Biological Diversity (COP 12) and the United Nations Sustainable Development Goals (SDGs).

The SDGs serve as an important tool in reaching the LDN. While some targets focus on solely the biophysical indicators, the others, concentrate on the socio-economic structure. Among them, only SDG 15 (Life on Land) has a direct relation with the LDN. The sub-goal 15.3 of the SDG 15 is particularly effective in determining the targets for extensively degraded countries

(FAO, 2019; UN, 2021). Otherwise, SDG 5 (Gender Equality) is also related to implemented activities of soil conservation and management (UN Women, 2019; Topcu, 2019) (Figure 1). In particular, research on the LDN can advocate for the women's land ownership and contribute to SDG 5 (Feng et al.,2022).

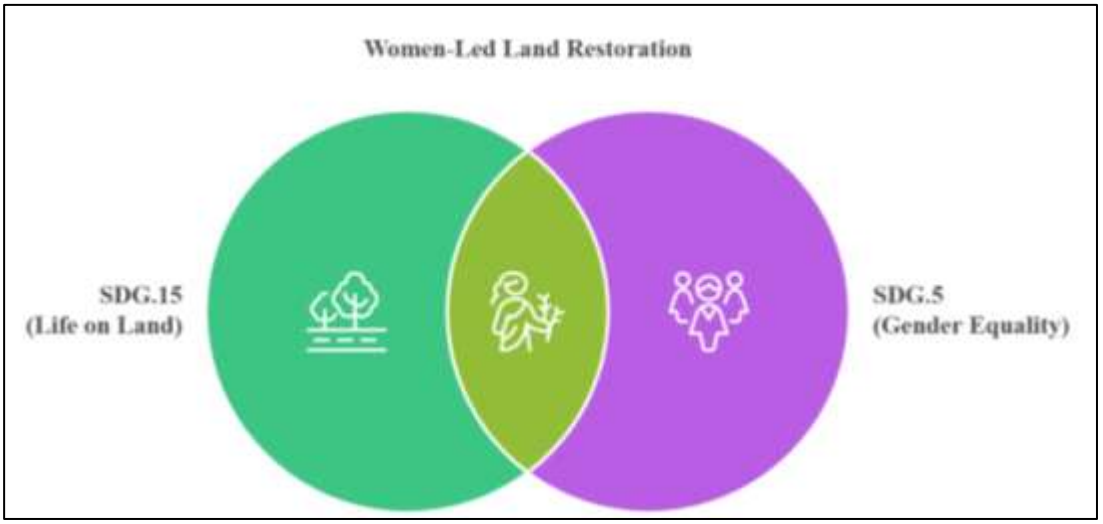

Figure 1. Empowering the women farmers for land restoration

As emphasized by Liniger et al. (2019), Sustainable Land Management (SLM) helps to halt the land degradation. Ensuring that the land users are supported by financial resources, an enabling legal framework and, in particular, information on the SLM practices is the most rational way to promote the adoption of the SLM and ultimately the achievement of the LDN.

Considering the pressure of the agricultural sector on natural resources, it is important to strengthen the effectiveness of the women farmers, who play an active role in the agricultural sector, in the field for the sustainable management of the agricultural lands. However, the male farmers mainly have been worked with in projects and activities aiming to improve the living standards of local people living in rural areas and to reduce the problems in the agricultural sector, and the women farmers have been mostly neglected in this process. Consequently, as a result of the acceptance of the women only as "agricultural labourers", "land" has come to be seen only as a "working area" for the women farmers. This has caused natural assets, which should be used and managed with a sustainable approach, to become a commercial commodity.

Furthermore, it is known that the women farmers who are actively involved in agriculture are seen as agricultural labourers and do not have any role in the land ownership or decision-making processes in production. Indeed, the women farmers need to be more active in the field due to the pressure of the agricultural sector on natural resources in order to ensure sustainable management of the agricultural lands. In this context, trainings on awareness-raising activities should be organized to include the women farmers and projects for the women should be diversified. Regarding this issue in Türkiye, projects and programmes such as Contribution of Women's Cooperatives in Rural Areas to Social Change: The Case of Ankara Province, Women Farmers Agricultural Extension Project, Safe Agriculture and Healthy Nutrition Project with Women and Their Families are being carried out.

The purpose of this study is to reveal the role of the women in land acquisition and sale transactions, their place in agricultural education and extension services, their activity in agricultural production and their status in decision-making processes by utilising the survey findings applied to two districts with intensive land use due to agricultural activities.

MATERIAL AND METHOD

The study area is particularly selected in central Anatolia where rainfed agriculture under semi-arid climate prevails as irrigated agriculture concentrates at the valley bottoms of mountainous topography. The main reason of choosing Kırbaşı and Tacettin villages was that the areas have a high-to-moderate sensitivity to desertification, and agricultural production activities are actively carried out by local farmers. The study was conducted in these villages in Beypazarı district located 100 km from the Ankara province in the Central Anatolia Region of Türkiye.

The economy of these villages is mainly based on the plant production. The average landholding size is 8-10 ha per household and significant numbers of scattered plots are very common. The reason for the fragmentation of these lands is that they are divided by inheritance, which has devastating effects on the stability of the agricultural sector in central Anatolian villages. The women farmers of the villages, who work as unpaid agricultural labourers, take an active part in almost all agricultural activities, mainly in crop production, as well as fulfilling household responsibilities such as child and elderly care. A survey of the study was conducted these two villages. The questionnaire was divided into seven main sections (Figure 2). In the scope of this study, the questions belonging to sections 1 (General Information), 2 (Household Structure) and 6 (Gender Roles) were utilized.

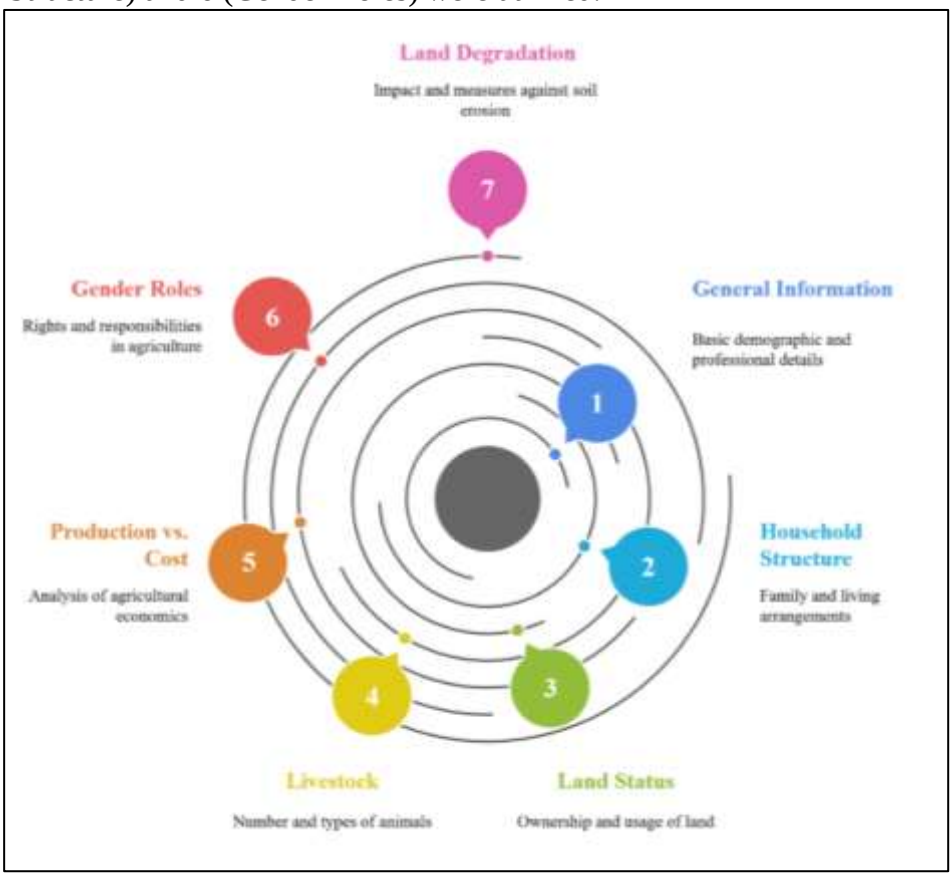

Figure 2. Basic parts of the questionnaire

Face-to-face interviews were held with the 206 farmers who voluntarily accepted to do the survey. Particular attention was paid to the participation of female farmers as much as possible in the survey application. The study was based on 5% error margin and 95% confidence

level. In the analysis of the data, it was evaluated as percentage and frequency by using frequency analysis.

RESULTS AND DISCUSSION

In the "General Information" section of the questionnaire, "gender" and "age" information of the farmers in the two villages were included (Table 1). When the respondents are analyzed in terms of gender, 56.3% of the farmers are "men" and 43.7% are "women". The minimum age of the interviewed farmers is 31, the maximum age is 74 and the average age is 52.8.

The household structure in the villages is presented in Table 2. When the household data are examined, it is seen that the weight of the household is mostly in families of "5" to "6" people. This shows that "family farming" is dominant in these villages. The family farmers have a great impact on the sustainable use and management of natural resources as well as their other role in society (Shaban et al., 2021). In particular, it is effective in the protection of soil ecosystems and the transfer of knowledge and culture between generations (FAO and IFAD, 2019).

The LDN-gender relationship was determined in the 6th section of the questionnaire (Table 3.). To the first question "The role of women in land acquisition and sale transactions" asked to the farmers operating in two villages, 72.8% answered "none" and 27.2% answered "yes". Although the continuity of women-land-productivity is the life assurance of Anatolian society, an analysis of statistics over time reveals that the women engaged in agricultural activities are unpaid "agricultural labourers" rather than "land owners" (Sabbağ, 2017). However, the fact remains that empowering the women farmers by strengthening and enforcing land use and land decision-making rights can ensure long-term productivity thereby achieving the purpose of the LDN targets (Collantes et al. 2018).

On the other hand, studies show that the women farmers' participation in agricultural extension services helps to reduce poverty, increase food security, improve family health and nutrition status, and create new job opportunities. In Table 3., 52.4% of the respondents answered "yes" and 47.6% answered "none" to the question "Involvement of the women in agricultural training and extension services".

As Sharma and Badodiya (2016) emphasised, the women farmers face some challenges in carrying out their agricultural activities, such as access to finance. A reflected pattern of similar situations was observed for the farmers in these two villages. All of the farmers who participated in the survey (n: 206) answered "none" to the question "Women's access to agricultural financial resources", even though there are various agricultural support payments of the T.R. Ministry of Agriculture and Forestry and also Instrument for Pre-Accession Assistance for Rural Development (IPARD) supports with a special scope for Beypazarı district. This shows that these financial resources are available but access is mostly by the male farmers.

The women farmers continue to account for a large share of the labour force devoted to the agriculture. It indicates that the women farmers constitute about 43% of the agricultural labour force globally and in developing countries (FAO, 2011). Continuing in this section (Table 3.), 68.4% of the respondents answered "yes" and 31.6% answered "none" to the question "Women's activism in agricultural production".

All of the farmers responded as "none" to the question of "women having agricultural or non-agricultural business contacts". This adverse situation once again highlights the fact that women are employed as family workers and that they need to be empowered in rural areas by increasing their opportunities to engage in individual work, particularly through cooperatives.

Lastly, regarding the role of women in decision-making processes within the scope of agricultural activities, 84% of the farmers stated "none" and 16% stated "yes". Asking the farmers

who answered "yes" to this question, it was found that the women have a say in the decision-making process was stated by all of the farmers (n: 33) as "consulted/speaking in land sales".

Table 1. General information of farmers (n=206)

Variable	**Category**	**f**	**%**
Gender	Women	90	43.7
	Men	116	56.3
	Total	**206**	**100.0**
Age	Mean age 52.8		

Table 2. Household structure (n=206)

Variable	**Category**	**f**	**%**
Family size (household size as person)	3	24	11.7
	4	33	16.0
	5	55	26.7
	6	49	23.8
	7	30	14.6
	8	12	5.8
	9	3	1.4
	Total	**206**	**100.0**

Table 3. Gender roles (n=206)

Variable	Category	f	%
The role of the women in land acquisition and sale transactions	Yes	56	27.2
	None	150	72.8
	Total	**206**	**100.0**
- If yes, why?	Consulted in buying and selling	42	75.0
	Has a say in buying and selling	14	25.0
	Total	**56**	**100.0**
Involvement of women in agricultural education and extension services	Yes	108	52.4
	None	98	47.6
	Total	**206**	**100.0**
- If yes, why?	Participates in trainings-courses	108	100.0
Access to agricultural financial resources	None	206	100.0
Active in agricultural production	Yes	141	68.4
	None	65	31.6
	Total	**206**	**100.0**
- If yes, why?	Works in the field	141	100.0
Having agricultural or non-agricultural business contacts	None	206	100.0
	Yes	33	16.0

The role of women in decision-making processes	None	173	84.0
	Total	**206**	**100.0**
- If yes, why?	Consulted/speaking in land sales	33	100.0

CONCLUSIONS

The findings show that the women farmers are still active in the agricultural lands, but they face some difficulties despite this vital role within the scope of the questions asked in the survey. Nevertheless, it is a fact that in order to ensure the SLM and thus the achievement of the LDN targets it is critically important that they have a say in the production phase, especially in the land ownership, and have easy access to financial resources. Therefore, any initiative on this subject will contribute to the achievement of this goal.

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ENDOPHYTIC BACTERIA FROM ECHIUM AMOENUM FISCH & MEY. AS A NEW ELICITOR OF PHENOLIC BIOSYNTHESIS

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ABSTRACT

The *Echium amoenum* Fisch & Mey., as a native plant of Iran, has unique properties in traditional Iranian medicine. E. *amoenum* is rich in numerous phytochemical compounds such as rosmarinic acid, anthocyanidins, and flavonoids that are utilized as a new source of materials in different industries such as drug, food, and cosmetic. These bioactive compounds are used for treatment of many infectious and fungal diseases such as herpes, oral infections, colds, pneumonia, congestion, coughs and stress. Microorganisms can interact with plants and play a significant role to produce plants with high nutritional value. Recently, the isolation of endophyte bacteria from some medicinal plants and their application as an elicitor, has attracted the attention of researchers. Hence, in this study, a novel strain of endophytic bacterium *Bacillus amyloliquefaciens* was isolated from the E. *amoenum* plants. After that, the elicitation effects of autoclaved bacteria were investigated on phenolic production of E. *amoenum* plant *in vitro*. Based on the results, total phenolic content and total flavonoid content were positively increased under the influence of different concentrations of bacterial extract. The results also showed that *Bacillus amyloliquefaciens* extract can be used as a new elicitor for the biosynthesis of phenolic compounds in future research.

Keywords: *Bacillus amyloliquefaciens*, Flavonoid, Interaction, Microorganism, Phenol

INTRODUCTION

Echium amoenum Fisch & Mey. (Gol-e-Gav-Zaban) belongs to the *Boraginaceae* family. This perennial plant, as a medicinal plant native to Iran, grows wild in the mountainous regions of northern Iran and the Caucasus. E. *amoenum* is a valuable medicinal plant that has been used for more than a thousand years in the treatment of many infectious and inflammatory diseases (Jamnani et al., 2023).

Phytochemical studies have indicated that E. *amoenum* has bioactive compounds, such as polyphenols, flavonoids, anthocyanins, shikonin derivatives, rosmarinic acid, and sterones. These phytopharmaceutical compounds possess a range of beneficial activities, including antibacterial, anti-mutagenic, anti-carcinogenic, cytotoxic, anti-inflammatory, antidepressant, and anti-proliferation properties. They are commonly used to treat various conditions such as herpes, oral infections, colds, pneumonia, sore throat, and cough. In addition, the extract of E. *amoenum* is used as a sedative in the treatment of stress and anxiety (Amjadi et al., 2023).

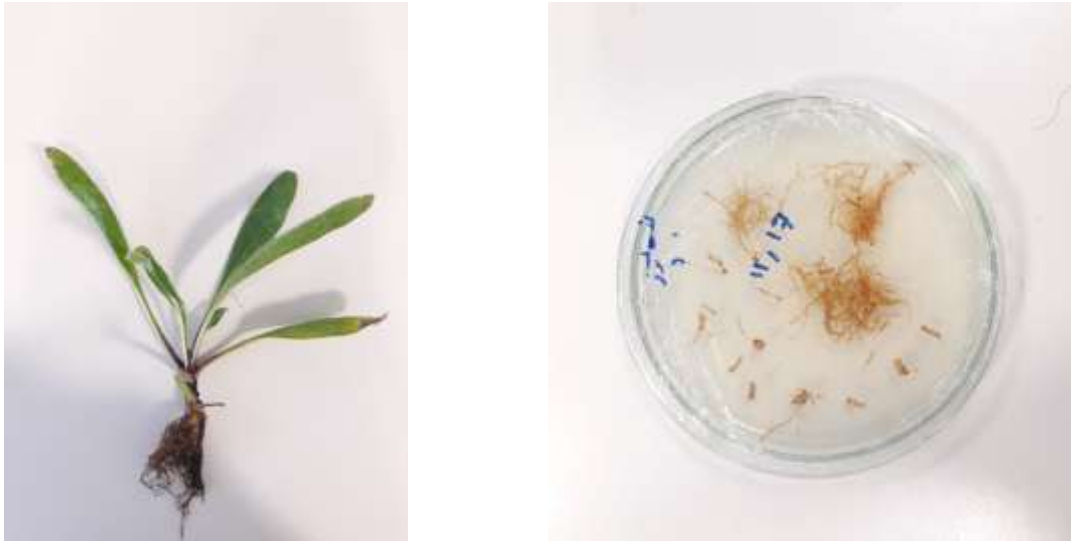
Medicinal plants are great reservoirs of microorganisms and valuable sources of specialized metabolites. The previous studies have revealed that endophytes can produce some secondary metabolites in their host plants (Ptak et al., 2022). However, data on the isolation of endophytes from the *Boraginaceae* family and their ability to biosynthesize polyphenols are limited. In a previous study, the endophytic bacteria belonging to the *Actinobacteria* phylum have been isolated

from E. *amoenum* plants. However, the Bacillus genus has not yet been isolated from E. *amoenum* (Oloumi et al., 2023).

Endophytic bacteria can be used as elicitors of metabolite biosynthesis in plants. Biotic elicitors can be either raw or autoclaved endophytic bacterial cells. According to previous studies, the autoclaved endophytic *Bacillus megaterium* was able to stimulate the production of quinone-methide triterpene in adventitious *Peritassa campestris* root cultures (Ptak et al., 2022). To our knowledge, no biotic elicitors have been studied in *in vitro* E. *amoenum* cultures. Therefore, in this study, the *Bacillus amyloliquefaciens* was identified and isolated from plants of E. *amoenum*. After that, the ability of *Bacillus amyloliquefaciens* to biosynthesize total phenolics and flavonoids was investigated.

MATERIAL AND METHOD

This study was conducted at the University of Mohaghegh Ardabili, Ardabil, Iran, in 2025. The *Echium amoenum* Fisch & Mey plants (Fig. 1A) were collected from the mountain regions of Ardabil. First, the roots were washed with water and surface disinfected with ethanol and hypochlorite. Finally, they were rinsed 3 times with sterile water, and the last rinse was cultured in LB agar plates to ensure the removal of non-endophytic bacteria. Then, the roots were cut into smaller pieces and placed in LB and PDA media and incubated at 37°C (Fig. 1B). After bacterial growth, several subcultures were performed, and bacterial colonies were isolated from each other.



(A) (B)

Figure 1. A, collected *Echium amoenum* plants, B, root pieces cultured in PDA medium

Bacteria obtained from LB plates were used for DNA extraction. A single bacterial colony was inoculated into 10 mL of LB medium and placed in a 37 °C incubator on a shaker at 100 rpm. The cells were isolated by centrifugation at 6000 × g for 10 min. Pellet was used for genomic DNA extraction using the Ptak et al. method (Ptak et al., 2022). Taxonomic analysis of the isolated strain and search for the most similar species were done using the NCBI web server.

For elicitor preparation, briefly, one colony of isolated bacteria was incubated in 10 mL of LB liquid medium and placed at 37 °C on a shaker at 100 rpm for 24 h. After bacterial quantification and centrifugation, the supernatant was removed, and the cell sediment was resolved in sterile distilled water and applied for elicitation (Ptak et al., 2022).

Echium amoenum plants 1-month old were obtained from *in vitro* seed culture. These plants were transferred to a solid MS medium. Autoclaved *Bacillus amyloliquefaciens* group sp. strain

EA-1 was added to the medium at concentrations of 0% (as a control), 0.01%, 0.02%, and 0.04%. The pH of all media was adjusted to 5.8. This study was established in five replications. The plants were investigated for phytochemical analyses after 30 days.

Methanolic extract from leaf tissue was prepared using the Wang et al. method (Wang et al., 2015). The total phenolic content was quantified according to the Folin-Ciocalteu method (Singleton et al., 1965). The absorbance of the samples was read at 765 nm. Gallic acid was considered the standard (Fig. 2A). The total phenolic content was expressed as mg gallic acid equivalent (GAE) per 1 g FW (fresh weight). Total flavonoid content was measured using the aluminum chloride method. The sample's absorbance was read at 510 nm. Quercetin was used as a standard (Fig. 2B). Total flavonoid content was expressed as µg quercetin equivalent (QE) per 1 g FW (Wang et al., 2015).

This study followed a completely randomized design (CRD) with five replications. All quantitative data were subjected to one-way ANOVA using SAS 9.1 to indicate significant differences. Means were separated at a 5% level of probability by Dun- can's Multiple Range Test. All graphs were drawn with Excel.

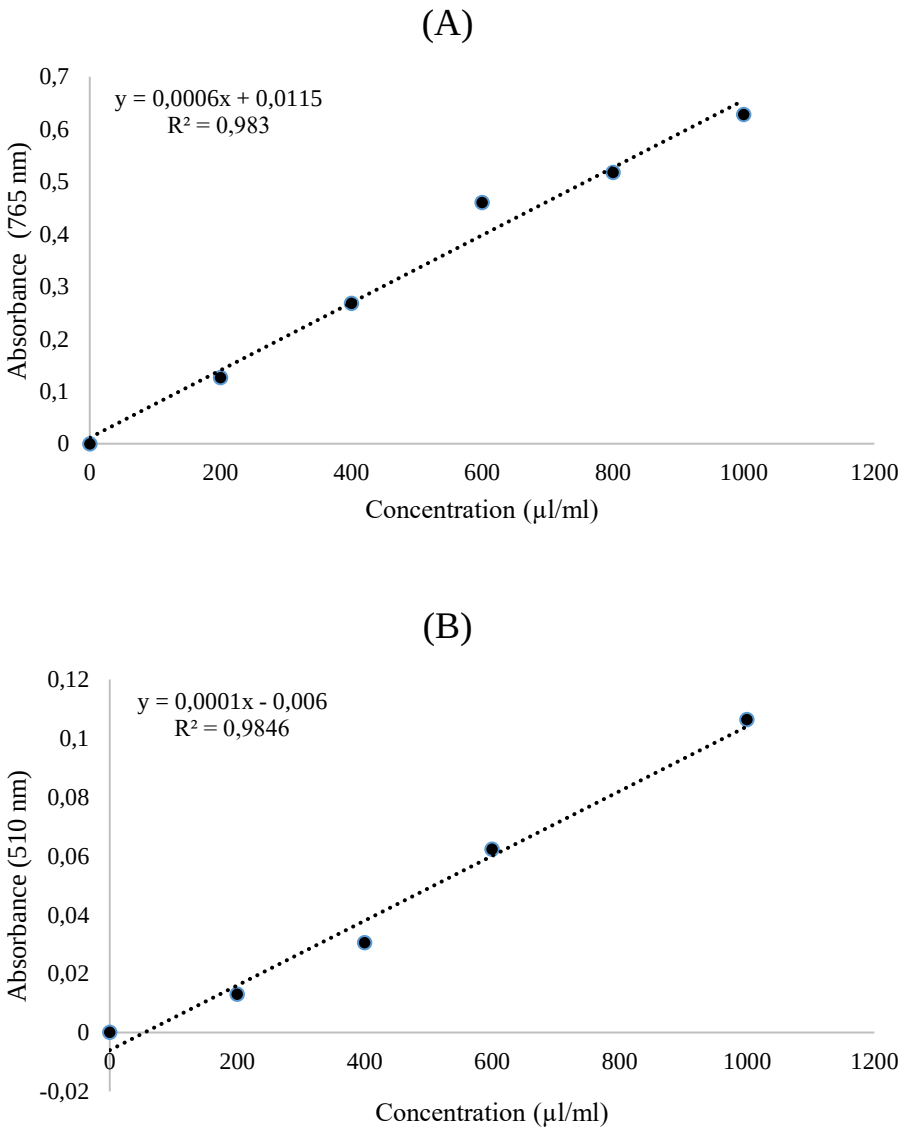


Figure 2. A, gallic acid standard curve, B, quercetin standard curve.

RESULTS AND DISCUSSION

In this study, *Bacillus amyloliquefaciens* group sp. strain EA-1 was isolated from *Echium amoenum* plant roots (Fig. 3). Two 16S forward and reverse primers, including AGAGTTTGATCCTGGCTCAG and CGGTTACCTTGTTACGACTT, were used for PCR analysis, respectively. Genome sequencing of the isolated strain resulted in 1094 (forward) and 1114 bp (1114). Assemblies were finally deposited in the GenBank under the Bio Project number PV731489.1, and the strain was labeled as EA-1. Based on Blast in EzBioCloud and NCBI sites, it was found (when considering 16S rDNA sequence) that the most related species was *Bacillus amyloliquefaciens* group sp. To our knowledge, this is the first study to report the isolation of *Bacillus amyloliquefaciens* group sp. from *Echium amoenum* plant roots. Previously, Oloumi et al. isolated bacteria belonging to the *actinobacteria* from *Iris persica* and *E. amoenum* plants (Oloumi et al., 2023).

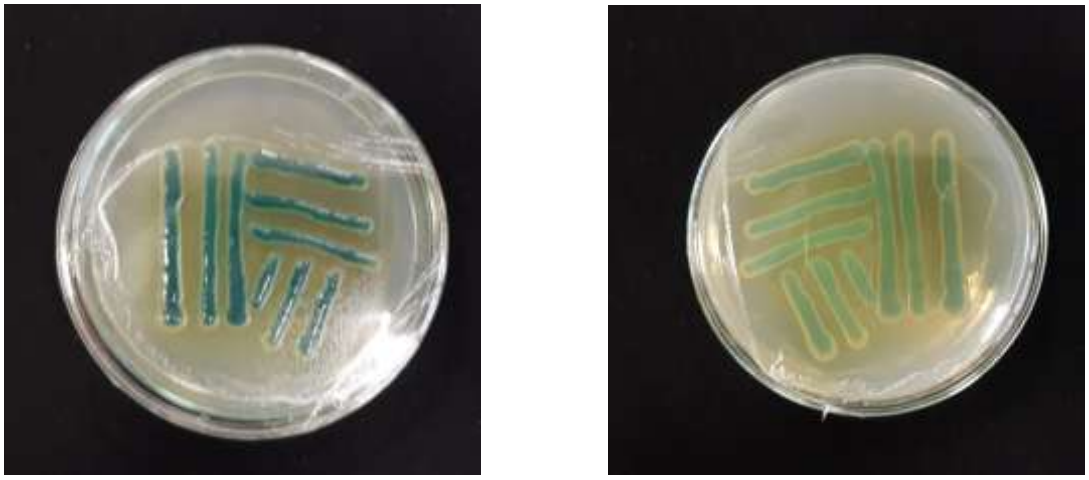


Figure 3. The isolated *Bacillus amyloliquefaciens* group sp. strain EA-1 from *Echium amoenum* plants on PDA medium

Endophytic bacteria live inside plant tissues without the plants exhibiting symptoms of disease. The result of this study indicated that endophytic *Bacillus amyloliquefaciens* group sp. strain EA-1 isolated from *Echium amoenum* plant roots increases the synthesis of phenolic compounds and flavonoid content. Based on this experiment, the autoclaved endophytic bacteria of *Bacillus amyloliquefaciens* group sp. strain EA-1 acted as an elicitor and increased the total phenolic content (88.5333 GAE mg/g FW) and total flavonoid content (2153 QE µg/g FW) (Fig. 4A and 4B). Previous studies have revealed that the autoclaved endophytic P. *lautus* strain PLV increases the production of galanthamine and lycorine in *Leucojum aestivum* L. tissue cultures (Ptak et al., 2022). Liu et al. indicated that inoculation of in planta *Lycoris radiata* with live endophytic bacteria increased the plant dry weight, enhancing the total yield of alkaloids (Liu et al., 2020). Inacio et al. indicated that eliciting *Peritassa campestris* culture with autoclaved endophytic *Bacillus megaterium* increased the biosynthesis of 22 β-hydroxymaitenin (Inácio et al., 2017).

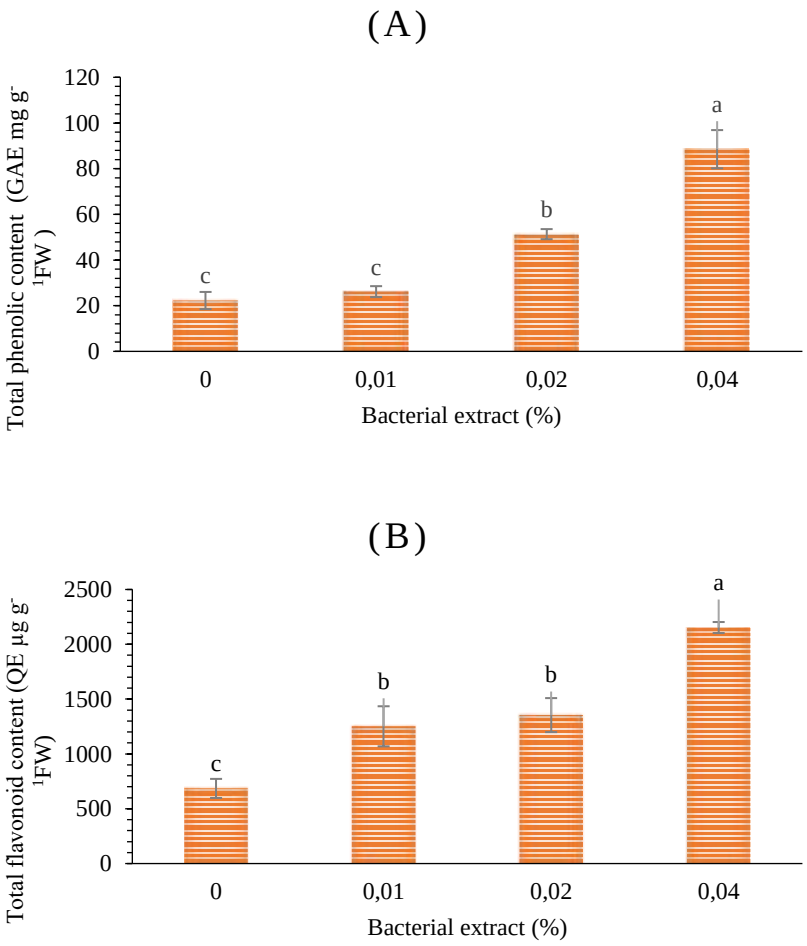


Figure 4. Effect of *Bacillus amyloliquefaciens* group sp. strain EA-1 elicitation on total phenolic content and total flavonoid content in E. *amoenum* in vitro plants. A, total phenolic content, and B, total flavonoid content. Different letters indicate significant differences determined using Duncan's multiple range test ($p < 0.05$). Error bars represent ±SE, n=5.

CONCLUSION

This research was conducted to isolate endophytic bacteria and investigate the effects of autoclaved endophytic bacteria as an elicitor on the synthesis of phenolic and flavonoid content in in vitro *Echium amoneum*. In this study, a new strain of *Bacillus amyloliquefaciens*, designated EA-1, was identified from the roots of E. *amoneum*. The obtained results revealed that this new elicitor increased the total phenol and total flavonoid content. Furthermore, it may elicit the biosynthesis of other secondary metabolites that can be investigated in future research.

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THE IMPACT OF DILL LEAVES ON THE SENSORY QUALITY OF TUNA PATE

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ABSTRACT

Canned tuna is one of the most traded food products around the world due to its availability in processing and consumption. Similar to other seafood processing methods, many by-products are generated during the tuna canning process and utilization of these by-products in the alternative value-added products production is a promising approach for circular economy and the environment. In this research, tuna pate was produced with the usage of chunks and trimming of canned tuna products. For these aims, two tuna pate groups were produced from without or dill leaf addition. Then each pate group was separated into three sub-groups in terms of packaging material; flint glass (F), amber glass (A), and plastic container (P). Packaged tuna pate groups are stored in the refrigerator during six months. Sensory attributes such as colour, odour, taste, texture, and general acceptance were also determined. The results showed that the sensory quality of dill leaf enriched tuna pate groups had higher sensory scores than control tuna pate groups. The results has also revealed that the tuna pate groups in the glass jar, especially in amber glass groups compared to plastic container groups had been evaluated more appropriate by panelists.($P<0.05$). These results revealed that the usage of dill leaf prolonged the shelf life of tuna pate within sensory quality of tuna pate. The structure and colour of packaging materials also impacted the quality of tuna pate sensory quality.

Keywords: Seafood, quality, sensory attributes, dill leaves, tuna, spreadable pate

INTRODUCTION

With increasing demand of healthy food products globally, alternative products have become popular in the food industry. In particularly,ready-to-eat food items are most commonly preferred by consumer due to can be easily consumed and do not required any process. Pate is one of the ready to eat food products containing meat, spice, additional oil and other food additives. Traditionally, pate prepared with goose liver which is known as "foie-grass", the increasing demand to this product and the price had to tended to producer usage to alternative sources. From pork livers and chicken livers to different animals offal have been used for pate production (Estévez et al., 2007; Polak et al., 2011). The market size of pate was more than a billion USD in 2018, and it is estimated to increase total size by 1.3 % up to 2025 (Grand View Research, 2019). Recently fatty fish species have used in pate production due to their functional characteristics resulted by fat content and health benefits. Higher bioavailability rate and containing 20% more protein than meat pate make fish pate a popular seafood product (Branciari et al., 2019).

Especially, fatty fish species such as tuna and anchovy are used for pate production owing to their desirable taste and other sensory quality parameters.

Tuna species known as a large group of important fishes that belong to the Scombridae family, are classified into three genera, Katsuwonus, Sarda, and Euthynnus and one of the most consumed species globally (Abdullah and Rehbein, 2016). Especially canned tuna has been accepted as one of the traded food products due to its efficiency to proccssing, easy availability, and sensory attributes to consumer preference (Dantas et al., 2021). Sames as other seafood processing methods, a wide range by-products such as tuna trimming and tuna chunk generated during canning process (Malaweera and Wijesundara, 2014). Valorisation of these by- products in value-added novel food products offers economic and eco-friendly production with contributing to sustainable development goals and sustainable production. Since, pate products are highly susceptible to oxidation as a result of containing external high lipid rate and commonly fatty species preferred. Different processing techniques, using of natural antioxidants, packaging materials are robust approaches to avoiding chemical quality deterioration and improving sensory quality. Utilization of several plants directly using leaves or indirectly using extracts leads to delaying to oxidation in addition to improvement of the sensory attributes of food products. Thus, this research aimed to investigate dill leaf usage as taste enhancer and sensory quality improver of refrigerated tuna pate in different packaging materials during 6 months.

MATERIAL AND METHOD

Materials

Tuna (Katsuwonus pelamis) by-products (chunks and trimmings obtained after canned tuna processing) in pre-cooked form used as raw material for tuna pate production. The chunks and trimmings were obtained from seafood processing plant (Sasu Ltd., Adana, Turkey) the other ingredients (milk powder, spices, starch, and lemon juice) were obtained from local market located in Adana, Turkey in fresh form. Dill leaves (Anethum graveolens) were also obtained from the local market as fresh form and then washed, dried and grinded. All reagents used were of analytical grade and obtained from Sigma-Aldrich, Inc. (St. Louis, MO, USA) and Merck (Darmstadt, Germany).

Tuna pate preparation and storage

For the production of the tuna pate, tuna chunks and other indigents were mixed in two different formulations. Frozen tuna trimmings and tuna chunks were thawed and chopped then mixed with mush potato, milk powder, salt, starch, and sunflower oil, spices and other ingredients in a dough mixer (AHE.PM.5, Alveo,Turkey) at 4°C until obtaining a homogeneous tuna trimmings dough (10 min). Mixed pate was divided into 2 groups: Control group (C) without any other ingredient, and for dill leaf enriched (DE) group, dried dill leaves and lemon juice were added to control groups while mixing slowly. Then, each pate group was divided into three sub-groups depending on packaging material (F: flint glass jar, A: amber glass jar and P: plastic container). Different tuna pate groups (C and DE) in different packaging materials classified as CF, CA, CP, DEF, DEA, DEP. The control and dill leaves enriched pate sample were manually full filled into the glass jars and plastic container as around 100 g. Then, the packaged tuna pate pasteurized at 80 °C for 45 min laboratory-type oven (Tefal model; 18L; Tefal, Barcelona, Spain) with slight modification the method described by Estévez, and Cava,(2004).Pate groups were stored under refrigerated conditions at 2±1 °C during 6 month. For each groups, sensory analysis were carried out on week 0, 7, 12, 16, 20, 22 and 24 of storage.

Sensory attributes of tuna pate during storage

The sensory evaluation of the tuna pate (CF, CA, CP, DEF, DEA, DEF) was conducted with 10 experienced panelists (5 women, 5 men). Panelists assessed flavor, odour, taste texture and general acceptance of pate groups. Sensory assessment performed with nine-point descriptive scale described by UNE 87-020-93 (UNE, 1993). According to scale, higher scores were evaluated as "good quality", and lower scores evaluated as deterioration of quality.

RESULTS AND DISCUSSION

Sensory Attributes of tuna pate

The descriptive sensory attributes of tuna pates during storage period shown in Figure 1. Significant differences were detected related to sensory quality in terms of taste, odour, texture, general acceptance ($P < 0.05$). Dill leaf enrichment lead to improvement of sensory attributions of tuna pate regardless of packaging material especially for odour and taste parameters. Control groups reached the rejection limit at the 22th week of storage and the dill leaves enriched groups reached the same limit at the end of storage (24th week).

Dill leaves enrichment extended the shelf life of tuna pate stored in refrigerated storage by 2 weeks compared to the control tuna pate groups. Similar findings reported by Özoğul et al., (2017) who declared the usage of plant based material on the sensory quality of fish products.

Packaging material also impacts the sensory quality of tuna pate. While, tuna pate stored in plastic packaging material reached at the rejection limit at the 22th week, the tuna group stored in the glass jar achieved at the 24 th week of storage.

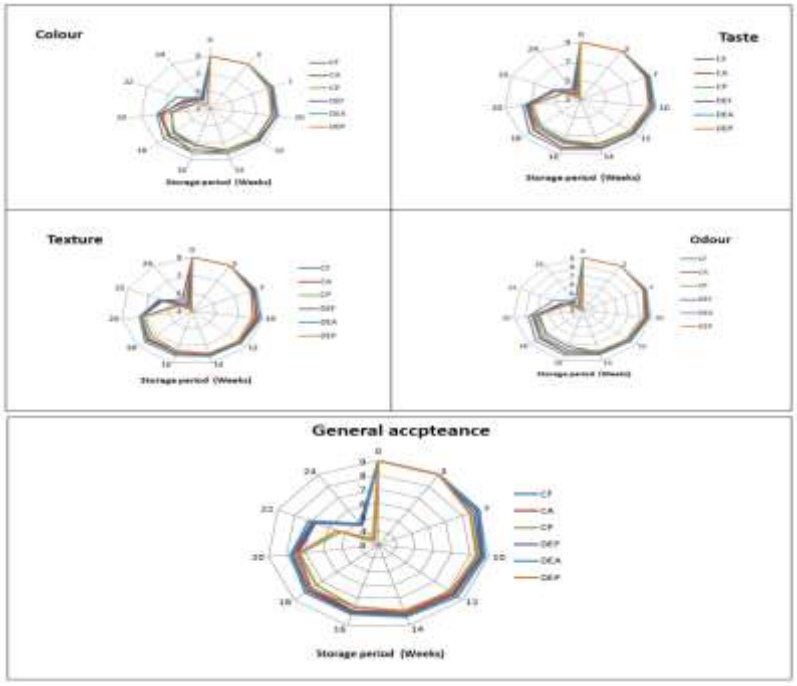

Figure.1. Sensory attributions of tuna pate during refrigerated storage

Groups: CF: Control tuna pate stored in flint glass jar, CA: Control tuna pate stored in amber glass jar, CP: Control tuna pate stored in plastic container, DEF: Dill leaf enriched tuna pate stored in flint glass jar, DEA: Dill leaf enriched tuna pate stored in amber glass jar, DEP: Dill leaf enriched tuna pate stored in plastic container

CONCLUSIONS

Due to sensory quality impacts on the product acceptability and the feasibility of processing of any food products, this quality is so important.Tuna pate enriched with dill leaf proved the sensory quality which could be related to lower oxidation capacity. In terms of packaging material, stored in glass jar especially in amber glass jar lead to higher sensorial acceptance. In terms of sensory attributes, texture, color, odor and general acceptance among tuna pate groups, dill leaf enrichment protect the sensory quality. This report recommended that dill leaf could be an useful taste and odor enhancer for other food items have high fat content such as pate products. Glass jar could be a more suitable packaging material for pate type products, especially during refrigerated storage. The findings of research revealed that usage of plant allows better quality of seafood in addition to improving of taste and the packaging material impact on the quality of food product, especially high lipid contained food.

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FROM INVASION TO CONSUMER TABLE: AS A MODEL: LIONFISH

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ABSTRACT

While the importance of marine sources for not only food and feeding but also for biomaterial and sustainability of fisheries has become more essential for the public, decision-makers and researchers; the threat of invasive species on the marine sources has still been an important problem. According to the research, the main effects that threaten the coastal system are invasive species, tourism, destruction of the coasts and the lack of awareness of people. Alien species, which are one of these effects, face very different ecological factors in the geography where they have just moved. Some of these alien species spread rapidly, using the environment's available resources better than other species that already existed in the environment, and over time, they become invasive species, replacing natural species in the environment and invading new ecosystems to which they are moved. The lionfish, which is naturally found in the Pacific Ocean, has attracted the attention of researchers in recent years with its spread in different seas of the Earth. This invasive species has become prevalent in Turkey from Eastern Mediterranean to the Aegean Sea. Using and valorization of natural sources for maximum benefits are one of the important parts of sustainability goals globally. Therefore the usage of invasive marine species as an alternative for food and feed sources is a promising approach for converting a threat into benefits. This review will evaluate the potential of utilization of lionfish as a food or feed sources model for invasive species in Turkey. The proximate composition, nutritional benefits and potential risks such as bioaccumulation of heavy metals or other environmental pollutants will be criticized. The economical feasibility will also determine within previous research. The results could be useful for the food industry and fisheries science.

Keywords: Lionfish, seafood, invasive fish species, valorization, and nutritional value

INTRODUCTION

Invasive species that settle in the area where they have just moved, cause damage by competing with native species, change the balance in the ecosystem, and have negative effects on human health and economy are classified as IS. Circular Bioeconomy is defined as "the conversion of biological residues (residual) into bio-based products that can be shared/reused/reproduced, recycled or safely released into the biosphere through organic and nutrient cycles".Depending on Circular Economy and bioeconomy: some of the main aims accepted as ;prevention and reduction of urban biological waste, conversion of inevitable urban biological waste into valuable resource management, Climate change mitigation: Reducing greenhouse gas emissions by avoiding landfill and capturing carbon in bio-waste-based products, Industrial symbiosis : Linking the bio-waste and wastewater sectors with other sectors such as bio-based, chemicals, plastics, food and feed,

etc., Resource efficiency and safety: Conversion of municipal biological waste into valuable substances such as Nitrogen (N), Phosphorus (P) and Potassium (K), Sustainable food and nutrition security: Improving local food systems through bio-based solutions such as the production of feed (and ultimately food) components from urban biological waste

All species of Pterois, which belong to the family of scurvyes (Scorpaenidae), have poisonous dorsal, anal and pelvic spines (Frose and Pauly, 2019). The poison of these spines is effective enough to cause injury and death for fishermen and divers (Schofield, 2009). Due to its poisonous spines, it has few natural predators. P.miles of Indo-Pacific origin live in hard ground structures, longoses, seagrass meadows, coral reefs, artificial reefs, cliffs, muddy bottoms and sandy habitats in temperate waters deep between 1-300 feet (Khalaf and Kochzius, 2002; Albins and Hixon, 2008; Ferrieira et al., 2015). They hide on rocks and in hollows (Hare and Whitfiel, 2003).

The lesepian species entering the Mediterranean ecosystem, the evaluation of ecological niches that are not evaluated by native species, their easy population formation and the dominance on native species in bilateral competition are interpreted as an indication that this migration will be continuous (Ergüden and Doğdu, 2020). While the process is still experienced in the form of pressure of foreign species on native species, it is expected that new foreign species will replace the foreign species that have become dominant in areas where native species are found. Studies on biodiversity in the region show that this change can only be experienced in a few years (Gürlek, Erguden, Dogdu and Turan, 2016). For these reasons, the importance of combating the species that valorization of invasive species in circular economy is so important since the easiest and most functional way to combat these species is to bring the species into the economy (Zenetos et al., 2012).

VALUE-ADDED PRODUCTS FROM LIONFISH

The lionfish, whose spiny fins are poisonous, but whose meat does not contain poison, was taken to the menu of social facilities in order to spread its consumption. Lionfish are generally served after their spines are cleaned and processed into soup, grilled, steamed and fried. For the processing and consumption of an animal source as safe food; Physicochemical suitability (Nutrient composition, fat protein ratios) ,Textural properties Heavy metal, Pesticide, Pcb content should be tested to be safe in terms of Cytokoxydede and in terms of allergen.Purification of protein isolate or other biomaterial from lionfish is another alternative for processing of this species.

CONCLUSIONS

It is important to investigate the availability of fish species that show invasive characteristics in the waters of our country as functional products together with their nutritional composition, suitability for processing techniques and other food matrices. It affects the feasibility of processing during the use of invasive species as raw materials as food products, and the composition of nutrients, which differ from species to species and even from individual to individual in a bear species, leads to differences in the accumulation of aquatic pollutants in fish muscle.

The processing, packaging and introduction of invasive aquatic species, which are one of the leading environmental risk factors and which cannot have economic importance, to the aquaculture processing sector with different methods will give a new perspective to the aquaculture circular economy, which is one of the leading adequate and balanced nutrition, which has become increasingly important recently.With this application, the AIM is to hunt the existing invaders in an environment, process them and bring them to the economy and turn them into high value-added

products. The risk of invasive species moving to a new location and showing their effect there is the most important part of feasibility studies.

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USE OF ORIGANUM SPECIES ESSENTIAL OILS IN THE CONTROL OF PLANT DISEASES

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ABSTRACT

Origanum is a significant medicinal and aromatic plant belonging to the Lamiaceae family. In parallel with the developments in studies on the isolation and determination of various bioactive substances contained in *Origanum* species, it has been determined that they contain many different compounds. The main groups of these compounds are terpenoids, triterpene acids, hydroquinones, flavonoids, phenolic acids, hydrocarbons containing carvacrol, thymol, and various extracts as the main bioactive components. Studies on the effects of these different components against plant diseases have been the focus of attention of researchers in recent years. In studies on the impact of essential oils obtained from *Origanium* species on plant diseases, the effects of essential oils obtained from *Origanum vulgare, O. compactum, O. heracleoticum, O. onites, O. syriacum, O. dictamnus, O. acutidens, O. rotundifolium,* and *O. majorana* species were mostly in investigated. Essential oils obtained from *Origanum* species are used as antifungal agents against *Alternaria* spp, *Aspergillus* spp., *Penicillium* spp*., Rhizopus* spp., *Fusarium* spp*., Phytophthora* spp., *Pythium* spp., *Colletotrichum* spp., *Monilinia* spp*., Geotrichum citri-aurantii, Verticillium dahliae*, *Botrytis cinerea, Plasmopara viticola, Sclerotium rolfsii, Rhizoctonia solani, Macrophomina phaseolina, Sclerotinia sclerotiorum, Phoma tracheiphila, Stemphylium beticola* and *Aphanomyces euteiches.* They also have been observed to be effective as antibacterials in research on *Clavibacter* spp*., Xanthomonas*spp*., Pseudomonas spp., Erwinia spp., Ralstonia solanacearum and Pectobacterium carotovorum. The* essential oils from the *O. vulgare* plant were effective against *Zucchini yellow mosaic virus* coat protein (ZYMV-CP) and tomato leaf curl New Delhi virus (ToLCNDV) infections. Further studies are needed to evaluate the efficacy of the model products of these essential oils.

Key words: *Origanum* spp., antifungal, antibacterial, antiviral, plant diseases

INTRODUCTION

Origanum is an important medicinal and aromatic plant belonging to the Lamiaceae family. In parallel with the developments in studies on the isolation and determination of various bioactive substances contained in Origanum species, it has been determined that they contain many different compounds. The main groups of these compounds are terpenoids, triterpene acids, hydroquinones, flavonoids, phenolic acids, hydrocarbons containing carvacrol, thymol and various extracts as the main bioactive components. Studies on the effects of these different components against plant diseases have been the focus of attention of researchers in recent years.

Essential oils (EO) are aromatic oily liquids obtained from various plants, generally localized in temperate and hot countries. Essential oils, as secondary metabolites in nature, play an important

role in the protection of plants as antibacterial, antiviral, antifungal and insecticide (Bakkali et al., 2008).

As observed from antifungal trials in different literature, essential oils have a great antifungal effect on many plant pathogens and have been determined to inhibit most of the plant pathogens tested in the laboratory (Omar and Kordali, 2019).

EFFECT of *Origanum* spp. ESSENTIAL OILS AGAINST FUNGAL DISEASES

Guzmán-Guzmán et al. (2003) reported that the preventive effect of various concentrations of peppermint, eucalyptus, laurel, clove, sweet marjoram, rosemary, cinnamon, pepper, grapefruit and origanum spp. essential oils (1,250, 2,500 and 3,750 ppm) was possible to control *Fusarium oxysporum* f. sp. *phaseoli.* Eight substances, three of which are the main components of essential oils, from Mediterranean aromatic plants (*Verbena officinalis, Thymus vulgaris* and *Origanum vulgare*), were tested in vitro against some phytopathogenic fungi and postharvest fruit rot agents such as *Botrytis cinerea, Penicillium italicum, P. expansum, Phytophthora citrophthora* and *Rhizopus stolonifer*, which were previously found to be active against them. The tested compounds, β-phellandrene, β-pinene, camphene, carvacrol, citral, o-cymene, γ-terpinene and thymol exhibited fungicidal activity (Camele et al., 2012). The essential oil and various extracts obtained from *Origanum acutidens* and methanol extracts were evaluated for their antioxidative, antimicrobial and antiviral properties obtained from callus cultures (MeOH). In particular, the essential oil exhibited strong antimicrobial activity with a significant inhibitory effect against 27 of 35 bacteria (77%), 12 of 18 fungi (67%) and tested. GC and GC-MS analyses resulted in the identification of 38 components of the oil, the main component being carvacrol. MeOH Extracts obtained from plant parts showed better antioxidative activity than butylated extracts (Sökmen et al., 2004). *Origanum onites* and other essential oils were evaluated for their antibacterial and antifungal properties. The investigated oils showed antimicrobial activity and provided antifungal activity at a high concentration of 200 µg/mL. The fungi that *Origanum onites* is active against can be listed as follows; *Colletotrichum acutatum, C. fragariae and C. gloeosporioides* (Altintas et al., 2013). 83.37% carvacrol was observed as the main component in the essential oil obtained from *Origanum vulgare*. Germination of *Aspergillus flavus, A. parasiticus, A. terreus, A. ochraceus, A. fumigatus* and *A. niger* agents was inhibited by the obtained oil (Carmo et al., 2008; Mitchell et al., 2010). *Origanum vulgare* essential oil was found to be effective against *Colletotrichum gloeosporoides, F. oxysporum, Rhizoctonia solani, P. ultimum, Botrytis cinera* (Sun et al., 2007) (Table 1).

Plant essential oils can potentially replace synthetic fungicides in the management of postharvest fruit and vegetable diseases. This study evaluated the in vitro and in vivo activities of thymol, carvacrol, linalool and trans-caryophyllene, the single components of *Origanum vulgare* essential oil, against *Monilinia laxa, M. fructigena* and *M. fructicola,* which are important phytopathogens and causative agents of brown rot in pome and stone fruits before and after harvest. Furthermore, the possible phytotoxic activities of these components were evaluated and their minimum inhibitory concentrations (MICs) were determined. In vitro experiments showed that thymol and carvacrol had the highest inhibitory concentrations. The results of in vivo experiments confirmed the potent activity of thymol and carvacrol against brown rot of peach fruits. The MIC value of thymol was found to be 0.16 lg/lL against *M. laxa* and *M. fructigena* and 0.12 lg/lL against *M. fructicola*, while for carvacrol it was found to be 0.02 lg/lL against the first two *Monilinia* species and 0.03 lg/lL against the third. The results of this study showed that thymol and carvacrol can be used after appropriate formulation to control postharvest fruit diseases caused by the examined *Monilinia* species (Elshafie et al., 2015). In addition, the oil obtained from the *Origanum compactum* plant, which contains carvacrol and thymol in its components, was found to be

effective against *Alternaria alternata, Bipolaris oryzae, Fusarium graminearum, Fusarium equiseti, Fusarium verticillioides* agents (Santamarina et al., 2015) (Table 1).

It has been found to be quite successful against *Origanum majorana, Aspergillus niger, Monilinia fructicola, Penicillium expansum*. Its components include terpinen-4-ol, δ-2-carene, y-terpinene, carvacrol (Della Pepa et al., 2019). *Origanum acutidens* and its components are carvacrol, thymol, p-cymene; In vitro studies against *Alternaria alternata, A. solani, Botrytis* sp., *Fusarium acuminatum, F. culmorum, F. equiseti, F. nivale, F. oxysporum, F. sambucinum, F. semitectum, F. solani, Monilinia* sp., *Pythium ultimum, Phytophthora capsici, Rhizoctonia solani, Sclerotinia minor, Verticillium dahliae* have shown that thymol and carvacrol, which contain free hydroxyl groups in the p-cymene skeleton, are more fungitoxic than p-cymene (Kordali et al., 2008) (Table 1).

In the study, an alternative to the synthetic fungicides used was sought. *Botrytis cinerea*, a destructive fungal pathogen that causes gray mold disease in tomatoes. Antifungal activities of essential oils obtained from the aboveground parts of aromatic plants belonging to the Lamiacea family. Origanum (*Origanum syriacum* L. var. *bevanii*), lavender (*Lavandula stoechas* L. var. *stoechas)* and rosemary (*Rosmarinus officinalis* L.) were investigated against *B. cinerea*. It was found that the contact and volatile phase effects of different concentrations of essential oils inhibited the growth of *B. cinerea* in a dose-dependent manner. It was found that the volatile phase effects of essential oils were more effective on fungal growth than the contact phase effect. It was found that the volatile vapor of Origanum oil in air at 0.2 μg/ml completely inhibited the growth of *B. cinerea* (Soylu et al., 2010).

Plasmopara viticola is the most important pathogen in vine cultivation. Alternatives to reduce fungicides are needed to ensure a fully sustainable vineyard ecosystem, consumer health and public acceptance. Essential oils are among the most promising natural plant protection alternatives and have demonstrated antibacterial, antiviral and antifungal properties in various products. In the study, grape leaves were infected with *P. viticola* and then continuously fumigated. *Origanum vulgare* essential oil was proven to be sufficient to reduce the development of the pathogen by 95% 24 hours after infection during the steam treatment (Rienth et al., 2019) (Table 1).

Legumes products such as chickpeas, lentils and dry peas are widely grown for human and animal consumption. It is quite difficult to combat pathogens that cause yield losses. Alternative methods should be resorted to due to the negative effects of fungicides used on human and environmental health. Safe and environmentally friendly plant-derived essential oils have been reported to be effective against some pathogenic fungi. Growth in essential oil supplemented growth media and inverted petri plate assay were used to determine the effects of 38 oils and their volatiles on mycelial growth and spore germination of important pathogenic fungi: *Aphanomyces euteiches, Botrytis cinerea, Colletotrichum lentis, Didymella pisi, D. rabiei, D. lentis, Fusarium avenaceum, Stemphylium beticola, Sclerotinia sclerotiorum* and *Pythium sylvaticum.* Addition of palmarosa, thyme, clove, cinnamon, lemongrass, citronella and oregano oils to the media inhibited the mycelial growth of all pathogens by 100% at 1:1,000 to 1:4,000 dilutions (Parikh et al., 2021) (Table 1).

The antioxidant and antimicrobial activities of methanol or dichloromethane extracts obtained from wild and organic culture samples of *Origanum dictamnus* were determined. Rancimate and malondialdehyde (MDA) were used to measure the antioxidant activity by HPLC methods in comparison with common commercial antioxidants butylated hydroxytoluene (BHT) and α-tocopherol. Extracts showing high antioxidant activity were encapsulated in liposomes and their antioxidant activity was estimated again by differential scanning calorimetry (DSC). Thermal-oxidative decomposition of samples (pure liposomes and encapsulated extracts) and

modification of the main transition temperature for lipid mixture and splitting of the calorimetric peak in the presence of antioxidants were also investigated by DSC method. All extracts showed antioxidant and antimicrobial activities. Their effects were proved to be superior to α-tocopherol. Methanol extract (240 ppm) of organically grown *O. dictamnus* showed higher activity than butylated hydroxytoluene. After encapsulation in liposomes, antioxidant and antimicrobial activities were proven to be higher than the same extracts in pure form (Gortzi et al., 2007) (Table 1).

In the study, in vitro antifungal activities of essential oils and their main components (carvacrol, trans-etol and 1,8-cineole) obtained from taxonomically different medicinal plants such as thyme (*Origanum syriacum* L.), fennel (*Foeniculum vulgare* Mill.) and laurel (*Laurus nobilis* L.) growing naturally in different regions of Hatay province were investigated. The effectiveness of essential oils investigated against soil-borne fungal disease agents of bean plants, *Sclerotium rolfsii, Rhizoctonia solani* and *Macrophomina phaseolina*, has been determined to be quite high (Türkölmez and Soylu, 2014) (Table 1).

EFFECT of *Origanum* spp. ESSENTIAL OILS AGAINST BACTERIAL DISEASES

Origanum heracleoticum, whose main component is carvacrol, has been found to be effective against fungal agents such as *Aspergillus niger, Botrytis cinerea, Monilinia fructicola, Penicillium expansum* and bacterial agents such as *Clavibacter michiganensis, Xanthomonas campestris, Pseudomonas fluorescens, Pseudomonas syringae* pv. *phaseolicola* (Della Pepa et al., 2019). *Clavibacter michiganensis* subsp. *michiganensis* (Smith) Davis et al. (Cmm) and *Ralstonia solanacearum* Yabuuchi et al. (Smith) (Rs) are important seed-borne bacterial pathogens of tomato (Solanum lycopersicum) listed as A2 pests in the EPPO (European and Mediterranean Plant Protection Organization) region. Currently, there are few strategies to control these pathogens and seed control with eco-compatible approaches is widely promoted. In this study, essential oils (EOs) of oregano (*Origanum vulgare*), garlic (*Allium sativum*), basil (*Ocimum basilicum*), cinnamon (*Cinnamomum zeylanicum*), clove buds (*Syzygium aromaticum*), thyme (*Thymus vulgaris)* were tested in vitro for their antimicrobial activities against Cmm and Rs. The tested essential oils caused significant inhibition of bacterial growth with very promising MBC (minimum bactericidal concentration) and MIC90 (minimum inhibitory concentration that causes 90% growth inhibition) values. Furthermore, in vivo germination test showed that there was no significant reduction in seed germination when the substances were applied as seed treatments. To test the antimicrobial activity of thyme and cinnamon oil in seed treatment in vivo, a rapid molecular screening method was developed for the specific quantification of Cmm in the presence of a plant matrix by real-time PCR without resorting to time- and space-consuming whole plant experiments (Orzali et al., 2020) (Table 1).

Hydrodistilled essential oils from *Origanum acutidens, O. rotundifolium* and *O. vulgare*. showed a broad spectrum of antibacterial activity on *Xanthomonas axonopodis*. At the same time, other main components such as carvacrol, thymol and terpinen-4-ol and linalool were found to have antimicrobial activity (Dadaşoğlu et al. 2011) (Table 1).

In vitro activity of *Origanum heracleoticum* L. essential oil compounds was tested against *Verticillium dahliae, Fusarium oxysporum, Phoma tracheiphila, Phytophthora cactorum* and *Botrytis cinerea* using four different concentrations (1×104 to 1×10-2 µg.ml-1) by well diffusion method. Carvacrol and plain essential oil showed inhibitory effect against all tested pathogens at higher concentrations (Salomone et al., 2008). In this study, essential oils of four aromatic plants were tested. The compositions and antimicrobial effects of the essential oils of cinnamon (*Cinnamomum zeylanicum*), thyme (*Thymus vulgaris*), oregano (*Origanum vulgare*) and clove (*Syzygium aromaticum*) were investigated against plant pathogenic bacteria (*Pectobacterium* spp. and *Pseudomonas* spp.). Both are commonly associated with diseased fruit trees. As a result, it

was concluded that some essential oils can be used for the control of postharvest bacterial pathogens. The findings of the present study showed that essential oils have the potential to be used as antibacteial agents (Božik et al., 2017) (Table 1).

Erwinia amylovora was found to be susceptible to essential oils obtained from *Melissa officinalis, Mentha arvensis, Origanum compactum, O. vulgare, Thymus vulgaris, Eugenia caryophyllata, Mentha pulegium* plants (Kokoškova and Pavela, 2007) (Table 1).

EFFECT OF *Origanum* spp. ESSENTIAL OILS AGAINST VIRAL DISEASES

A study aimed to evaluate the antiphytoviral activity of applications with three essential oils obtained from *Origanum vulgare, Thymus vulgaris* and *Rosmarinus officinalis* and their corresponding hydrosols on *Zucchini Yellow Mosaic Virus* and *Tomato Leaf Curl New Delhi Virus* infected squash plants. Applications were applied after or simultaneously with virus inoculation to detect an inhibition or curative activity, respectively. Symptoms were observed and weekly samplings were done. Virus titer and expression levels of phenylalanine ammonia lyase gene (PAL) were measured in treated and untreated infected plants by real-time PCR. PAL gene plays an important role in plant defense response as it is involved in tolerance/resistance to phytopathogens. A significant inhibition was observed with *O. vulgare* essential oil and hydrosol, resulting in a 10-4 fold reduction in virus titer after 3 weeks of treatment (Taglienti et al., 2022) (Table 1).

Table 1. *Origanum* species found effective in controling plant diseases and the pathogens they affect

Origanum sp. from which essential oil is obtained	**Fungal Agent**	**Bacterial Agent**	**Viral Agent**	**References**
Origanum spp.	*Fusarium oxysporum f. sp. phaseoli*			Guzmán-Guzmán et al., 2003
Origanum vulgare	*Botrytis cinerea* *Penicillium italicum* *Penicillium expansum* *Phytophthora citrophthora* *Rhizopus stolonifer*			Camele et al., 2012
Origanum acutidens	*Alternaria solani* *Aspergillus flavus* *Aspergillus niger* *Fusarium oxysporum* *Fusarium solani* *Moniliania fructicola* *Penicillum spp.* *Rhizopus spp.* *Rhizoctonia solani* *Sclorotinia sclerotiorum*	*Clavibacter michigan ense* *Pseudomonas syringa e* pv.tomato *Xanthomonas campes tris*		Sökmen et al.,2004
Origanum onites	*Colletotrichum acutatum* *Colletotrichum fragariae* *Colletotrichum gloeosporioides*			Altintas et al.,2013
Origanum vulgare	*Aspergillus flavus* *Aspergillus parasiticus* *Aspergillus terreus* *Aspergillus ochraceus* *Aspergillus fumigatus* *Aspergillus niger*			Carmo et al., 2008 Mitchell et al., 2010
Origanum vulgare	*Colletotrichum gloeosporoides* *Fusarium oxysporum,* *Rhizoctonia solani* *P.ultimum* *Botrytis cinera* *Monilinia laxa* *M..fructigena* *M. fructicola*			Sun et al., 2007 Elshafie et al., 2015
Origanum compactum	*Alternaria alternata* *Bipolaris oryzae* *Fusarium graminearum* *Fusarium equiseti* *Fusarium verticillioides*			Santamarina et al., 2015
Origanum majorana,	*Aspergillus niger* *Monilinia fructicola* *Penicillium expansum*			Della Pepa et al., 2019
Origanum acutidens	*Alternaria alternata* *Alternaria solani* *Botrytis* sp. *Fusarium acuminatum* *Fusarium culmorum* *Fusarium equiseti* *Fusarium nivale*			Kordali *et al.*,2008

	Fusarium oxysporum *Fusarium sambucinum* *Fusarium semitectum* *Fusarium solani* *Monilinia sp.* *Pythium ultimum* *Phytophthora capsici* *Rhizoctonia solani* *Sclerotinia minör* *Verticillium dahliae*			
Origanum syriacum	*Botrytis cinerea*			Soylu et al., 2010
Origanum vulgare	*Plasmopara viticola*			Rienth et al., 2019
Origanum vulgare	*Aphanomyces euteiches* *Botrytis cinerea* *Colletotrichum lentis* *Didymella pisi,* *Didymella rabiei* *Didymella lentis* *Fusarium avenaceum* *Stemphylium beticola* *Sclerotinia sclerotiorum* *Pythium sylvaticum*			Parikh et al., 2021
Origanum syriacum	*Sclerotium rolfsii* *Rhizoctonia solani* *Macrophomina phaseolina*			Türkölmez ve Soylu, 2014).
Origanum heracleoticum	*Aspergillus niger* *Botrytis cinerea* *Monilinia fructicola* *Penicillium expansum*	*Clavibacter michiganensis* *Xanthomonas campestris* *Pseudomonas fluorescens* *Pseudomonas syringae pv. phaseolicola*		Della Pepa et al., 2019
Origanum vulgare		*Clavibacter michiganensis subsp. Michiganensis* *Ralstonia solanacearum*		Orzali et al., 2020
Origanum acutidens *O. rotundifolium* *O. vulgare*		*Xanthomonas axonopodis*		Dadaşoğlu ve ark. 2011
Origanum heracleoticum	*Verticillium dahliae, Fusarium oxysporum, Phoma tracheiphila, Phytophthora cactorum* *Botrytis cinerea*			Salomone et al., 2008
Origanum vulgare		*Pectobacterium spp.* *Pseudomonas spp.*		Božik et al., 2017
Origanum compactum *O. vulgare*,		*Erwinia amylovora*		Kokoškova ve Pavela, 2007
Origanum vulgare			*Zucchini Yellow Mosaic Virus, Tomato Leaf Curl New Delhi Virus*	Taglienti et al., 2022

CONCLUSIONS

In recent years, interest in the biological activities of natural compounds has been increasing due to the increasing use of fungicides that pose a risk to human and environmental health. In plant protection, essential oils are reported to exhibit antiviral, antifungal and antibacterial activities and are considered promising for the formulation of safe antimicrobial agents. Essential oils are considered as an alternative method due to their low cost and availability, and further research is required to make their use widespread.

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FROM SOIL TO SURVIVAL: PGPB-TRIGGERED DEFENSE AND ADAPTATION IN PLANTS

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ABSTRACT

Plants and beneficial soil bacteria engage in dynamic and reciprocal interactions that influence plant development, nutrient acquisition, and stress resilience. Among these, plant growth-promoting bacteria (PGPB) have garnered considerable attention due to their ability to support plant health through a range of biochemical and physiological mechanisms. These bacteria produce a variety of bioactive compounds that enhance plant stress tolerance, improve nutrient availability, and offer protection against phytopathogens.

PGPB influence plant performance through both direct and indirect mechanisms. Direct pathways include the biosynthesis of phytohormones (such as indole-3-acetic acid), solubilization of essential nutrients like phosphate, zinc, and potassium, ammonia production, and atmospheric nitrogen fixation. Indirectly, they contribute by secreting siderophores, lytic enzymes, hydrogen cyanide, and antibiotics, which suppress harmful microorganisms and enhance plant immunity.
These functional traits position PGPB as valuable components in sustainable agriculture, especially for the development of bioformulants including biofertilizers, biopesticides, and biofungicides. Such alternatives reduce dependency on chemical inputs and contribute to environmentally responsible crop management. Nonetheless, despite the expanding repertoire of beneficial strains, the practical implementation of PGPB in agriculture remains challenging. Factors such as microbial survival, strain specificity, plant-microbiome compatibility, and fluctuating environmental conditions can influence their efficacy in field applications.

This review explores recent advances in understanding PGPB-mediated plant adaptations to abiotic and biotic stressors, with emphasis on molecular mechanisms, signaling pathways, and metabolite production. It also discusses formulation strategies and delivery systems designed to maximize their stability and performance. Ultimately, leveraging PGPB potential requires a deeper integration of microbiology, plant physiology, and environmental science to ensure their consistent and scalable use in sustainable agricultural systems.

Keywords: Plant Growth-Promoting Bacteria, stress tolerance, sustainable agriculture, biocontrol

INTRODUCTION

Through the release of bioactive chemical signals, bacteria and plants interact and communicate in a dynamic and ongoing co-evolutionary relationship. Both positive and negative interactions are possible (Wille et al., 2019). The plants gain from positive relationships because they help them acquire minerals, phytohormones, and other nutrients. By releasing a number of bioactive chemicals, these helpful bacterial species can also combat phytopathogens and help plants withstand a variety of stressful situations. Conversely, detrimental interactions are detrimental because infections infiltrate plant tissues, which causes the host plants to die (Dolatabadian, 2021; Adedayo et al., 2022). Utilizing these advantageous microbes can therefore help plants withstand stress that reduces their yield.

As part of the soil ecosystem, microorganisms have a major impact on the nitrogen cycle, soil fertility management, and plant diversity preservation. According to Zhou et al. (2020), a crucial area for important biological interactions between plants and microorganisms is the rhizosphere, a tiny area that surrounds the plant roots. Microorganisms such as bacteria, actinobacteria, fungus, algae, and protozoa aggressively compete for food and space in this productive area (Manghwar et al., 2023). Both the host and the microorganisms benefit from the ability of the plant growth promoting microorganism (PGPM) to live in and interact with plant roots; a population of rhizospheric bacteria and fungi may also serve as a home for other microbes (dos Lopes et al., 2021). The most prevalent of all the helpful microorganisms are bacteria, which are followed by fungus and actinobacteria (Poria et al., 2021). Through direct or indirect methods of nutrient absorption and assimilation, they have a beneficial effect on the plant (Kumari et al., 2018).

Food security is threatened by the world's population growth, which increases the need of fertilizers based on inorganic chemicals, which is bad for the environment and human health (Mitter et al., 2021). Because of the numerous environmental stressors that also contribute to low crop yields, organic farming that relies on microflora such as PGPM guarantees food availability while improving crop quality, productivity, and environmentally friendly farming practices (Jalal, et al., 2023). Therefore, by reducing the use of chemical fertilizers, crop production using PGPM provides sustainability and protects soil biodiversity (dos Lopes et al., 2021).

The majority of plant growth-promoting bacteria (PGPB) are proteobacteria. It includes genera like *Pantoea, Thiobacillus, Pseudomonas, Micrococcus, Rhodococcus, Azospirillum, Azotobacter, Acinetobacter, Acetobacter, Klebsiella, Enterobacter, Alcaligenes, Arthrobacter, Burkholderia, Azorhizobium, Achromobacter, Serratia, Bradyrhizobium, Flavobacterium, Mesorhizobium, Microrhizobium, Streptomyces, Bacillus, Azoarcus, Aeromonas, Azoarcus, Caulobacter, Chromobacterium, Delftia, Frankia, Flavobacterium, Gluconacetobacter, Paenibacillus, Rhizobium* and *Streptomyces* (Table 1).

In addition to helping plants tolerate a variety of biotic and abiotic stressors, the PGPB also promotes plant growth by promoting the solubility of various inorganic mineral nutrients, fixing nitrogen, releasing plant growth regulators, and producing a number of other biochemicals that either directly or indirectly increase plant productivity. Figure 1 shows the several ways that bacteria can promote plant growth.

Table 1. Plant growth promoting bacteria and their common host plant family (from Fanai et al., 2024).

Family	**Plant**	**Plant growth-promoting bacteria**
Fabaceae	*Phaseolus vulgaris*	*Rhizobium acidosoli, R. endophyticum, R. esperanzae, R. etli, R. hidalgoense, R. mesoamericanum, R. tropici, Acinetobacter* sp.
Poace	Rice, wheat, maize, soirghum, sugarcane	*Azospirillum* sp.
Asteraceae	Puticaria	*Bacillus cereus, Agrobacterium fabrum, Brevibacillus brevis, Bacillus subtilis, Paenibacillus* sp., *Acinetobacter radioresistant, Burkholderia* sp.
Solanaceae	*Artemisia annua*	*Brevibacillus* sp., *Bacillus* sp., *Pseudomonas* sp., *Azospirillum* sp., *Klebsiella* sp., *Enterobacter* sp., *Alcaligenes* sp., *Azotobacter* sp., *Streptomyces* sp., *Pantoea* sp., *Bacteroides* sp., *Proteobacteria* sp., *Radiobacter* sp., *Stenotrophomonas* sp.
Brassicaceae	*Brasica oleraceae*	*Pseudomonas* sp., *Enterobacter* sp., *Arthrobacter* sp., *Pantoea* sp.
Crasulaceae	*Echevari laui*	*Erwinia* sp., *Pantoea* sp.

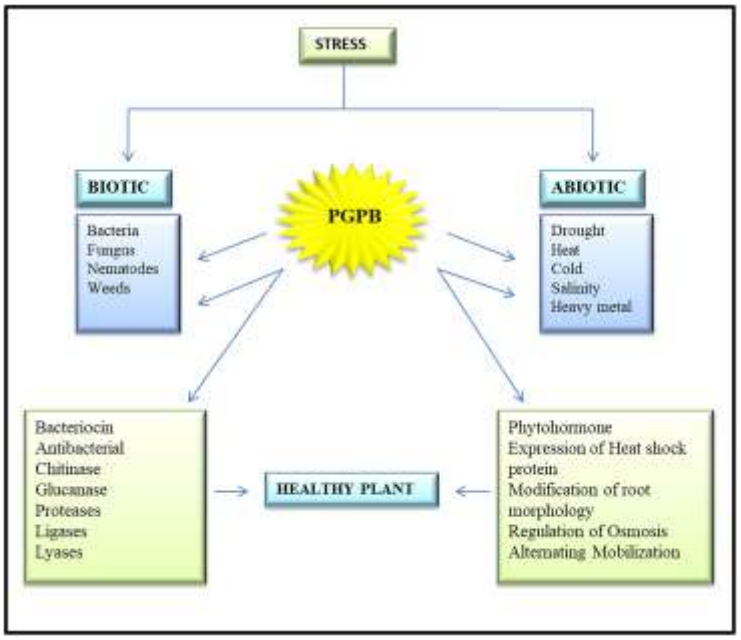

Figure 1. Functions that plant growth-promoting bacteria provide. The impacts of both biotic (caused by living things) and abiotic (induced by environmental variables) stress on plants demonstrate how PGBP helps to lessen the effects of stress. Through processes including siderophores, lytic enzymes, HCN, antibiotics & nitrogen fixation, hormone synthesis, etc., PGBP improve plant development, nutrient absorption, and stress tolerance (from Fanai et al., 2024).

One possible approach to a sustainable future is the use of helpful microorganisms as bioinoculants in agricultural systems. As an effective, environmentally friendly, and productive fertilizer that could help meet the growing demand for food from the world's population, it is a

good substitute for chemical fertilizers. They improved the soil microbiota and preserved soil fertility in addition to promoting plant growth.

ABIOTIC AND BIOTIC STRESS FACTORS

Continuously subjecting plants to biotic and abiotic stressors has a negative effect on their growth and development, which lowers yield and quality (Singh et al., 2021). With the help of a naturally occurring PGPB that increases resistance against different phytopathogens by generating biochemicals and improving soil fertility, plants subsequently develop particular kinds of defense mechanisms for stress response (Leontidou et al., 2020). Figure 2 shows the various stressors and bacterial stress tolerance systems.

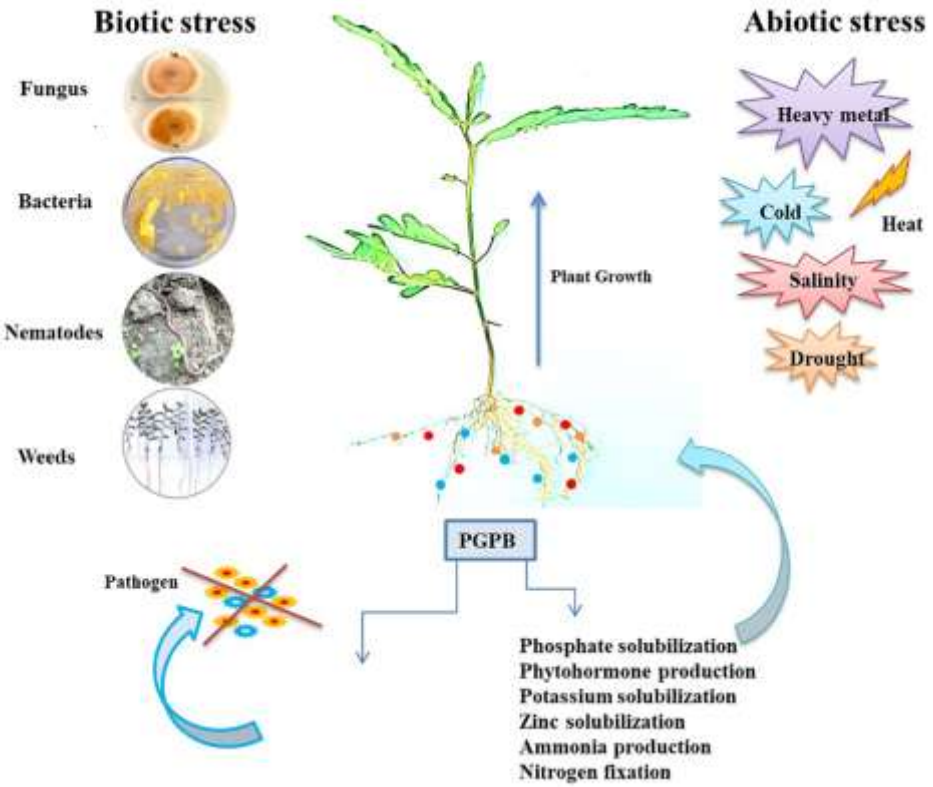

Figure 2. Various stressors and bacteria that support plant growth react to provide plant growth promotion. The impacts of both biotic (caused by living things) and abiotic (induced by environmental variables) stress on plants demonstrate how PGBP helps to lessen the effects of stress. By means of processes like nitrogen fixation, hormone synthesis, and biocontrol, PGBP improve plant growth, nutrient absorption, and stress tolerance (from Fanai et al., 2024).

Drought stress is categorized into hydrological, socio-economic, meteorological, and agricultural types, each leading to soil moisture deficits and the accumulation of reactive oxygen species that damage plant structure and function (Ahluwalia et al., 2021). Traditional mitigation measures—such as biochar amendments, nanoparticle treatments, film farming, and drought-resistant cultivars—have shown only limited benefits in real agricultural systems (Fadiji et al., 2022). By contrast, plant growth-promoting bacteria (PGPB) enhance drought tolerance through improved water uptake, root architecture modification, and production of protective phytochemicals (Khan and Bano, 2019). For example, inoculation with *Bacillus pumilus* and

Pseudomonas putida improved maize drought resilience and nutrient acquisition (Kálmán et al., 2024), while *Bacillus subtilis* and *Azospirillum brasilense* increased osmolyte synthesis to support wheat germination under water deficit (Ilyas et al., 2020). Strains such as *Pseudomonas lini* and *Serratia plymuthica* enhance soil aggregate stability, and *Bacillus albus* together with *Bacillus cereus* boost seed vigor, pigment concentration, and antioxidant activity, collectively strengthening photosynthetic efficiency and stress defenses (Zhang et al., 2020; Ashry et al., 2022; Saleem et al., 2021). Other notable drought-tolerant bacteria include *Achromobacter xylosoxidans*, *Pseudomonas aeruginosa*, *Lelliottia adecarboxylata*, *Enterobacter cloacae*, *Pseudomonas fluorescens* S3X, and *Staphylococcus sciuri* (Danish et al., 2020; Khalilpour et al., 2021).

A rise in average global temperatures poses a significant abiotic challenge for crop productivity (Desaint et al., 2021). Heat-tolerant microbes—including *Bacillus cereus, Serratia liquefaciens, Pseudomonas putida, P. fluorescens* (Mitra et al., 2021), *Burkholderia phytofirmans*, *Curvularia protuberata* (Rana et al., 2021), *Paraburkholderia phytofirmans*, and various *Bacillus* and *Pseudomonas* spp. (Ahmad et al., 2023)—mitigate thermal damage by modulating plant hormone levels (e.g., cytokinins, ACC deaminase) and activating antioxidant enzymes that control water uptake and induce heat-shock protein expression (Moumbock et al., 2021). Exogenous application of specific amino acids further alleviates heat-stress effects by enhancing stress-responsive pathways (Santos et al., 2022). Inoculation with *B. cereus* has been shown to boost overall biomass, chlorophyll content, and heat-shock protein accumulation (Khan et al., 2020b), while *Enterobacter* sp. SA187 increased wheat and *Arabidopsis* heat endurance, leading to higher grain yield, plant height, and seed weight. Moreover, *B. cereus* enhances carotenoid, protein, ascorbate peroxidase, chlorophyll, and superoxide dismutase levels (Bisht and Mishra, 2020), and strains such as *B. thuringiensis, B. subtilis, P. brassicacearum* (Ashraf et al., 2019), and *B. velenzensis* further demonstrate the promise of microbial inoculants for improving plant thermotolerance.

Soil salinization, driven by water scarcity and the accumulation of NaCl-rich compost from sewage and waste treatment, and the build-up of soluble ions—bicarbonate, magnesium, sodium, chloride, sulfate, carbonate, and calcium—alters soil composition, diminishes fertility, impairs germination, and disrupts chloroplast structure, leading to reduced pigment synthesis, ion toxicity, and osmotic imbalance (Ahmed et al., 2020; Krishnamoorthy et al., 2022). Salinity stress further reduces plant development, fruit yield, biomass, stomatal conductance, and water movement (Ansari et al., 2019). Plant growth-promoting bacteria counteract these adverse effects by inducing stress-responsive pathways that lower reactive oxygen species production, synthesizing Na^+-binding exopolysaccharides, and producing phytohormones to enhance root growth and water uptake (Subramaniam et al., 2020). For instance, *Bacillus amyloliquefaciens* GB03 releases volatile organic compounds that boost stress mitigation sixfold (Cappellari et al., 2020). Additionally, halotolerant strains of *Streptomyces*, *Aneurinibacillus aneurinilyticus*, *Paenibacillus* spp., *Pseudomonas azotoformans*, and other genera such as *Bacillus, Enterobacter, Klebsiella, Agrobacterium*, and *Ochrobactrum* can tolerate NaCl up to 150 g/L, highlighting their promise in reclaiming saline soils (Liu et al., 2021).

Plant growth-promoting microbes (PGPM) suppress biotic stresses from pathogenic fungi, bacteria, nematodes, insects, and weeds by activating systemic acquired resistance (SAR) and induced systemic resistance (ISR). SAR arises when PGPM directly inhibit pathogens, whereas ISR is triggered by bacterial elicitors such as volatile organic compounds (VOCs), microbe-associated molecular patterns (MAMPs), and bioactive secondary metabolites (Dubey et al., 2020). These microbes employ lytic enzymes, antibiotics, and VOCs for direct antagonism and enhance plant defenses indirectly through nitrogen fixation, siderophore production, phosphate solubilization, and phytohormone signaling (Migunova and Sasanelli, 2021). For instance, *Pasteuria penetrans* and *Brevibacillus laterosporus* secrete proteases that inhibit *Heterodera glycines* (Mohan et al., 2020), while *Bacillus megaterium* combats *Meloidogyne graminicola* similarly. Strains like *Pseudomonas aeruginosa, P. cepacia*, and *P. fluorescens* exhibit broad-

spectrum antimicrobial and anthelmintic activities (Bhavya and Geetha, 2021), and *Bacillus subtilis* and *B. pumilus* produce chitinases and antifungal VOCs that disrupt nematode chitin synthesis. *Bacillus amyloliquefaciens* FZB42 and *Arthrobacter nicotianae* release bacteriocins and VOCs that inhibit *Meloidogyne* spp. By colonizing the rhizosphere and producing siderophores, phytohormones, and antibiotics, PGPM also inhibit soil-borne fungi, solubilize phosphate, and promote plant growth via indole-3-acetic acid synthesis. These combined actions outcompete pathogens for nutrients and induce ISR against bacterial genera—such as *Erwinia*, *Pectobacterium*, *Pantoea*, and *Xanthomonas*—that cause wilts, blights, galls, and root rots (Nazari and Smith, 2020; Nabila and Kasiamdari, 2021).

Table 2. Overview of Plant Growth-Promoting Rhizobacteria functions (from Priyanka et al., 2020)

Function of PGPR	**Description**	**PGPR strain**
Nitrogen fixation	Conversion of free atmospheric nitrogen into oxide form that can be used	*Enterobacter, Klebsiella, Burkholderia,* and *Stenotrophomona*
Phosphate solubilization	Conversion of insoluble phosphate into the soluble form absorbed by the plants	*Achromobacter, Agrobacterium, Bacillus, Enterobacter, Erwinia, Escherichia, Flavobacterium, Mycobacterium, Pseudomonas* and *Serratia*
Potassium solubilization	Conversion of potassium in the useful form	*Acidothiobacillus ferrooxidans*, *Bacillus edaphicus*, *Bacillus mucilaginosus*, *Burkholderia*, *Paenibacillus* sp. and *Pseudomonas*
Production of siderophore	Enhancing the source of iron in the soil that can be absorbed by the plants	*Aeromonas*, *Azadirachta*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Pseudomonas*, *Rhizobium*, *Serratia* and *Streptomyces* sp.
Phytohormone production	Production of phytohormones such as auxin, cytokinins, gibberellic acid and ethylene	*Azotobacter*sp., *Rhizobium* sp., *Pantoea agglomerans*, *Rhodospirillum rubrum*, *Pseudomonas fluorescens*, *Bacillus subtilis* and *Paenibacillus* sp.

DIRECT AND INDIRECT MECHANISMS OF PGPB IN ENHANCING PLANT PERFORMANCE

Indirect mechanisms

Siderophore production: Bacteria secrete ferric-specific ligands (hydroxamates, phenolates, carboxylates) to solubilize iron for themselves and host plants, enhancing root colonization and outcompeting pathogens; common producers include *Bacillus*, *Chryseobacterium*, *Phyllobacterium*, and *Pseudomonas* spp. (Bhatt et al., 2019).

Protease secretion: Microbial proteases (alkaline, acidic, neutral)—notably from *Bacillus* spp.—hydrolyze pathogen proteins and generate antifungal metabolites (e.g., serine proteases,

subtilisins), serving as an indirect plant defense; activity is screened on skim-milk agar by clear halo formation (Castaldi et al., 2021).

Catalase activity: Catalase-positive strains (e.g., *Bacillus marinus, B. sphaericus, Staphylococcus aureus*) detoxify H_2O_2 at root interfaces, mitigating oxidative damage under stress; detected by bubble formation in 3% H_2O_2 (Talaiekhozani, 2022).

Amylase production: Alpha-amylases from endophytes like *Bacillus amyloliquefaciens* and *B. licheniformis* degrade pathogen cell walls and contribute to industrial applications (detergents, stain removal); screened on starch agar via iodine-stained halos (Ismail et al., 2021).

Urease activity: Ureolytic bacteria hydrolyze urea into plant-available ammonium and nitrates, supporting nitrogen nutrition and biomineralization (soil enrichment, concrete repair); measured by pH-dependent color change in urea broth (Cui et al., 2022).

Hydrogen cyanide (HCN) release: Rhizobacteria (mostly *Pseudomonas* and *Bacillus* spp.) emit HCN to inhibit fungi, nematodes, insects, and weeds without harming the host plant; detected via alkaline-picrate colorimetric assay (Alemu, 2016).

Table 3. Mechanisms and functions of PGPB (adapted from Olanrewaju et al. (2017) and Fanai et al. (2024))

Mechanism	**Category**	**Primary Function**	**Reference**
Indole-3-acetic acid (IAA)	Direct	Stimulates root initiation, cell division, lateral root formation	Olanrewaju et al. (2017)
ACC deaminase activity	Direct	Degrades precursor of ethylene; alleviates abiotic-stress inhibition	Olanrewaju et al. (2017)
Cytokinin production	Direct	Promotes shoot growth, delays leaf senescence	Fanai et al. (2024)
Nitrogen fixation	Direct	Converts atmospheric N_2 into bioavailable NH_4^+	Fanai et al. (2024)
Phosphate solubilization	Direct	Releases organic acids to convert insoluble P into plant-available forms	Fanai et al. (2024)
Siderophore production	Indirect	Sequesters Fe^{3+}, suppresses pathogen growth, improves iron nutrition	Olanrewaju et al. (2017)
Lytic enzyme secretion	Indirect	Produces chitinases/proteases to degrade fungal and nematode walls	Olanrewaju et al. (2017)
Hydrogen cyanide (HCN) emission	Indirect	Volatile compound that inhibits soil-borne pathogens and weeds	Olanrewaju et al. (2017)
Antibiotic metabolite release	Indirect	Synthesizes antimicrobial compounds against bacteria and fungi	Olanrewaju et al. (2017)
Induced systemic resistance (ISR)	Indirect	Elicits plant defense pathways via MAMPs and VOCs	Fanai et al. (2024)

Direct mechanisms

Phytohormone production: Plant growth–promoting bacteria (PGPB) synthesize cytokinins, gibberellins, and especially indole-3-acetic acid (IAA), which regulate cell division, root initiation, and stress responses. Strains such as *Paenibacillus polymyxa, Rhizobium leguminosarum, Pseudomonas fluorescens* produce cytokinins (Mekureyaw et al., 2022), while *Bacillus pumilus* and *B. licheniformis* generate multiple gibberellins (Gutiérrez-Mañero et al., 2001). IAA producers, including *Azospirillum, Arthrobacter, Bradyrhizobium, Pantoea, Rahnella,* and *Enterobacter*, enhance root elongation and nutrient uptake (Rehman et al., 2020).

Phosphate solubilization: PSB release organic acids (citric, gluconic) that chelate cations and convert insoluble phosphates (tricalcium, rock phosphate) into bioavailable forms. Key taxa include *Pseudomonas putida, Azospirillum lipoferum, Bacillus firmus, B. polymyxa, Serratia*

marcescens, and *Arthrobacter aureofaciens*. PSB inoculation boosts phosphorus availability and activates stress-responsive genes in crops like potato and sugarcane (Lin et al., 2023).

Ammonia production: Ammoniagenic PGPB such as *Pseudomonas putida*, *Klebsiella* spp., and *Enterobacter asburiae* generate NH_3, raising soil pH to suppress pathogens and supply inorganic nitrogen, which enhances root/shoot growth and biomass (Gohil et al., 2022).

Nitrogen fixation: Symbiotic genera (*Bradyrhizobium, Mesorhizobium, Sinorhizobium, Azorhizobium, Neorhizobium*) and free-living diazotrophs (*Azotobacter, Azospirillum, Burkholderia, Pseudomonas*, cyanobacteria) convert atmospheric N_2 into plant-available forms, supporting growth and yield (Basile and Lepek, 2021). Microbial consortia of *B. subtilis* and *A. brasilense* enhance root/shoot development, gas exchange, and grain yield in wheat, while co-inoculation of *Bradyrhizobium* and *Bacillus* improves nodulation and *Vigna unguiculata* productivity (Galindo et al., 2024; Gaspareto et al., 2023).

Zinc solubilization: Zinc is a vital enzyme co-factor, with optimal plant tissue levels at 30–100 $mg \cdot kg^{-1}$; deficiency impairs growth and causes necrotic lesions. Zinc-solubilizing bacteria secrete organic acids and iron-chelating enzymes to lower rhizosphere pH and release Zn^{2+} (Eshaghi et al., 2019). Key taxa include *Pseudomonas aeruginosa, Gluconacetobacter diazotrophicus, P. striata, P. fluorescens, Burkholderia cenocepacia, Serratia liquefaciens, S. marcescens, Bacillus thuringiensis, B. aryabhattai, B. subtilis, Thiobacillus thioxidans*, and cyanobacteria. *Rhizobium, Pseudomonas*, and *Bacillus* spp. enhance Zn translocation and grain biofortification, improving wheat quality via exopolysaccharide and siderophore production (Jalal et al., 2024). Co-inoculation of *B. subtilis* or *P. fluorescens* with foliar ZnO further boosts maize chlorophyll, amino acids, glutelin, and prolamin content. Bacterial Zn nanoparticles inhibit biofilm formation in pathogens and augment human oral microbiome antimicrobial activity (Lallo da Silva et al., 2019).

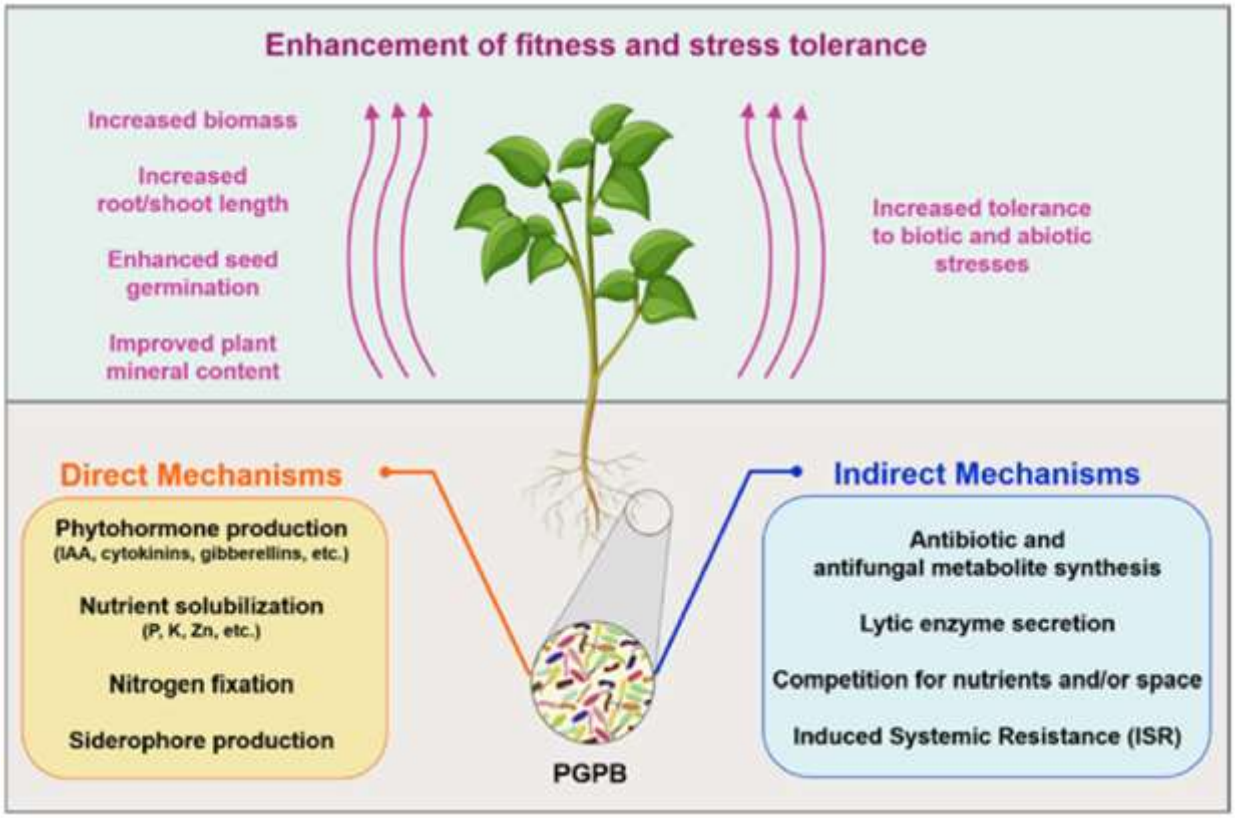

Figure 3. Overview of the main mechanisms used by PGPB to improve plant growth (from Vuolo et al., 2022)

Potassium solubilization: Although abundant in soils, K often exists in insoluble forms; PGPB release organic acids (citric, oxalic, tartaric) to chelate and solubilize K^+, facilitating uptake (Olaniyan et al., 2022). Potassium activates over 60 plant enzymes, regulates stomatal dynamics, enhances disease resistance, fiber strength, and produce quality, and mitigates water-stress sensitivity (Rawat et al., 2022). Notable K-solubilizers include *Acidithiobacillus, Burkholderia, Pseudomonas* spp., *Bacillus megaterium, Arthrobacter, Pantoea ananatis, Rahnella aquatilis, Enterobacter* sp., *Bacillus/Paenibacillus mucilaginosus, Bacillus licheniformis, P. azotoformans, B. edaphicus*, and *Pseudomonas putida* (Bakhshandeh et al., 2017).

CONCLUSION

Plant growth–promoting bacteria represent a cornerstone of sustainable agriculture by naturally supplying key metabolites—phytohormones, siderophores, lytic enzymes, hydrogen cyanide, and ammonia—and by solubilizing essential nutrients (P, Zn, K) or fixing atmospheric nitrogen. Their multifaceted modes of action not only enhance crop yield and quality under both biotic and abiotic stresses but also reduce reliance on synthetic agrochemicals, thereby protecting soil health and the broader environment.

To translate this promise into practice, future work must prioritize the development and field-ready formulation of robust biofertilizers and biocontrol agents tailored to specific crops and stress conditions. Equally critical is the systematic evaluation of PGPB–host compatibility to safeguard beneficial traits during root colonization, as well as the design of microbial consortia whose members coexist synergistically without compromising individual functionality. Addressing these challenges will pave the way for precision applications of PGPB, driving both productivity gains and ecological resilience in modern farming.

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ASSESSMENT OF CATAGLYPHIS NODUS (BRULLÉ, 1833) IN THE ECOSYSTEMS AROUND NARTA LAGOON AS ENVIRONMENTAL INDICATOR

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ABSTRACT

Ants (Family Formicidae) are essential eusocial insects crucial for ecosystems, often used as environmental indicators due to their sensitivity to ecosystem changes. This study examines the specific role of the species Cataglyphis nodus (Brullé, 1833), known for its adaptation to dry and high-temperature environments, within the wetland ecosystem of Narta Lagoon, an internationally important Protected Area in Albania. Through sampling with "Pitfall Traps" at 11 stations during June-July 2025, we analyzed the distribution of *C. nodus* based on soil moisture, soil type, vegetation, and anthropogenic impacts. The results clearly showed that the highest density of individuals was found in areas with sandy/gravelly, dry soil and herbaceous vegetation. Zones with agricultural activities and those used as pastures also showed a high presence, suggesting that human interventions might create suitable habitats for this xeric species. This highlights its role as a valuable ecological indicator in the lagoon ecosystem, signaling xeric (drought) conditions, open and sandy microhabitats, and anthropogenic impact. In conclusion, *C. nodus* is an important indicator species for monitoring drought trends, changes in habitat structure, and assessing the impact of human activities in lagoon ecosystems. Continued studies of this nature are recommended to gain clearer information on ecosystem adaptations and their services to climate change.

Keywords: Formicidae, indicators, ecological, xeric species, Cataglyphis nodus, lagoon.

INTRODUCTION

The family Formicidae, commonly known as ants, is a diverse and successful group of eusocial insects belonging to the order Hymenoptera. Ants are a dominant group of insects in many terrestrial habitats and play a key role in the functioning of ecosystems. Ants are extremely adaptable and successful insects, and their study has provided deep insights into social behavior, communication, and ecology. As predators, scavengers, seed dispersers, and ecosystem engineers, changes in ant populations can reflect larger changes in ecosystem functioning. Different ant species have different levels of tolerance to environmental changes (Tibcherani et al., 2018). Some are "opportunistic" species that thrive in altered environments, while others are "specialists" that are sensitive to disturbances. These characteristics make formicidae good indicators of environmental change and indicators of microhabitat conditions (Keshamma 2022). *Cataglyphis nodus* (Brullé et al., 1833) is a species of ant known to survive and thrive in extreme, arid environments (Agosti et al., 1990). It is able to physiologically adapt to harsh conditions, especially high temperatures (Boulay et al., 2017). This species is mainly found in arid and semi-arid regions of the Mediterranean basin, the Middle East and North Africa. They prefer sandy,

gravelly or clayey soils with good drainage, where they can build their underground nests (CERDA 2013). *Cataglyphis nodus* favors open habitats, with little dense vegetation, which allows maximum exposure to sunlight and increased surface temperatures. This makes them common in dunes, dry grasslands and degraded areas. They are among the few species that are active and hunt during the hottest hours of the day, tolerating ground temperatures that often exceed 50°C, when most other creatures hide. Their long legs help them keep their bodies off the hot ground. They have also developed specific hydrocarbons in their cuticle that minimize water loss and protect them from dehydration. They move at high speed and in an irregular manner to minimize contact time with the hot ground, and often seek out small objects such as leaves or pebbles to cling to and cool off for a few moments. They feed mainly on dead insects and other arthropods, which have often died from the heat. This makes them important scavengers in desert ecosystems. They may also consume live insects, as well as other food sources such as nectar, seeds, depending on availability. Due to their specific requirements for dry and hot environments, the presence and density of "*Cataglyphis nodus*" can serve as an indicator of xeric (drought) conditions, habitat degradation (creation of open and exposed areas), and the impact of anthropogenic activities (such as grazing or agriculture that favor dry and open lands) within ecosystems that may normally be wetter, as is the case with wetland ecosystems (antwiki.org). Overall, "Cataglyphis nodus" is an interesting species that highlights the power of natural adaptation to environmental challenges and provides valuable data on the health and changes of the ecosystems where it is found.

MATERIALS AND METHODS

Study Area

The Narta Lagoon is a key wetland ecosystem in Albania, of great national and international importance. Located north of the city of Vlora, it covers an area of approximately 42 km² and is part of the “Pishë Poro – Nartë” Protected Landscape, as well as the “Karaburun-Sazan” National Marine Park (Protected Landscape “Vjosë-Nartë”).

Narta is an area of particular importance for biodiversity. It has been declared a Ramsar Wetland (a wetland of international importance), as well as an Important Bird Area (IBA) (Bino et al., 2019). These statuses reflect its high ecological value.

As a coastal lagoon, Narta is characterized by brackish and semi-braked waters, which are influenced by the connection to the Adriatic sea and the freshwater inflows from small streams and canals. Its depth is generally shallow, which creates ideal conditions for many aquatic and wetland species.

The lagoon is a key resting and feeding point for thousands of migratory birds, including globally endangered species such as the flamingo and the dalmatian pelican. It also supports a rich variety of fish, amphibians, reptiles and mammals (Saçdanaku et al., 2020).

The plant landscape around Narta is diverse. In addition to typical wetland vegetation such as reedbeds, there are also areas with halophilic (salt-tolerant) vegetation, Mediterranean shrubs, sandy areas (dunes) and areas with herbaceous vegetation, especially on the outskirts and small islands (Meço et al., 2024).

Despite its protected status, the lagoon is affected by human activities such as traditional fishing, livestock grazing, and agricultural activities in the surrounding areas. These impacts can cause changes in habitats and pose challenges to sustainable ecosystem management.

Fig.1 Map of the study area and sampling points

As an area with a mediterranean climate (hot, dry summers), the Narta Lagoon and its coastal areas exhibit interesting interactions with drought-adapted species, such as *Cataglyphis nodus*, which serve as potential indicators of microhabitat shifts towards more xeric conditions.

Sampling method

Sampling was carried out at 11 stations (St.I to St.XI) within the lagoon ecosystem of the Narta lagoon. Sampling was carried out during June and July 2025. The method used for sampling is "Pitfall Traps" (Borowiec et al., 2022). The method consists of placing cups or containers on the ground, with the rim level with the ground surface (Jiménez-Carmona 2020). A preservative solution (water with detergent, 70-95% ethyl alcohol, or propylene glycol) is placed inside to capture and preserve the ants that fall inside. The traps are fitted with a small cover that can be placed on top of the trap to prevent rainwater from entering and reduce evaporation (antwiki.org). The determination of the samples was carried out based on the determination keys for the family formicidae for the European region (Key to Cataglyphis of Greece).

Fig.2 Sampling stations

Fig.3 Photo during laboratory determinations

RESULTS AND DISCUSSION

Data Analysis

Based on the data analysis, the following conclusions were reached (Table 1):

Station IV stands out immediately with 38 individuals (0.372%), while the other points have significantly lower numbers (2-12 individuals). This point is sandy, has herbaceous vegetation with few pine trees and is partially dry. This matches well with the preferences of Cataglyphis nodus.

Stations IX and X have sandy and clayey/gravelly soil, herbaceous vegetation, and are areas used as pastures. The soil moisture condition is "dry". These characteristics are very favorable for Cataglyphis nodus.

Station XI has sandy soil, herbaceous vegetation and agricultural trees, has agricultural activities and is "dry". This point also meets many of the ants' requirements.

Stations II and III, although "dry" and with pine forest, are clayey (not ideal) and have low numbers of individuals. The forest fire at St.III may have changed the conditions (perhaps making it more open and hotter) but the fact that the soil is clay remains a disadvantage.

Stations V and VI are sandy but "wet", explaining the low numbers.

Station VIII is sandy and clay, with vegetation and pine forest, and dry. We would expect more individuals here based on the dryness, but perhaps the dense pine forest vegetation affects things differently.

Table 1. General data collected in the field

Sampling Point	No. of Individuals	D (%)	Soil Type	Vegetation Types	Observations on Pollution/Changes	Soil Moisture Condition
St.I	6	0.06%	Clay	Herbaceous	No	Moist
St.II	4	0.04%	Clay	Pine Forest	No	Dry
St.III	2	0.02%	Clay	Pine Forest	No (forest burned a few years ago)	Dry
St.IV	38	0.37%	Sandy	Partially Pine and Herbaceous Vegetation	No	Partially Dry
St.V	5	0.05%	Sandy	Pine Forest and Herbaceous Vegetation	No	Moist
St.VI	5	0.05%	Sandy	Herbaceous Vegetation	No	Moist
St.VII	2	0.02%	Sandy	Herbaceous Vegetation	Moist, with water during the winter season	Moist
St.VIII	5	0.05%	Sandy and Clay	Herbaceous Vegetation and Pine Forest	Area used as pasture	Dry Soil
St.IX	12	0.12%	Sandy and Clay	Herbaceous Vegetation	Area used as pasture	Dry Soil
St.X	12	0.12%	Gravelly and Clay	Herbaceous Vegetation	Area used as pasture	Dry Soil
St.XI	11	0.11%	Sandy	Herbaceous Vegetation and Agricultural Trees	Agricultural activities	Dry

Analysis of the distribution of *Cataglyphis nodus* according to soil moisture condition

To assess the impact of moisture, the stations were grouped according to moisture condition and the average number of individuals was calculated:

Wet: Average individuals 4.5 (St.I, St.V, St.VI, St.VII)

Partially dry: Average individuals 38 (St.IV)

Dry: Average individuals 7.67 (St.II, St.III, St.VIII, St.IX, St.X, St.XI)

This distribution shows a clear trend: the points with lower humidity (partially dry and dry) exhibit a significantly higher number of *Cataglyphis nodus* individuals, with St.IV significantly dominating in this respect. Although the "dry" stations have a lower average than St.IV, they are the group with the second highest density, highlighting the preference for dryness.

Analysis of *Cataglyphis nodus* distribution by soil type

To understand soil preferences, stations were analyzed by substrate type:

Clay: Average of individuals 4 (St.I, St.II, St.III)

Sandy: Average of individuals 12.2 (St.IV, St.V, St.VI, St.VII, St.XI)
Sandy and Clay: Average of individuals 8.5 (St.VIII, St.IX)
Gravel and Clay: Average of individuals 12 (St.X)
The results show that soil types with sandy or gravelly content (Sandy, Sandy and Clay, Gravel and Clay) support larger populations of *Cataglyphis nodus* compared to clay soils. This is in line with the ecological requirements of desert species that prefer soils with good drainage and high drying rates.

Analysis of the impact of vegetation and anthropogenic changes

Herbaceous Vegetation: Stations with high numbers of individuals (St.IV, St.IX, St.X, St.XI) display dominant herbaceous or mixed vegetation. This indicates a preference for open areas, where sunlight can warm the soil surface without obstacles, a key requirement for thermophilic species.
Anthropogenic Impacts: The high-density points (St.IX, St.X) are located in areas used as pastures, and St.XI has agricultural activities. These activities may contribute to the creation of a more open and potentially drier habitat, favoring the presence of Cataglyphis nodus through the reduction of plant cover and exposure of the soil surface. The observation at St.III of the "burnt forest" suggests a change in habitat, but the clayey nature of the soil and the possible residual moisture may have limited the number of individuals.

Analyzing the Role of *Cataglyphis nodus* as an Ecological Indicator

Based on the above analyses, *Cataglyphis nodus* appears as a valuable ecological indicator in the wetland ecosystem, signaling the following directions.
Indicator of xeric conditions:
The presence and high density of *Cataglyphis nodus* is directly related to "dry" or "semi-arid" soil conditions. This suggests that areas with large populations of this species are experiencing habitat drying and may be under the influence of climatic or hydrological changes leading to more xeric conditions.
Indicator of open and sandy microhabitat:
The species' preference for sandy/gravelly soils and areas with herbaceous vegetation indicates that they serve as indicators of open, sun-exposed, and rapidly draining microhabitats. This is particularly significant within a wetland ecosystem that is typically characterized by humidity.
Anthropogenic Impact Indicator: High density in areas of pastures and agricultural activities suggests that human interventions may create or expand suitable habitats for Cataglyphis nodus, altering the local ecosystem.

CONCLUSIONS AND RECOMMENDATIONS

Sampling data clearly show that *Cataglyphis nodus* prefers and is found in dry, sandy and open microhabitats within the wetland ecosystem. This makes it an important indicator species for:

- Monitoring drought or desertification trends in coastal areas.
- Identifying changes in habitat structure that favor xeric species.
- Assessing the impact of human activities such as grazing and agriculture on the ecosystem.

The presence and high density of *Cataglyphis nodus* is directly related to "dry" or "semi-arid" soil conditions. The species' preference for sandy/gravelly soils and areas with herbaceous vegetation indicates that they serve as indicators of open, sun-exposed and rapidly draining microhabitats. This is particularly significant within a wetland ecosystem that is typically characterized by humidity
The use of environmental indicators provides us with clearer information on long-term environmental conditions and the state of microhabitats.
This study provides a solid basis for understanding the ecological role of *Cataglyphis nodus* and

their interaction with environmental conditions in a wetland ecosystem.
To protect our ecosystems in the future, it is recommended to continue such studies, as we obtain clearer information on ecosystem adaptations related to climate change.

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CURRENT STATUS OF SOME FISHERY COOPERATIVES IN GIRESUN (EASTERN BLACK SEA REGION) AND SOCIO-DEMOGRAPHIC CHARACTERISTICS OF ARTISANAL FISHERMEN

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ABSTRACT

In Giresun, one of the important fishing cities of the Black Sea with a total coastline of 122 km, the fishing gears used intensively in small-scale fishing are gillnet and trammel net and various hand lines. With these, fish species such as whiting, turbot, horse mackerel, anchovy, red mullet, garfish, bluefish are mainly caught. In addition, trout production is also abundant in the pools along the rivers in the region. The present study was conducted in the central district of Giresun with the objective of ascertaining the present status of fishery cooperatives and certain socio-demographic characteristics of fishermen, in addition to the problems with which they are confronted. The present study was conducted in the fishing ports of Giresun Fishery Cooperative, Üçkayalar Fishery Cooperative and Aksu Neighbourhood Fishery Cooperatives between October 2023 and April 2024. Thirty-two fishermen were interviewed in person. The participants were boat owners who were members of cooperatives in the ports. The status of fishery cooperatives in the region was determined, as were the regional fisheries and problems.

Keywords: Small-scale fisheries, marketing, cooperative, Eastern Black Sea

INTRODUCTION

As in all Eastern Black Sea cities, fishing is one of the most important occupations for the local population. Fishing in Giresun is conducted in a coastal manner, based on observing schools of fish. Anchovy, whiting, turbot, horse mackerel, sea bream, scorpionfish, red mullet, grey mullet, bluefish, and bonito are the most commonly targeted fish species. 3% of the total registered fishing vessels in our country are in Giresun, and a total of 563 boats operate in the fishing sector throughout the province, 545 of which are between 0-12 m in length and 18 of which are >12 m in length (Erdoğan Sağlam and Çalık, 2016a).

Fisheries cooperatives are a form of organization that brings together stakeholders engaged in fishing, processing, aquaculture, or marketing activities within the sector. These cooperatives are focused on the development of the fishing industry and the improvement of fishermen's welfare. They aim to increase income, improve living standards, and increase production. Their primary purpose is to rationally and economically meet and provide for the professional and livelihood needs of individuals lacking sufficient financial resources through mutual aid, solidarity, and guarantees (Ünal and Yercan, 2006).
The Black Sea Region, where fishing is widespread, accounts for 75% of the fish production (TRGM/TEPGE, 2024). Eastern Black Sea region is providing 58% of total production. Giresun is one of the most important fishing centers of the Eastern Black Sea Region. There are not many studies on the status of the fisheries cooperatives and fishermen operating in the city center. Some studies have been found on a general overview of fisheries in Giresun (Erdoğan Sağlam and Çalık, 2016a), an examination of fisheries associations and

cooperatives in Giresun (Erdoğan Sağlam and Çalık, 2016b), an economic analysis of fishing activities in Giresun (Mutlu et al., 2018), and the fishing coastal structures of Giresun Province (Tokmak Kırkses and Samsun, 2020). The purpose of this study is to examine the functioning of three fisheries cooperatives located in the central district of Giresun, to determine some socio-demographic characteristics of fishermen, and to identify current problems of the fishermen and cooperatives.

MATERIAL AND METHOD

This study was conducted at three different Fisheries Cooperatives and their fishing ports in the central district of Giresun between November 2023 and April 2024. Face-to-face interviews were conducted with 32 fishermen, members of the cooperatives at the fishing ports of the Giresun Fishery Cooperative, Üçkayalar Fishery Cooperative, and Aksu Neighbourhood Fishery Cooperative. The fisheries in the region, the socio-demographic characteristics of the fishermen, the current status of the cooperative, and their problems were identified on-site.

RESULTS

Fish auction is not held in the cooperatives studied. The catch fish is sold early in the morning to retailers at the fishing port. In addition, fish are sold to buyers from other provinces, especially Samsun. There is also a fish market in central Giresun, built by the Municipality and allocated the fishmongers. During anchovy season, fish are transport throughout Türkiye, but mostly to Ankara and Istanbul.

Giresun Fishery Cooperative

The oldest cooperative in Giresun province, the Giresun Fisheries Cooperative, was established in 1955. The fishing port, operated by the cooperative, has 150 registered members. Fishing boats in the port ranged in length from 4 to 48 m. The most commonly used fishing gear includes purse seines, gill / trammel nets, and fishing lines for fish such as horse mackerel, anchovy, red mullet, garfish, and bluefish.

Üçkayalar Fishery Cooperative

It is located in central Giresun. Also known as Gemilerçekeği, this place has the second largest fishing port and boatyard in Giresun. It was built in 1974. The cooperative was established in 2015. The main breakwater is 270 m long. The infrastructure includes electricity and fresh water connections, while the superstructure includes an administrative building and restrooms. It is a boatyard with a high density of boats. There are 130 registered boats using the boatyard. The lengths of the boats ranged from 4 to 10 m. Fishing is done with gill / trammel nets and fishing lines. Depending on the season, the catch is mostly horse mackerel, whiting, red mullet, bluefish, and garfish.

Aksu Neighbourhood Fishery Cooperative

The Aksu fishing port and boatyard are located east of Giresun city center. The cooperative was established in 2014. The main breakwater is 127 m long. There are no infrastructure or superstructure facilities. The length of vessels varies between 4 and 7 m. Fishing activities use gill / trammel nets, and the catch is generally horse mackerel, garfish, bonito, whiting, and bluefish.

Socio-Demographic Characteristics of Fishermen

During the interviews with the fishermen, questions were asked about their personal characteristics, level of education, fishing experience, and why reasons for choosing fishing (Table 1).

Table 1. Some socio-demographic characteristics of fishermen in Giresun (min-max values in the right parenthesis).

Age of fisherman (mean ± SD)	56.6 ± 10.6 (29–80)
Fishing experience (year, mean ± SD)	30.4 ± 13.3 (4–60)
Number of days spent fishing (year, mean ± SD)	128.3 ± 55.2 (4–60)
Number of crew on board (mean ± SD)	2.1 ± 5.5 (0–25)
Those all income comes from fishing (%)	31.2
Married (%)	87.5
Going fishing with his wife (%)	18.7
Wants their child to become a fisherman (%)	25.0
Reason for choosing fishing (%)	
-Sea passion	53.0
-Father's profession	35.0
-Subsistence	9.0
-Other	3.0
Has social security (%)	
-SSK	62.5
-Bağ-Kur	21.9
-Emekli Sandığı	15.6
Crew payment method (%)	
-Share	44.4
-Per diem	11.2
-Other	44.4
Marketing type (%)	
-Middleman	68.8
-Self	15.6
-Retailer	3.1
-Wholesale market	3.1
-Other	9.4
Income satisfaction (%)	
-Very good	9.4
-Good	15.6
-Average	21.9
-Poor	53.1
-Other	3.0

When we look at how the fishermen market their products, we see that, due to the lack of a fish whole sale market in the province, they largely sell their products to middleman. Some of their products are sold at the fish market built by the Giresun Municipality, while the rest is sent to other provinces.

When looking at the educational background of the fishermen, 60% have completed elementary school, 34% have completed high school, and 6% have completed college education.

Considering the fishermen's professional experience, 34.4% have less than 20 years, 21.9% have 21-30 years, and 28.2% have 31-40 years.

When we asked fishermen, "Do you make your living solely from fishing?" Because the region is predominantly populated by amateur fishing vessels, we found a large proportion of boat owners with diverse income sources. It appears that 69% of boat owners also earn money from non-fishing sources (55% are retired, while the remainder are workers, tradesman, teachers, and farmers).

A significant portion of fishermen are amateur fishermen and own small boats, so their sea passion (53%) is the primary reason for choosing fishing. 35% continue their father's profession.

When working hours are examined, it is seen that 59.4% of fishermen work between 4 and 6 hours.

Fishermen's Opinions and Problems About the Cooperative

In this section, fishermen were asked about their opinions about cooperatives, whether they had any problems with other fishermen, and whether they had any problems with the adequacy of the fishing ports they were in.

When fishermen were asked about their reasons for becoming members of the cooperative, they stated that their reasons for becoming members were largely to secure a place in the port (43.8%) and to benefit from bureaucratic conveniences (34.4%).

When we asked the fishermen, "Do you think cooperatives are successful?", most of the fishermen (63%) think that cooperatives are unsuccessful.

We asked the fishermen, "Would fish be sold at a lower price if the cooperative didn't exist?" All of the fishermen stated that the cooperative had no impact on fish prices.

Sixty-three percent of fishermen report that their fishing ports are inadequate.

We asked the small-scale fishermen if they had any problems with large-scale fishermen, and 56% said they did. These problems generally arise from competition for fishing in the same area. Small-scale fishermen say large-scale fishermen are overfishing and decreasing fish stocks. As a solution, they suggested that fish-finding devices like sonar and echo-sounders should be avoided and that fisheries authorities should implement stricter inspections.

When we asked fishermen, "Do you have any problems with dolphins and marine mammals?" 56% of them said yes. According to them, dolphins (65%) are the most problematic species. 35% of fishermen reported having problems with both dolphins and rays, while 17% reported having problems with both dolphins and rays, plus sharks.

DISCUSSIONS

This study conducted interviews with fishermen in three different cooperatives located in the central district of Giresun to determine some of the socio-demographic characteristics of the fishermen in the region and their opinions about the cooperatives. The majority of the fisheries in Giresun are small-scale and recreational fishermen. The most important target species in the region are horse mackerel, whiting, horse-mackerel, red mullet, garfish, bonito, bluefish, and anchovy. Sea snail (*Rapana venosa*) fishing is also very common. Fishermen generally use gillnets, beam-trawls, and various hand lines.

Coastal fishermen sell their fish through middlemen in fishing ports. Income from fish sales is received weekly (Mutlu et al., 2018).

All three cooperatives in the city center face significant problems. The fishing ports are inadequate and poorly maintained, the cooperatives' impact on fisheries is weak, they lack sustainable fishing initiatives, auctions are not even held, and they lack cold storage facilities. Similar problems have been confirmed by Erdoğan Sağlam and Çalık (2016b). Furthermore, almost all cooperatives report insufficient dues and rental income, leading to financial difficulties due to their inability to secure a source of income (Erdoğan Sağlam and Çalık, 2016b). Furthermore, it has been reported that none of the coastal facilities have pre-cooling storage, ice production facilities, or retail outlets (Tokmak Kırkses and Samsun, 2020).

This study suggests that the primary reason fishermen join cooperatives is to secure or retain their port space. A significant portion of the region's fishermen are amateur fishermen. They engage in this work either because they enjoy the sea or as a side hustle. Consequently, only a small portion of fishermen earn their entire living from fishing.

Most fishermen work 4-6 hours a day. Because there is no wholesale fish market in the province, they sell their products through middlemen or on their own. Most fishermen fishing during 100-200 days a year. The majority of fishermen are between 51 and 61 years old. Most fishermen are dissatisfied with their income. Despite this, while they have no intention of quitting fishing, they do not want their children to take up this profession.

Fishermen in Aksu fishing port are disturbed by the fact that the Giresun Municipality's zoning plan to remove the garbage dump next to the port includes their own port borders, and they say the newly built port poses a risk of flooding in adverse winds.

As a result of interviews conducted with fishermen in the region, it was determined that they have many problems. These are:

-Fishermen state that dolphins and rays pose a significant threat to them, tearing apart their nets and fishing lines, and that action must be taken to resolve this problem.

-Fishermen, especially in Kumyalı port, stated that the port is insufficient and small for boats and that it needs to be enlarged.

-Small-scale fishermen claim that large-scale fishermen are depleting the fish stocks in the region and that they are not adequately monitored. They want their use of fish-finder devices such as sonar and echo-saunders to be banned as a solution to this.

-Fishermen also complain that the facilities in the province are far behind those in the surrounding provinces and that there is no wholesale fish market.

-They state that garbage is still being thrown into the seaside garbage area and that this is polluting the sea.

In conclusion, it is expected that the necessary regulations, controls and inspections will be made in order to ensure the sustainability of small-scale fishing, especially in the region with fishing grounds suitable for fishing. For the development of the region's fisheries, cooperatives must be more effective and address their shortcomings. Establishing cold storage and holding auctions are also expected within the cooperatives. Furthermore, cooperatives' commitment to providing solutions to fishermen's problems and training in responsible fishing will significantly contribute to the development of the region's fisheries. Support can be obtained from the relevant ministries and universities in this regard.

Giresun definitely needs a wholesale fish market for its fishing industry to thrive.
Establishing a market would help fishermen access better marketing opportunities.

One of the fishermen's greatest expectations is for the fisheries management authority and the academicians to come together and find a solution to overcome the problems with species such as dolphins, sharks and rays.

Giresun Municipality should reconsider the development plan it will create in place of the garbage dump, taking into account the opinions and suggestions of the fishermen. Furthermore, garbage is still being dumped on the seashore, it should stop this in order to protect our seas and prevent environmental pollution.

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PHYTOCHEMICAL ANALYSIS AND IDENTIFICATION OF BIOACTIVE COMPOUNDS IN SPINACH LEAVES (*SPINACIA OLERACEA L.*)

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ABSTRACT

Green vegetables contain various phytochemicals in suitable amounts, which are very helpful in preventing and fighting numerous diseases. They also have different types of vitamins and minerals for the effective functioning of the body system. Spinach is a leafy green flowering plant with edible leaves. The bioactive components and phytochemicals, such as flavonoids, polyphenols, carotenoids, and ascorbic acid present in the spinach (*Spinacia oleracea* L.) leaves, are responsible for their nutritional and medicinal properties. The study analyzed the bioactive compounds and phytochemicals present in spinach leaves. Phytochemicals in the spinach, including saponins, steroids, tannins, glycosides, flavonoids, phenols, phlorotannin, and ascorbic acids, were determined and screened according to the standard method using extracts from different solvents like water, ethanol and ethyl acetate. The leaf samples were collected, dried, and ground for extraction. The solvents, water, ethanol and ethyl acetate used for the extracts were incubated for 72 hours, then filtered and centrifuged. The centrifuged extracts were subjected to Gas Chromatography-Mass Spectroscopy (GC-MS) for further analysis of the bioactive compounds present. This analysis was achieved on a GC-MS Shimadzu GC–MSQP2010 Plus system equipped with an RTX-5 M.S. capillary column (0.25 mm x 30 m x 0.25 lm). The result established from the GC-MS analysis of the ethyl acetate extract and the ethanol extract of the spinach leaves was identified from 25 compounds in the chromatogram of each extract. The active compounds' names, compound structures, and molecular weights in the ethyl acetate and ethanol extracts were identified. This GC-MS spectrum proved the similarity percentage of these components as compared to the Wiley online library (WILEY8.LIB), which was the library source. This phytochemical composition's medicinal and nutritional value makes the plant highly essential for good health. However, these plant phytoconstituents have not been lost due to cooking. As a result of these positive effects seen in spinach, it is a green vegetable consumed everywhere in the world.

Keywords: Bioactive compounds, Gas Chromatography-Mass Spectroscopy, Phytochemicals, Spinach

INTRODUCTION

Agriculture is a major sustainable aspect that has immensely contributed to TRNC in terms of production, and commercial revenue. Cyprus is a semiarid island with insufficient rainfall. Out of about 329,890 hectares in northern Cyprus, 56% that is equivalent to 187,068 hectares is adequate for agricultural practices. The climatic conditions and to some extent shortage of water have been the major constraint. However, irrigation of lands is supposedly done for the growing of fruits and vegetables of different varieties. From 1985 to 2001, the land for Agricultural purpose has decreased by 6% and this was as a result of the urban development that has occurred over time (Agricultural Statistics, 2001). The farmers who grow fruits and vegetables have extensively adopted the consistent use of the modern irrigation systems with the most appropriate method of irrigation (Metochis and Eliades, 2002).

In Africa, western region especially and using Nigeria as a case study, Agriculture is still and will continue to stand as the most important sector that boost the economy of Nigerian. Not less than 65% of Nigerian population in general is evaluated to survive on agricultural products for adequate living, while 35% of the Gross Domestic Product are accompanied with agricultural contribution through the sector of modern agriculture. (FAO, 2006). Agriculture right from time immemorial has pose relevance in the development of Nigeria especially in the areas of economy and commerce. The merits of this has assisted in the availability of food for the vast population, employment for more than half of the entire population with the conversion of raw material for the earnings foreign exchange in the fast growing sector of industry (Adeboye, 1996).

Spinach **(***Spinacia oleracea L.*) is a leafy and at the same time highly nutritious vegetable, which is adequately rich in nutrients and phytochemicals. Vitamins A (from β-carotene), C and K are the micronutrients that are present in spinach, with common minerals like calcium, iron and potassium. The phytochemicals present includes carotenoids, flavonoids, steroids, phlobatannins, saponins and phenolic compounds just to mention but a few of them (Bergquist, 2006). Various researchers have worked on identifying the bioactive compounds that are present not just in vegetables but also in fruits when considering the pre-harvest and postharvest factors.

One of the main reasons why the consumption of spinach is annually increasing is because people are now being conscious and more sensitive with their health than before and spinach which has always remains as one of the healthy vegetables rich in most of these essential nutrients needed for a healthy living. The vegetable is easy to grow with a short life span, which makes it possible for the vegetable to be grown all through the year.

The top five countries in the world that grow spinach include China, which is leading with the production of 27,540,167 tons per year. This is followed by the United States of America with a total production of 435,721 tons annually and followed by Turkey with a production of 229,793 tons. While Japan is the fourth with a production of 226,865 tons and Kenya the fifth and leading in Africa with an annual production of 178,707 tons annually. Nevertheless, Cyprus id quite rich in wild spinach.

Thus, the intention of this research is to investigate the different bioactive compounds and identify the phytochemicals present in the spinach leaves which could have a great impact on our nutrition and health benefits.

MATERIAL AND METHOD

The Bloomsdale variety leaves belong to the savoy species, which is the most popularly grown in Cyprus usually 30cm long and 15cm broad on the average, was selected and collected with plastic zip lock bags from a vegetable farm in northern Cyprus and taken to the laboratory. The spinach leaves were washed four times with water from the tap; this is to remove any debris from the leaves. This is then rinsed with distilled water and leave to be well dried to be suitable for extraction (Olasupo et al., 2018, Tiwari et al., 2005).

The qualitative studies of the bioactive compounds from plant materials depends mostly on the selection of the proper extraction method (Smith, 2003, Sasidharan et al., 2011). This extraction is the initial step taken in any medicinal or nutritional study of plants because it has significance on the result of the research. Sample preparation techniques are also known as the extraction methods.

The collected samples that are already well dried through the use of oven for 24 hours at 100 degree celcius were grinded to powder form. 5 grams of this powdered sample were then soaked in centrifuge bottles with 50 ml of water, ethanol, and ethyl acetate separately. The whole mixture was then incubated at 4°C for 72 hours. Immediately after this incubation period, the mixtures were carefully filtered with the use of the filter paper and centrifuged at 6,000 rpm at 4°C for a period of 60 minutes. The extracts were then concentrated to dryness in a rotary evaporator (IKA-RV 10 Control) at and were stored at 4°C for further use (Romanik et al., 2007, Moldoveanus and David 2015, Roshanak et al., 2016).

Phytochemical analysis of the test samples were carried out according to standard methods (Trease and Evans, 1989; Harborne, 1998) on test for;

1. Saponin

5ml of each of the plant extracts were added to 20ml of distilled water in a test tube. They were vigorously shaked and then mixed with 3 drops of olive oil. A stable persistent froth forms an emulsion. (Ejikeme et al., 2014).

2. Taninns

1 ml of each extract were boiled in 20 ml of water in a test and then filtered. A few drops of 0.1% ferric chloride were added and once green or a blue-black coloration is observed, it confirms the presence of tannin (Alhakmani et al., 2013).

3. Phenol

5 ml of the extract will be pipetted into a 30 ml test tube, then 10 ml of distilled water will be added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol will also be added and left to react for 30 min. Development of bluish green colour is a positive presence of phenol. (Alhakmani et al., 2013).

4. Flavonoids

3 ml of 1% Aluminium chloride solution was carefully added to 5 ml of each extract. A persistent yellow coloration appeared, indicating the presence of flavonoids. 5 ml of dilute ammonia solution was further added to the above mixture followed by the addition of concentrated H2 SO4. The yellow coloration disappeared. The yellow coloration which disappeared indicates a positive test for flavonoids (Chang et al., 2002),

5. Glycosides

5 ml of each extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution and underplayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicates a deoxysugar characteristic of cardenolides which confirms a positive presence of cardenolides. A violet-green ring appearing

below the brown ring, in the acetic acid layer, indicates that glycoside is present (Rangari 2002, Roshanak et al., 2016).

6. Steroids,

2 ml of acetic anhydride were added to 2 ml extract of each sample followed by careful addition of 2 ml H2 SO4. The color will change from violet to blue or green which confirms the presence of steroids (Rangari 2002).

7. Terpenoids (salkowski test),

5 ml of each extract were mixed with 2 ml of chloroform, and followed by a 3 ml concentrated H2 SO4 carefully added to form a layer. A reddish brown coloration of the interface was formed showing the positive presence of terpenoids (Rangari 2002).

8. Phlobatannins

2 ml of extract of each plant samples was boiled with 1% aqueous hydrochloric acid and a red precipitate showed the presence of phlobatannins (Rangari 2002, Roshanak et al., 2016).

RESULTS AND DISCUSSION

The results of the phytochemical test carried out on each of the extracts were recorded as shown in Table 1. This preliminary screening on each of the extracts show significant quantity of the phytochemical constituents, revealing the presence of saponins, flavonoids, terpenes, phenols, tannins, alkaloids, phlobatanin, glycoside and steroids. However, the phytochemical constituents in the various part of the spinach plant vary as well (Uraku 2016, Altemimi et al., 2017).

This present investigation revealed that the aqueous extract of spinach shows abundant presence of terpenes, and flavonoids, with moderate presence of saponins and less presence of phlobatanin and cardenolides but steroids, glycosides, phenol, glycosides and tannins are absent in the aqueous extract.

For the ethanol extract, tannins and glycosides are moderately present, while steroids and phenol are less present but saponins, flavonoids, terpenes, cardenolides and phlobatanin are absent.

The investigation also revealed much abundant presence of saponins and cardenolides in ethyl acetate extract, with a moderate presence of phenol and less presence of tannins and flavonoids, but showed absence of phlobatanin, terpenes, steroids, glycosides.

Phytochemicals are non-nutritive plant chemicals that have disease preventive properties (Kumar et al., 2009). Different phytochemicals have been found to possess a wide range of activities. The phytochemicals are known to have antimicrobial activity (Ebana, 1995).

Different studies have proved that spinach has a high nutritional value (Maeda et al., 2005). Many researchers also reported that, glycosides play very crucial role in reducing blood pressure. They could also be used in treating heart failure (Nyarko AA & Addy M E 1990).

The spinach leaf extract with their phytoconstituents are reportedly known for anti-inflammatory, antidiarrheal, antimicrobial, antioxidant and insecticidal activities (Chouhan HA & Singh SK, 2011). Steroids are very much important in pharmacy because of their relationship with compounds like sex hormones and can be used for drug production (Okwu DE 2007).

Tannin and flavonoid are thought to be responsible for antidiarrheal activity (Enzo, 2007). Usman and Osuji reported that tannin has been widely used topically to sprains, bruises and superficial wounds as such. Similarly, Elmarie and Johan reported tannin to have antibacterial. Phytochemicals such as terpenoid, flavonoid, tannin, steroid, and alkaloid have antiinflammatory effects (Liu, 2003) Flavanoids show anti allergic, anti-inflammatory, anti-microbialand anti-cancer activity (Yamato and Gayor, 2002).

The result established from the GC-MS analysis that was carried out from the ethyl acetate extract and the ethanol extract of the spinach leaves were identified from 25 compounds from the chromatogram on each of the extract, and this is well summarized in Table 2 and Table 3 respectively. The active compounds name, compound structures and molecular weight in the ethyl acetate and ethanol extract were identified. This GC-MS spectrum confirmed the similarity percentage of these various components when compared to the Wiley online library (WILEY8.LIB) which was the library source.

Table 1. Phytochemical screening of spinach leaf in different solvent

Compounds	Water	Ethanol	Ethyl acetate
Cardenolides	+	-	+++
Flavonoids	+++	-	+
Glycosides	-	++	-
Phenol	-	+	++
Phlobatamin	+	-	-
Saponins	++	-	+++
Steroids	-	+	-
Tannins	-	++	+
Terpenes	+++	-	-

+++ (Abundant); ++ (Moderate); + (Less); - (Absent)

Table 2. GC-MS analysis of the Ethyl acetate extract of spinach leaf

Similarity Percentage	Name of Compound	Molecular Formular	Molecular Weight
1	1,2,2-Trimethylcyclopropylamine	$C_6H_{13}N$	99
2	Ethyl 1-Hexyl-4-Hydroxy-2(1h)-Oxo-3-Quinolinecarboxylate, 4-Hydroxy-3-(2-Oxo-2h-1-Oxa-3-Phenanthryl)-2(1h)-Quinolinone	$C_{22}H_{13}NO_4$	355
3	8,9,9,10,10,11-Hexafluoro-4,4-Dimethyl-3,5-Dioxatetracyclo[5.4.1.0(2,	$C_{12}H_{12}F_6O_2$	302
4	Didecyl1,4-Dihydro-2,6-Dimethyl-3,5-Pyridinedicarboxylate,3,4,5,6,9,10-Hexahydro-9-(4-Hydroxyphenyl)-3,3,6,6-Tetramethylacridine-	$C_{23}H_{27}NO_3$	365
5	2,2-dimethyl-4-(2-propyl)aminobutanone 2-Butanone,3,3-dimethyl-4-[(1-methylethyl)amino]-(CAS)	$C_9H_{19}NO$	157
6	N-(P-Anisidinomethyl)-4-Methylphthalimide 4-Bromo-N-[(6-Methyl-2-Pyridyl)Aminomethyl]Phthalimide	$C_{15}H_{12}BRN_3O_2$	345
7	2-isopropylthio-5-trifluoracetyl-1,3-oxathiolyium-4-olat. 1,3-Oxathiol-1-ium, 4-hydroxy-2-[(1-methylethyl)thio]-5-(trifluoroacetyl)-, hydroxide, inner salt (CAS)	$C_8H_7F_3O_3S_2$	272
8	3,3-Dimethyl-2-Phenyl-2-(1-Oxo-1,2,3,4-Tetrahydronaphthalen-2-Yl)Azirane,1(2h)-Naphthalenone, 2-(3,3-Dimethyl-2-Phenyl-2-Aziridinyl)-3,4-Dihydro- (CAS)	$C_{20}H_{21}NO$	291
9	5-Methoxy-1-Aza-6 Oxabicyclo(3.1.0)Hexane,6-Oxa-1-Azabicyclo[3.1.0]Hexane, 5-Methoxy- (Cas)	$C_5H_9N\ O_2$	115
10	1,2,5-Oxadiazole Furazan (CAS), Azoxazole, 1-Oxa-2,5-diazacyclopentadiene	$C_2H_2N_2O$	70
11	Borane, compd. with carbon monoxide (1:1) (CAS) Borane carbonyl,Borane, carbonyl- Carbon monoxide-borane,Borine carbonyl (BH3CO), Borane-carbon monoxide (1:1) Boron, carbonyltrihydro-, (T-4)- BH3CO	CH_3BO	42
12	Borane, triethyl- (CAS) Triethylborane Triethylboron (C2H5)3B, Triethylborine	$C_6H_{15}10B$	98
13	[10b]-Triethylborane	$C_6H_{15}10B$	98
14	pnz-sar,Glycine,N-[[(4 methoxyphenyl)methoxy]carbonyl]-N-methyl-(CAS)	$C_{12}H_{15}NO_5$	253
15	[1R*,2R*]-1-acetyl-1,2-dihydroxycyclohex-3-ene	$C_8H_{12}O_3$	156
16	4-(Mesyloxy)-3,3-dimethyl-2-butanone	$C_7H_{14}O_4S$	194
17	1,1'-bibicyclo(2.2.2)octyl-4-carboxylic acid [1,1'-Bibicyclo[2.2.2]octane]-4-carboxylic acid (CAS)	$C_{17}H_{26}O_2$	262
18	2-ethylsulfenyl-3,4-dimethoxycarbonyl-5-trifuoroacetyl-furane3,4-Furandicarboxylic acid,2-	$C_{12}H_{11}F_3O_6S$	340

	(ethylthio)-5-(trifluoroacetyl)-, dimethyl ester (CAS)		
19	3-Butyn-1-ol (CAS) 3-Butynol 1-Butyn-4-ol 3-Butyne-1-ol 3-Butynyl alcohol 4-Hydroxy-1-butyne 2-Hydroxyethylacetylene HC..CCH2CH2OH (2-Hydroxyethyl)acetylene 1-Hydroxy-3-butyne	C_4H_6O	70
20	3-chloromethylfuran,Furan,3-(chloromethyl)-(CAS)3-(Chloromethyl)furan,3-Furylmethyl chloride	C_5H_5CLO	116
21	2-[3'-(1''-Hydroxy-1''-methylethyl)-2',2'-dimethylcyclobutyl] ethanal	$C_{11}H_{20}O_2$	184
22	7-hydroxy-5,6,7,8-tetrahydroindoliziaine 7-Indolizinol, 5,6,7,8-tetrahydro- (CAS)	$C_8H_{11}NO$	137
23	1,2,5-Oxadiazole,Furazan(CAS), Azoxazole,1-Oxa-2,5diazacyclopentadiene	$C_2H_2N_2O$	70
24	3-Butyn-1-ol (CAS) 3-Butynol,1-Butyn-4-ol,3-Butyne-1-ol,3-Butynylalcohol,4-Hydroxy-1-butyne2Hydroxyethylacetylene HC.CCH2CH2OH(2Hydroxyethyl)acetylene1-Hydroxy-3-butyne	C_4H_6O	70
25	3-Butyn-1-ol (CAS) 3-Butynol 1-Butyn-4-ol $$ 3-Butyne-1-ol 3-Butynyl alcohol $$ 4-Hydroxy-1-butyne2Hydroxyethylacetylene HC.$.CCH2CH2OH(2Hydroxyethyl)acetylene $$ 1-Hydroxy-3-butyne	C_4H_6O	70

Table 3. GC-MS analysis of the Ethanol extract of spinach leaf

Similarity Percentage	Name of Compound	Molecular Formular	Molecular Weight
1	8,9,9,10,10,11-Hexafluoro-4,4-Dimethyl-3,5-Dioxatetracyclo[5.4.1.0(2,	$C_{12}H_{12}F_6O_2$	302
2	Didecyl1,4-Dihydro-2,6-Dimethyl-3,5-Pyridinedicarboxylate,3,4,5,6,9,10-Hexahydro-9-(4-Hydroxyphenyl)-3,3,6,6-Tetramethylacridine-	$C_{23}H_{27}NO_3$	365
3	Trimer From Isobutyroyl Pyrazine	$C_{24}H_{25}CLN_6O_3$	480
4	N-(P-Anisidinomethyl)-4-Methylphthalimide,4-Bromo-N-[(6-Methyl-2-Pyridyl)Aminomethyl]Phthalimide	$C_{15}H_{12}BRN_3O_2$	345
5	Ethyl 1-Hexyl-4-Hydroxy-2(1h)-Oxo-3-Quinolinecarboxylate,4-Hydroxy-3-(2-Oxo-2h-1-Oxa-3-Phenanthryl)-2(1h)-Quinolinone	$C_{22}H_{13}NO_4$	355
6	Tetradecane,2,6,10-trimethyl-(CAS),2,6,10-Trimethyltetradecane	$C_{17}H_{36}$	240

7	Cyclohexane,1,1'-[1-(2,2-Dimethylbutyl)-1,3-Propanediyl]Bis-(Cas) Heptan, 1,3-Dicyclohexyl-5,5-Dimethyl-	$C_{21}H_{40}$	292
8	1,2,2-Trimethylcyclopropylamine	$C_6H_{13}N$	99
9	Piperidine, 1-nitro- (CAS) N-Nitropiperidine 1-Nitropiperidine	$C_5H_{10}N_2O_2$	130
10	2,4(1H,3H)-Pyrimidinedione, 5-nitro-,5-Nitrouracil,2,4-Dihydroxy-5-nitropyrimidine Uracil, 5-nitro-	$C_4H_3N_3O_4$	157
11	1-(3-Fluorobenzyl)-2(1h)-Imino-3-Methylpyridine Hydrobromide 1,4-Dihydro-4-Imino-1-(4-Phenylbenzoylmethyl)Pyridine Hydrobromide,2-(4-Phenylbenzoylmethylthio)Benzoxazole Hydrobromide 2(3h)-Imino-3-	$C_{19}H_{17}BR$ N_2O	368
12	2-Tetradecanol (CAS) sec-Tetradecyl alcohol Tetradecanol-2	$C_{14}H_{30}O$	214
13	2-isopropylthio-5-trifluoracetyl-1,3-oxathiolyium-4-olat. 1,3-Oxathiol-1-ium, 4-hydroxy-2-[(1-methylethyl)thio]-5-(trifluoroacetyl)-, hydroxide, inner salt (CAS)	$C_8H_7F_3O_3$ S_2	272
14	1h-Imidazole, 1-(1-Oxooctadecyl)-	$C_{21}H_{38}N_2O$	334
15	2-Hexadecanol (CAS) Hexadecanol-2	$C_{16}H_{34}O$	242
16	Octadecane, 6-Methyl- (Cas) 6-Methyl Octadecane	$C_{19}H_{40}$	268
17	9,12-Octadecadienoic Acid (Z,Z)-, 2,3-Bis(Acetyloxy)Propyl Ester (Cas) 1-Linoleyl-2,3-Diacetin	$C_{25}H_{42}O_6$	438
18	2-(4,5-Dihydro-3-Methyl-5-Oxo-1-Phenyl-4-Pyrazolyl)-5-Nitrobenzoic Acid	$C_{17}H_{13}$ N_5O_5	367
19	Nonadecane (CAS) n-Nonadecane	$C_{19}H_{40}$	268
20	Thiophene, 3-methyl-2-pentadecyl-	$C_{20}H_{36}S$	308
21	2-Isononenal (Cas) Branched Chain 2-Nonenal	$C_9H_{16}O$	140
22	Tridecane, 6-methyl- (CAS) 6-Methyltridecane	$C_{14}H_{30}$	198
23	Piperidine, 1-nitro- (CAS) N-Nitropiperidine $$ 1-Nitropiperidine	$C_5H_{10}N_2O_2$	130
24	Octadecane, 3-ethyl-5-(2-ethylbutyl)-(CAS) 3-Ethyl-5-(2'-ethylbutyl)octadecane	$C_{26}H_{54}$	366
25	3-Tert-Butyl-5-Chloro-2-Hydroxybenzophenone	$C_{17}H_{17}CLO_2$	288

CONCLUSIONS

For this present research, the commonly consumed spinach variety in north Cyprus, the Bloomsdale variety leaves which belongs to the savoy species contains adequate quantity of phytochemicals that are helpful in the prevention deadly diseases to mankind.

The GC-MS analysis of these phytochemicals, and the studies of the bio active compounds, indicate positive results for ethyl acetate, ethanol and aqueous extract of the spinach leaves. The phytochemical screening also shows the presence of saponins, terpenoids, phlobatannins, flavonoids, taninns, glycosides, steroids and phenols.

This current research has also explained that the spinach is one of the vegetables in north Cyprus that is consumed on daily basis, not only because it is cheap to get, but it's a green vegetable that is very rich in most of the phytochemicals and bio active compounds present in leafy green vegetables. The medicinal and nutritional values which are of these phytochemical composition, makes the plant to be highly essential for good health and also with its properties that can be useful in combating health challenges. However, there have not been losses of these plant phytoconstituents, as a result of cooking, except for vitamins and minerals. As a result of these positive effects seen in spinach, it is a green vegetable that is consumed everywhere in the world. It is best consumed cooked, except for a very few places where little amounts are added to meals like salad and eaten raw.

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EFFECT OF SALINITY STRESS ON BIOCHEMICAL, GROWTH, AND YIELD CHARACTERISTICS OF WHEAT

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ABSTRACT

Wheat is crucial in providing food and nutritional security, but rapidly increasing soil and water salinity severely threatens its production worldwide. Salinity has a direct impact on soil productivity and limits global yield potential. It also deleteriously impacts wheat growth and development, reducing grain production and quality. Wheat plants use a variety of physiological, biochemical, and molecular mechanisms to adapt to salt stress at the cell, tissue, and whole plant levels to optimize growth and yield while mitigating the harmful impacts of the saline environment. An experiment was conducted at the Institute of Graduate Studies and Research Department of Plant Sciences and Technologies, Cyprus International University, to examine the effect of salinity stress. In the present experiment, wheat varieties V1, V2, and V3 were tested under EC control, 7.5 dS m^{-1}, 10 dS m^{-1}, and 12 dS m^{-1} following a completely randomized design (CRD) with factorial arrangement. The findings revealed that wheat growth and yield characteristics decreased dramatically as saline levels increased. Among V1, V2, and V3, the highest reduction in plant height, SFW, SDW, RFW, RDW, number of tillers, spike length, and grain yield were noted in V3 when treated with 12 dS m^{-1}. Furthermore, V1 performed best as compared to all other varieties.

Keywords: Salinity, Wheat, Antioxidants, Growth, Yield

INTRODUCTION

Earth contains different type of salts. Major portion of earth is covered with water and there is approximately 3% NaCl in that water (Breiman & Graur, 1995; Royo & Abió, 2003; Hajihashemi et al., 2009). However, the total area of the earth that becomes salt-affected is not precisely known according to an estimate about 930Mha of the earth is salt affected and distributed throughout the world (Robin et al., 2016).

Soil salinization is a severe environmental issue worldwide, primarily affecting soil and crop productivity in arid and semi-arid areas due to high temperature and low rainfall (de Sa et al., 2021). Over the previous 45 years, the salt-affected soil area has expanded by 77 million ha, becoming a severe hazard, particularly for irrigated agriculture (Munns, 2002). Salt-affected soils cover an area of around 1060.1 Mha in the world and their area is gradually increasing due to the influence of climate change; soil salinity tends to increase with increase in sea level intrusion and temperature, decrease in precipitation and improper irrigation management (Eswar et al., 2021). It is a major challenge to modern agriculture, impeding and impairing crop

growth and development, especially in coastal areas and most arid regions of the world (Zaman et al., 2018).

It has been reported that salt stress has affected 20% of agricultural land. However, this problem is enhancing gradually owing to anthropogenic activities as well as different changes within climatic conditions (Arora, 2019). Due to salinity stress according to an estimate 50% reduction in crop production has been recorded (Acquaah, 2007). While, there is need to increase food supply up to 70% by 2050 in order to fulfill the demands of continuously increasing population (FAO, 2009).

Salinity has a detrimental effect on the chemical, physical, and biological properties of soil because of the high concentration of salts of basic cations in the soil and yield because of osmotic and ionic stress besides inhibit seed germination, structure and functions of the photosynthetic machinery, resulting in less economical agricultural production and rural poverty (Jahan et al., 2020). However, soil parent material, anthropogenic activities, and arid and semiarid climatic conditions contribute to unfavorable soil conditions like low rainfall which limits leaching of salts, causing accumulation of Ca-minerals that strongly changing soil organic matter turnover and nutrient recycling resultantly crop production decrease (Acquaah ,2007; FAO, 2009.; Wiese, 1977). Improving future agricultural management demands that soil salinity should be taken into consideration (Ennaji et al., 2018).

Variation in climatic conditions, increase in temperature and low rainfall are the red signals indicating that there is need to bring salt affected soils under cultivation to fulfil day by day increasing demands of food (Akhiyarova et al., 2005; Akbari et al., 2007). So, it is important to examine the maximum adverse outcome of excessive salts in the crop growth along with isolate salt tolerant varieties for maximum yield (Huang et al., 2010; Turan et al., 2009; Çelik & Atak 2012).

The growth and production of wheat is reducing throughout the world due to several factors such a decrease in agricultural land, environmental variations, extensive application of chemical fertilizer and less application of organic fertilizers (Masuda, 2016). Many sections of the world are vulnerable to stressors such as salt, which has a harmful effect on plant growth and yield production(Hasanuzzaman et al., 2014a; Hasanuzzaman et al., 2014b; Shabala & Munns 2012) . Around 20% of agricultural fields are saline, and because of the significant issue of global warming, increasingly cultivable lands are becoming salty. Because many stressors, including salinity, result in yield decreases of up to 50% (Munns and Tester, 2008; Munns et al., 2019), while it is important to enhance food production up to 70% by 2050 (FAO, 2009).

Salinity effect plant growth and development via various mechanisms, among them two most important are discussed here (1) the harmful impacts of sodium (Na^+) and chloride (Cl^-) ions, and (2) the osmotic capacity influencing plant physiology. Though, plants can tolerate salinity stress (Rahman et al., 2016; Afzal et al., 2005). Based on their ability to tolerate salinity plants can be tolerant, moderately tolerant, and sensitive. Different researchers classified wheat as a tolerant crop. However, wheat can tolerate salinization to some extent, but its production decrease under heigh level of salinity stress (Poustini & Siosemardeh, 2004; James et al., 2006). Plant synthesis energy via photosynthesis, this energy is utilized in maintenance, plant growth, and to survive under several types of abiotic and biotic hassles during growth stages (Miransari & Smith, 2019).

Under salinity stress plants activate various type of signaling pathways. Salinity stress give rise to artificial drought conditions under such conditions water is present in high amount but due to high concentration of soluble salts plants are unable to absorb water. In response to salinity coupled with drought stress production of reactive oxygen species (ROS) increases. These ROS negatively effects plant metabolism and reduces its ability to cope under stress conditions (Miransari & Smith, 2019).

Keeping in view the above-mentioned adverse impact of salinity on crop yield. This trail aims to define the influence of salt stress on three wheat varieties. Our objectives are;

I. To examine the impact of soil salinity on biochemical parameters of wheat under different salinity levels.
II. To determine salinity level at which maximum yield can be obtained under climatic conditions of Nicosia.

MATERIAL AND METHOD

Experiment

A pot experiment was performed at Institute of Graduate Studies and Research department of Plant sciences and technologies, Cyprus International University. The wheat verities were grown in pots containing 20 kg by weight. Soil for the experiment was taken from the farm. The soil was air-dried and sieved through a 2 mm sieve. Before the start of experiment soil properties, such as ECe, pHs, saturation percentage, SAR, texture and NPK was determined following standard methods.

Development of salinity

After preliminary analysis, four different levels of soil salinity i.e. control (C), S1 = 7.5, S2 = 10 and S3 = 12 dSm^{-1} was prepared by using a calculated amount of NaCl, Na_2SO_4, $CaCl_2$, $CaSO_4$ salts and mixing along with the soil in a mixing tub to achieve maximum homogeneity so that the required salinity levels can be establish in soil samples (Muhammed and Ghafoor, 1992).

Pot filling, fertilization, seed sowing and irrigation

Each pot was filled with 10 kg soil, after developing the required salinity levels, and seeds of wheat shall be sown in each pot. As per requirement thinning was carried out 45 days after planting, leaving 4 plants in each pot. The recommended dose of NPK for wheat was be applied before sowing according to planned treatments. Each pot was treated as per the following treatments.

Treatments layout

Four salinity levels and wheat varieties were used to determine effect of salinity stress on wheat growth, and yield parameters. A completely randomized design with factorial arrangements of 12 treatments each with 3 replications was used.

Varieties = V1, V2, V3 (Perre, Cumhuriyet 75, Gori)

Salinity levels = C, S1, S2, S3

Number of pots = V*S*Replications = 3*4*3 = 36

Plant parameters

Following parameters of crop will be measured after harvesting the wheat crop.

Shoot length (cm)

To measure the length of wheat shoot, meter rod will be used. Later, mean of shoot length will be calculated.

Root length (cm)

Plants root length of all samples will be estimated with meter rod. After those values of mean shall be determined.

Shoot fresh weight (g)

Fresh weight of shoot will be measured using electronic weighing balance.

Shoot dry weight (g)

Weight of dry shoot will be measured after putting shoot samples in an oven at 65± 5°C temperature for 24 hours.

Number of tillers

At maturity after randomly selecting one plant per pot total numbers of tillers of that plant were counted and mean value for number of tillers per plant were calculated.

Spike length (cm)

Spike lengths of three randomly chosen plants were measured by using meter rod. For measuring spike length, length from start point of spike to the upper tip of the spike was noted and it's mean length was calculated.

Grains per spike

After harvesting the spikes were manually threshed and separately counted the number of grains per spike and then average was calculated.

100 grain weight

Grains were counted by using seed counter, weighted on an electrical balance at the time of harvesting from each replication. At the end mean value for 100 grain weight was estimated.

Grain yield

Grains of each pot were manually harvested, and their weight was recorded by weighing balance.

Straw yield

Plants of each pot were harvested manually, tied into bundled and sun dried for a week. By means of digital balance total biomass of sun-dried samples were recorded.

Harvest index

Harvest index % (HI%) can be calculated by the given formula.

$$\text{HI}(\%) = \frac{\text{Grain Yield(g)}}{\text{Straw yield(g)}} \times 100$$

Biochemical parameters

Total chlorophyll content

The chlorophyll content of fully mature wheat leaf will be determined using Chlorophyll meter (SPAD-value) at three different points (Welburn, 1994) and then averaged.

RESULTS AND DISCUSSION

Shoot length (cm)

Salinity stress adversely effects different physiological along with biochemical developments of plant in addition to reduces its growth along with improvement. ANOVA table 4.1.1 shows the impact of soil salinity on shoot length of three varieties of wheat i.e., V1, V2, and V3. ANOVA table showed that the interaction between salinity stress and plant height is highly significant in all wheat varieties. Furthermore, it can be depicted from the Fig 4.1.1 that with increase in salinity considerable reduction in shoot length was observed. However, highest shoot length (115cm) was observed in V1 under controlled conditions. While lowest shoot length i.e., was observed in V3 treated with 12 dS m^{-1} salt stress.

Moreover, the results of this research demonstrated that salinity stress reduced shoot length by 21.7%, 39%, and 52% in V1 treated with SL1, SL2, and SL3 respectively as compared to control. In V2 a reduction in shoot length was also observed. The results showed 25%, 40%, and 63% reduction in shoot length as associated to control. Lastly the outcomes of this study indicated a reduction of 29%, 44.8%, and 65.4% in shoot length in pots treated with 7.5 dSm^{-1}, 10 dS m^{-1}, and 12 dS m^{-1} correspondingly. According to our findings V1 performed best as linked to V2 and V3. The outcomes of our research demonstrated that with increasing level of salinity plant height was reduced. Our conclusions are also justified by Nazeer et al. (2021) who stated that salinity negatively effects growth of wheat by decrease in tissue water contents. Elevated absorption of salts in soil solution induces osmotic stress. Osmotic influence reduces activity of meristematic cells in shoot axis thereby cell elongation and consequently plant height (Qiong et al., 2016).

Siddiqui et al. (2021); EL Sabagh et al. (2021) also reported the adverse impact of salinity on plant height. Salinity stress increases the concentration of Na and Cl ions that leads to ionic stress which adversely effects biochemical and metabolic processes in older leaves that resultantly causes decrease in leaf expansion and photosynthetic capacity. Leaves are the production houses of plant and because of impaired functioning of leaves the ability of plant to generate energy decreases that ultimately decreases plant height (Fathalla and El-Mageed, 2020). In another research Kalhoro et al. (2016) reported reduction in plant height, spike size, quantity of spikelets $spike^{-1}$, 1000 grain mass, as well as yield. However, salinity level of 10 dS m-1 has more harmful effect as associated to 2.16, 4.0, 6.0, 8.0 dS m^{-1}. The impact of soil salinity on shoot arid weight, and wheat yield was observed by Elgharably et al. (2010). In this experiment wheat response to EC level 2.2, 6.7, 9.2 and 11.8 dS m^{-1} and four different

concentrations of N and P was recorded. The results showed that EC level 11.8 dS m^{-1} considerably decreased the shoot, and grain yield but application of N and P increased the growth and yield related components.

Root length (cm)

Salinity stress adversely effects different biological as well as bio-chemical methods of plant and reduces its growth and development. ANOVA table 4.2.1 shows the impact of soil salinity on root length of three varieties of wheat i.e., V1, V2, and V3. ANOVA table showed that the interaction between salinity stress and plant height is highly significant in all wheat varieties. Furthermore, it can be depicted from the Fig 4.2.1 that with increase in salinity significant an increase in root length was observed. However, highest root length (40 cm) was observed in V1 treated with 12 dS m^{-1} salinity stress. While lowest root length i.e., 19 was observed in V2 under controlled conditions.

In addition, the findings of this study showed that salinity stress increased root length by 29%, 54%, and 62% in V1 treated with SL1, SL2, and SL3 respectively as compared to control. In V2 an increase in root length was also observed. The results showed 29%, 46%, and 61% increase in root length as compared to control. Lastly the results of this study showed an increase of 22%, 60%, and 72% in root length in pots treated with 7.5 dSm^{-1}, 10 dS m^{-1}, and 12 dS m^{-1} correspondingly. According to our findings V1 performed best versus V2 and V3.

The outcomes of our study revealed that with increasing level of salinity plant height was reduced. Our outcomes are also justified by Nazeer et al. (2021) who described that salinity negatively effects growth of wheat by decrease in tissue water contents. High intensity of salts in soil mixture stimulates osmotic effect. Osmotic influence reduces activity of meristematic cells in shoot axis thereby cell elongation and consequently plant height (Qiong et al., 2016).

Siddiqui et al. (2021); EL Sabagh et al. (2021) also reported the adverse effect of salinity on plant height. Salinity stress increases the concentration of Na and Cl ions that leads to ionic stress which adversely effects biochemical and metabolic processes in older leaves that resultantly causes decrease in leaf expansion and photosynthetic capacity. Leaves are the production houses of plant and because of impaired functioning of leaves the ability of plant to generate energy decreases that ultimately decreases plant height (Fathalla and El-Mageed, 2020). In another research Kalhoro et al. (2016) reported reduction in plant elevation, spike size, quantity of spikelets $spike^{-1}$, 1000 grain mass, along with yield. However, salinity level of 10 dS m-1 has additional unfavorable effect as associated to 2.16, 4.0, 6.0, 8.0 dS m^{-1}. The impact of soil salt on shoot dry weight, root dry weight and wheat yield was observed by Elgharably et al. (2010). In this experiment wheat response to EC level 2.2, 6.7, 9.2 and 11.8 dS m^{-1} and four different concentrations of N and P was recorded. The results showed that EC level 11.8 dS m^{-1} considerably decreased dry weight of shoot and root and also reduced grain yield, but application of N and P increased the yield related components. So, from the previous studies it can be concluded that salinity reduces root size.

Grains per spike

Salinity stress adversely effects different physiological and biochemical processes of plant and reduces their growth and development. ANOVA table 4.9.1 shows the impact of soil

salinity on grains per spike of three varieties of wheat i.e., V1, V2, and V3. ANOVA table showed that the interaction between salinity stress and plant height is highly significant in all wheat varieties. Furthermore, it can be depicted from the Fig 4.9.1 that with increase in salinity significant reduction in grains per spike was observed. However, a highest grain per spike (51) was observed in V1 under controlled conditions. While a lowest grain per spike i.e., 22 was observed in V2 treated with 12 dS m^{-1} salinity stress.

In addition, the findings of this research showed that salinity stress reduced grains per spike by 8%, 37%, and 45% in V1 treated with SL1, SL2, and SL3 respectively as compared to control. In V2 a reduction in grains per spike was also observed. The outcomes showed 10%, 22%, and 55% reduction in grains per spike as associated to control. Lastly the results of this study showed a reduction of 4%, 25%, and 44% in grains per spike in pots treated with 7.5 dSm^{-1}, 10 dS m^{-1}, and 12 dS m^{-1} correspondingly. According to our findings V1 performed best as assessed to V2 and V3. The findings of our research demonstrated that with increasing level of salinity plant height was reduced. Our outcomes are also justified by Nazeer et al. (2021) who stated that salinity negatively effects growth of wheat by decrease in tissue water contents. High-level absorption of salts in soil mixture causes osmotic stress. Osmotic impact reduces activity of meristematic cells in shoot axis thereby cell elongation and consequently plant height (Qiong et al., 2016).

Siddiqui et al. (2021); EL Sabagh et al. (2021) also stated the adverse effect of soil salinity on plant height. Salinity stress increases the concentration of Na^+ as well as Cl^- ions that leads to ionic stress which adversely effects biochemical and metabolic processes in older leaves that resultantly causes decrease in leaf expansion and photosynthetic capacity. Leaves are the production houses of plant and because of impaired functioning of leaves the ability of plant to generate energy decreases that ultimately decreases plant height (Fathalla and El-Mageed, 2020). In another research Kalhoro et al. (2016) reported reduction in plant elevation, grains per spike , quantity of spikelets $spike^{-1}$, 1000 grain weight, as well as yield. Though, salinity level of 10 dS m^{-1} has additional unfavorable effect as linked to 2.16, 4.0, 6.0, 8.0 dS m^{-1}. The impact of salt on grains per spike, grains per spike and wheat yield was observed by Elgharably et al. (2010). In this experiment wheat response to EC level 2.2, 6.7, 9.2 and 11.8 dS m^{-1} and four different concentrations of N and P was recorded. The results showed that EC level 11.8 dS m^{-1} significantly reduced the shoot, grains per spike, and grain yield but application of N and P increased the yield related components. So, from the previous studies it can be concluded that salinity reduces grains per spike.

Grain Yield (g/pot)

Salinity stress adversely effects different physiological as well as biochemical processes of plant and reduces their growing along with development. ANOVA table 4.10.1 shows the impact of soil salinity on grain yield of three varieties of wheat i.e., V1, V2, and V3. ANOVA table showed that the interaction between salinity stress and plant height is highly significant in all wheat varieties. Furthermore, it can be depicted from the Fig 4.10.1 that with increase in salinity significant reduction in grain yield was observed. However, highest grain yield (38g) was observed in V1 under controlled conditions. While lowest grain yield i.e., 7g was observed in V3 treated with 12 dS m^{-1} salt stress. Additionally, the consequences of this investigation

indicated that salinity stress lowered grain yield by 19%, 25%, and 76% in V1 treated with SL1, SL2, and SL3 respectively as linked to control. In V2 a reduction in grain yield was also observed. The findings indicated 2.7%, 31%, and 76% reduction in grain yield as compared to control. Lastly the results of this study showed a reduction of 6%, 35%, and 78% in grain yield in pots treated with 7.5 dSm^{-1}, 10 dS m^{-1}, and 12 dS m^{-1} correspondingly. According to our findings V1 performed best as linked to V2 and V3.

The outcomes of our research demonstrated that with increasing level of salinity plant height was reduced. Our results are also explained by Nazeer et al. (2021) who described that salinity negatively effects growth of wheat by decrease in tissue water contents. High-level absorption of salts into soil solution stimulates osmotic stress. Osmotic impact reduces activity of meristematic cells in shoot axis thereby cell elongation and consequently plant height (Qiong et al., 2016). Siddiqui et al. (2021); EL Sabagh et al. (2021) also informed the unfavorable effect of soil salinity on plant height. Salinity stress increases the concentration of Na along with Cl ions that leads to ionic stress which adversely effects biochemical and metabolic processes in older leaves that resultantly causes decrease in leaf expansion and photosynthetic capacity. Leaves are the production houses of plant and because of impaired functioning of leaves the ability of plant to generate energy decreases that decreases plant height (Fathalla and El-Mageed, 2020). In another research Kalhoro et al. (2016) reported reduction in plant height, grain yield, number of spikelets $spike^{-1}$, 1000 grain weight, and yield. However, salinity level of 10 dS m^{-1} has additional unfavorable effect as linked to 2.16, 4.0, 6.0, 8.0 dS m^{-1}. The effect of salinity on grain yield, grain yield and wheat yield were observed by Elgharably et al. (2010). In this experiment wheat response to EC level 2.2, 6.7, 9.2 and 11.8 dS m^{-1} and four different concentrations of N and P was recorded. The results showed that EC level 11.8 dS m^{-1} significantly reduced the shoot, grain yield, and grain yield but application of N and P increased the yield related components.

In addition, the findings of this experiment showed that salinity stress reduced grain yield by 19%, 24%, and 76%, in V1 treated with SL1, SL2, and SL3 respectively as compared to control. In V2 a reduction in grain yield was also observed. The results showed 3%, 33%, and 77 % reduction in grain yield as associated to control. Lastly the results of this study demonstrated a reduction of 20.5 %, 25 %, and 76.9 % in grain yield in pots treated with 7.5 dSm^{-1}, 10 dS m^{-1}, and 12 dS m^{-1} correspondingly. In accordance with our findings V1 performed best as compared to V2 and V3.

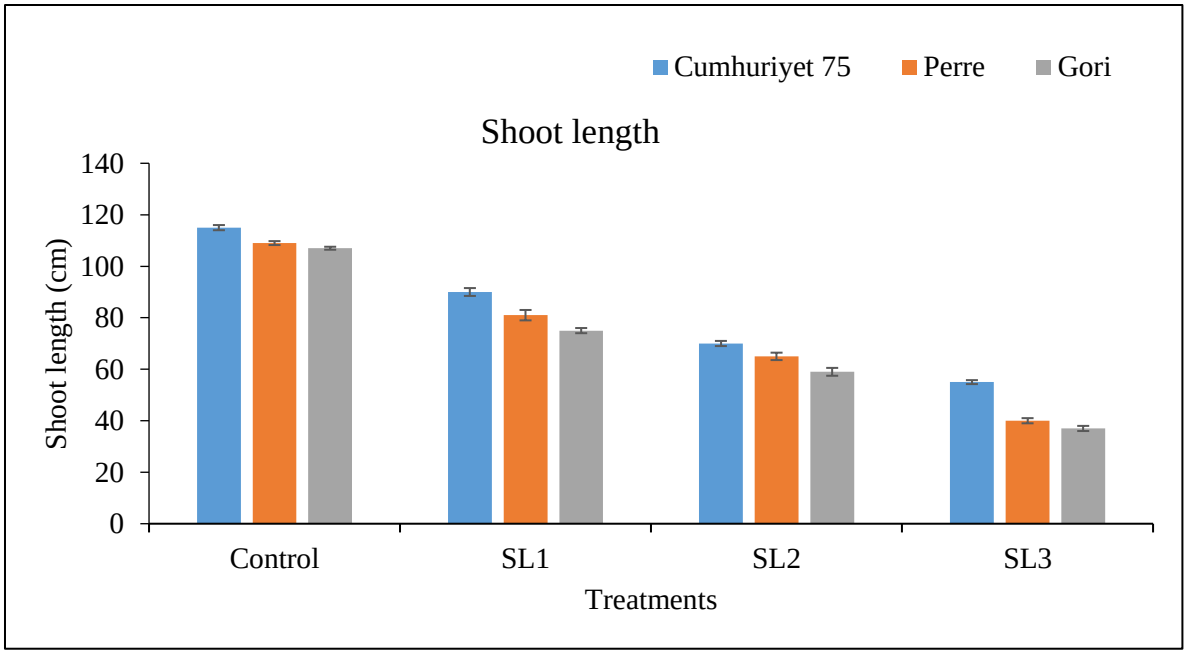

Figure 1. Effect of salinity on shoot length

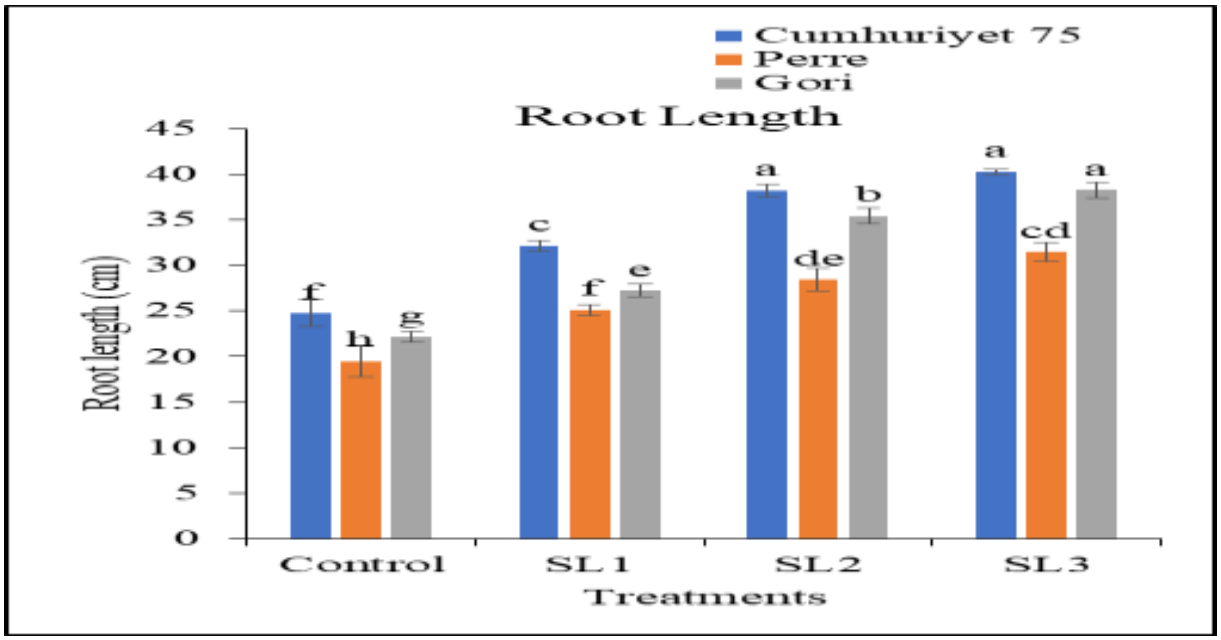

Figure 2. Effect of salinity on root length

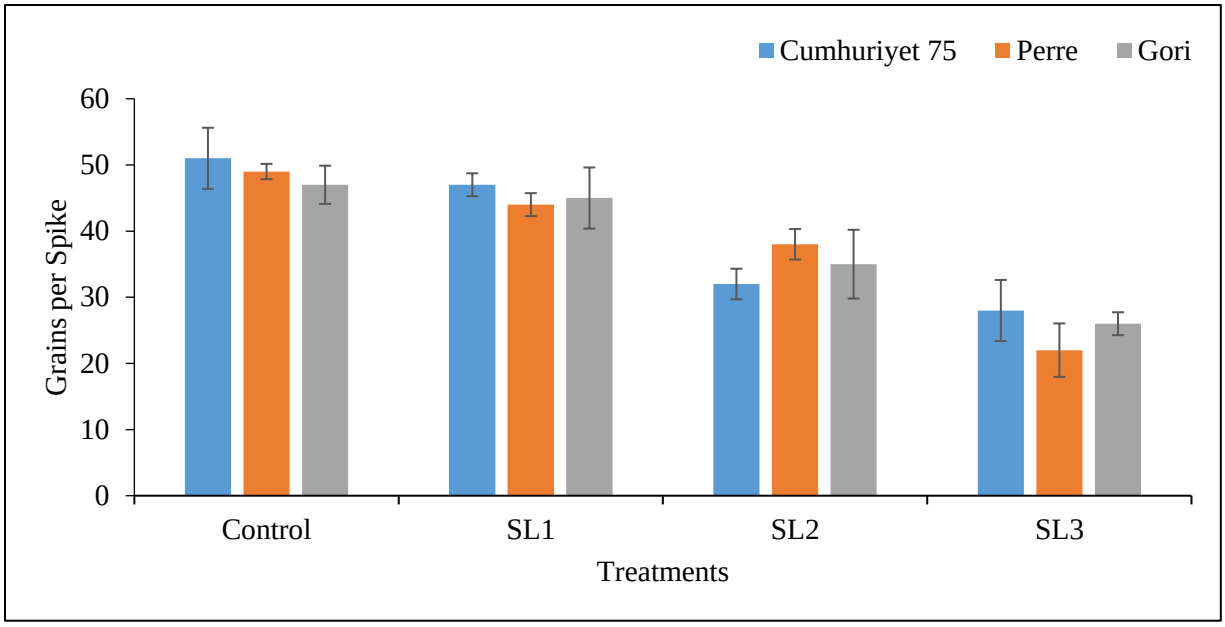

Figure 3. Effect of Salinity on grains per spike

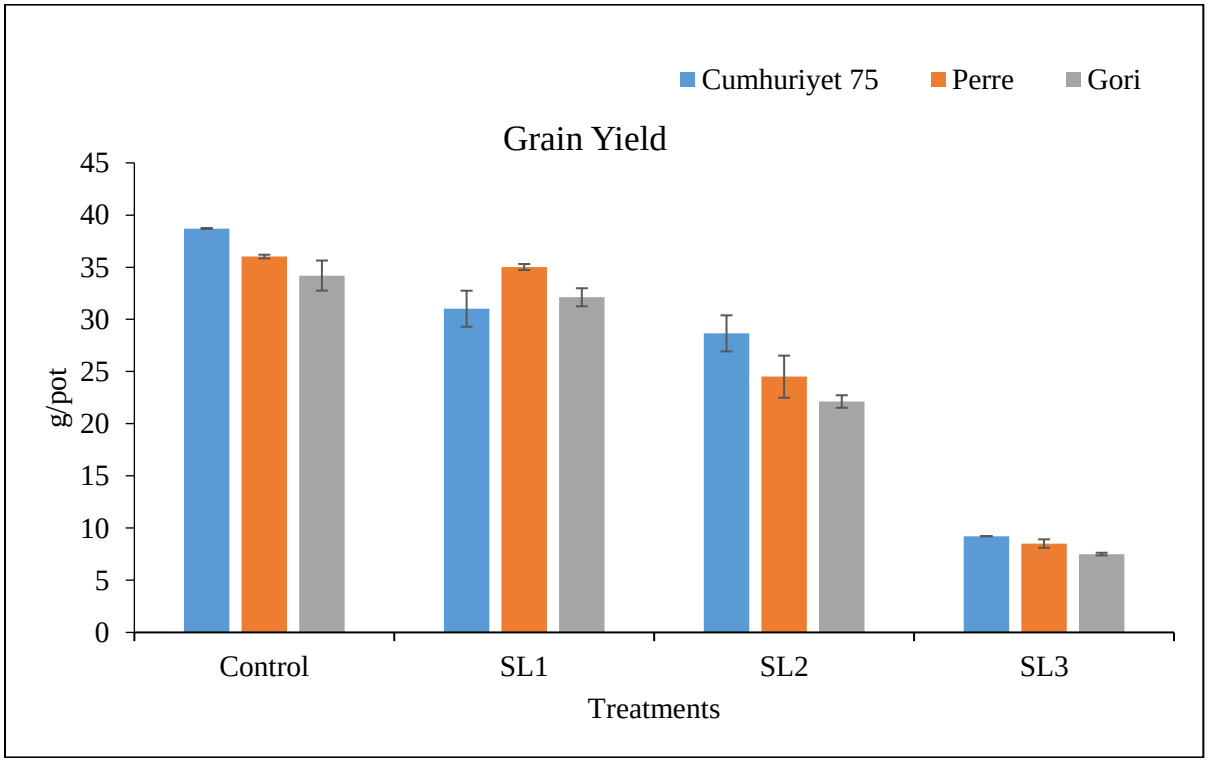

Figure 4. Effect of Salinity on grain yield of wheat

CONCLUSIONS

Keeping under consideration the interactive effect of salinity levels and varieties best results of plant height, spike length, number of tillers, 1000 grain weight, along with grain yield were observed in V1 under control conditions. However, this experiment suggested that improving salt acceptance of wheat varieties can be an effective strategy to increase crop growth

and production under saline conditions. While using salt tolerant varieties and applying organic amendments along with salt tolerant microbes can be more effective.

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ANTIOXIDANT ACTIVITIES OF DIFFERENT EXTRACTS OF INONOTUS CUTICULARIS (BULL. EX FR.) KARST. FROM SOUTHWEST ANATOLIA, TÜRKİYE

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ABSTRACT

Mushrooms are valuable natural sources of antioxidants due to their richness in phenolic compounds, polysaccharides, and other bioactive metabolites. Several species, such as *Ganoderma lucidum*, *Inonotus obliquus*, and *Trametes hirsuta* have exhibited remarkable antioxidant properties through radical scavenging, reducing power, and metal-chelating activities. These findings underscore the potential of mushrooms as functional food ingredients and natural alternatives in pharmaceutical formulations. Antioxidant mushrooms play a crucial role in reducing oxidative stress caused by free radicals, thereby protecting cellular components and contributing to the prevention of chronic diseases. Research on the biological activity of *Inonotus cuticularis* is still limited, but interest in its bioactivity and chemical composition has been increasing. In this study, *I. cuticularis*, which grows naturally in Türkiye and is traditionally consumed as tea, was extracted using solvents of increasing polarity (hexane, ethyl acetate, methanol, and water). The antioxidant activities of the extracts were evaluated using four standard assays: DPPH, ABTS, CUPRAC, and metal-chelating activity. Among the extracts, the water extract exhibited the strongest metal-chelating activity (IC_{50}: 63.25±0.56 µg/mL). Ethyl acetate and methanol extracts showed moderate CUPRAC (IC_{50}: 72.48±0.85 and 85.28±0.52 µg/mL) and ABTS (IC_{50}: 92.58±0.55 and 95.28±0.12 µg/mL) activities, while all extracts displayed weak DPPH activity (IC_{50} > 200 µg/mL). These results suggest that *I. cuticularis* possesses moderate antioxidant potential, particularly in terms of metal chelation and reducing power. However, further research is needed to optimize its activity and identify its active constituents for pharmaceutical or nutraceutical applications.

Keywords: *Inonotus cuticularis*, antioxidant activities, DPPH, ABTS, CUPRAC, metal chelate

INTRODUCTION

Free radicals are a major cause of disease and aging. Antioxidants reduce these harmful effects. Natural products contribute to health with their antioxidant effects and are gaining importance in the field of functional food and nutraceuticals (Duru et al., 2019; Abdelshafy et al., 2022).

Mushrooms are valuable natural sources of healthy nutrition, owing to their low calorie content, essential fatty acids, proteins, vitamins, and minerals. Many mushroom species are widely collected and consumed as nutritious food across different regions of the world (Duru & Çayan, 2015; Park et al., 2024; Korkmaz et al., 2024). Mushrooms exhibit over a hundred medicinal effects, mainly including antioxidant, anticancer, antidiabetic, immune-modulating, cardioprotective, antiviral, antibacterial, anti-inflammatory, and hepatoprotective activities (Chen and Seviour, 2007; Yu et al., 2009; Zhang et al., 2011; Finimundy et al., 2013; Chun et al., 2021; Çayan et al., 2021; Kuş et al., 2025).

Inonotus cuticularis (Bull. ex Fr.) Karst. belongs to the Hymenochaetaceae family (Sesli et al., 2020). It grows on rotten wood of *Quercus* species and broad-leaved trees (Amoopour et al., 2016; Guo et al., 2020; Vemić et al., 2024). It has a natural distribution in Asia, Africa, China, France, Iran, Italy and Türkiye (Amoopour et al., 2016; Guo et al., 2020; Sesli et al., 2020). In this study, *Inonotus cuticularis*, which is naturally distributed in our country, consumed as tea and has commercial potential, was extracted with increasing polarity (hexane, ethyl acetate, methanol and water) and antioxidant activities of the extracts were investigated. Antioxidant activities of *I.cuticularis* extracts were investigated by different methods (DPPH free radical scavenging, ABTS cation radical scavenging, CUPRAC and metal chelate activities).

MATERIAL AND METHOD

1. Mushroom Material

Inonotus cuticularis specimens were collected from *Eucalyptus* and *Quercus* species in their natural habitat in Datça, Türkiye. The collected samples were taxonomically identified using the identification keys provided by Ghobad-Nejhad and Kotiranta (2008), Sharma et al. (2013), and the updated List of Fungi of Turkey (Sesli et al., 2020). A voucher specimen was deposited in the Natural Products Laboratory of Muğla Sıtkı Koçman University under the code ME2405. Macroscopic images of *I. cuticularis* are shown in Figure 1.

Figure 1. Macroscopic views of *I. cuticularis*

2. Mushroom Extract

A total of 800 grams of dried *I. cuticularis* material was subjected to solvent extraction at room temperature using a sequential method with increasing solvent polarity: hexane, ethyl acetate, methanol, and water. Each extraction was performed three times (24 h × 3). After filtration, solvents were evaporated under reduced pressure using a rotary evaporator. The masses and yields of each extract are listed in Table 1.Table 1. Quantities and yields of *I. cuticularis* extracts

Mushroom	Extract	Code	Quantities	% Yields
Inonotus cuticularis	Hexane	ICH	1.92 g	% 0.24
	Ethyl acetate	ICE	11.65 g	% 1.46
	Methanol	ICM	7.35 g	% 0.92
	Water	ICS	5.60 g	%0.70

3. Antioxidant Activity

Four in vitro antioxidant assays were performed on all extracts of *I. cuticularis*:. These were:

- DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity,
- ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) cation radical scavenging activity,
- CUPRAC (Cupric Ion Reducing Antioxidant Capacity) and
- Metal-chelating activity.

All analyses were conducted following the method described by Taş et al. (2021). Butylated hydroxyanisole (BHA), α-tocopherol, and ethylenediaminetetraacetic acid (EDTA) were used as reference standards. The antioxidant capacity of each extract was expressed as the concentration required to inhibit 50% of radical activity (IC_{50}), determined from inhibition percentage versus concentration (µg/mL) plots.

RESULTS AND DISCUSSION

The antioxidant activities of Inonotus cuticularis extracts (hexane, ethyl acetate, methanol, and water) were evaluated using DPPH, ABTS, CUPRAC, and metal-chelating assays. The results were compared to those of standard antioxidants (BHA, α-tocopherol, and EDTA), and the IC_{50} values were calculated for each assay (Table 2).

Table 2. Antioxidant Activity Results of *I. cuticularis* Extracts ($p<0.05$)

Extracts / Reference	**DPPH**	**ABTS**	**CUPRAC**	**Metal Chelate**
	IC_{50} (µg/mL)	**IC_{50} (µg/mL)**	**$A_{0.50}$ (µg/mL)**	**IC_{50} (µg/mL)**
Hexane	>200	>200	>200	>200
Ethyl acetate	>200	92.58±0.55	72.48±0.85	>200
Methanol	>200	95.28±0.12	85.28±0.52	92.76±0.84
Water	>200	>200	>200	63.25±0.56
α-Tocopherol	38.20±0.50	34.50±0.55	60.45±0.37	-
BHA	19.50±0.40	12.70±0.10	25.40±0.22	-
EDTA	-	-	-	5.50±0.35

*Activity results expressed as mean ± standard deviation (SD) of 3 parallel tests ($p<0.05$)

The water extract of *I. cuticularis* exhibited the most significant metal-chelating activity (IC_{50}: 63.25 ± 0.56 µg/mL), whereas all extracts showed negligible DPPH radical scavenging activity (IC_{50} > 200 µg/mL). Ethyl acetate and methanol extracts demonstrated moderate ABTS and CUPRAC activities, with the ethyl acetate extract slightly outperforming the methanol extract.

When compared with reference compounds, all *I. cuticularis* extracts exhibited lower antioxidant efficacy. BHA was found to be the most effective in DPPH and ABTS assays, while EDTA showed superior metal-chelating activity.

These results suggest that the antioxidant activity of *I. cuticularis* varies significantly depending on the solvent used for extraction. The differences in extract efficacy may be attributed to variations in the composition and solubility of bioactive compounds, such as phenolics and terpenoids.

The antioxidant potential of *I. cuticularis* has been evaluated in a limited number of studies. Li et al. (2011) reported strong inhibitory activity of *I. cuticularis* extract against mouse sarcoma (S180) and esophageal carcinoma (Esc) cell lines, although no specific bioactive compound was isolated. Dokhaharani-Chaharmiri et al. (2023) studied Iranian *I. cuticularis* and *I. levis* extracts and reported DPPH IC_{50} values ranging from 144.98 to 333.52 µg/mL, and ABTS values between 128.06 and 290.23 µg/mL. Their findings indicated that ethyl acetate and acetone extracts showed the highest antioxidant activity. Similarly, Maaloul et al. (2023) investigated Mediterranean polypore species and observed that the ethanol extract of I. cuticularis showed the highest antioxidant activity in both ORAC and Folin–Ciocalteu assays. Their DPPH scavenging activity (EC_{50} = 0.10 ± 0.00 µg/mL) was notably higher than in the present study, possibly due to differences in extraction methods, geographical origin, or strain variability.

Although *I. cuticularis* showed relatively low DPPH scavenging activity, its water extract demonstrated promising metal-chelating capacity. This activity is particularly important in oxidative stress mitigation, as metal ions like Fe^{2+} and Cu^{2+} can catalyze the formation of harmful free radicals. These findings suggest that *I. cuticularis* could serve as a potential source of natural antioxidants, especially for pharmaceutical and nutraceutical formulations targeting metal ion-associated oxidative stress. However, further research is necessary to optimize extraction techniques, identify the bioactive constituents, and explore possible synergistic effects.

CONCLUSIONS

Further biological activity studies are needed to especially from the understudied *I. cuticularis*, which may offer new prospects in mycochemistry and pharmaceutical development.

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MACRO ELEMENTAL RISK ANALYSIS OF WATER IN TWO MAJOR DAM LAKES OF THE MARMARA REGION, TÜRKIYE

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ABSTRACT

Reservoirs play a crucial role in freshwater ecosystems, serving key functions such as irrigation, drinking water supply, and flood control. Despite their importance, these artificial lakes are increasingly threatened by anthropogenic pollution. Macro elements like sodium (Na), magnesium (Mg), potassium (K), and calcium (Ca) are essential for both aquatic life and human health, and their elevated levels may indicate ecological stress or contamination. Atikhisar and Alibey Dam Lakes, located in the Marmara Region of Türkiye, are among the region's most vital reservoirs, supplying water to the provinces of Çanakkale and İstanbul. In this study, surface water samples were collected during the dry season of 2022 from both the inlet and outlet zones of each reservoir (a total of four sites). The concentrations of major macro elements —sodium (Na), magnesium (Mg), potassium (K), and calcium (Ca)—were measured, and potential macro elemental risks were assessed. Results indicated that Alibey Dam exhibited higher average concentrations of these elements compared to Atikhisar Dam. Overall, the elemental abundance in both reservoirs followed the trend: Na (81.066 µg/L) > Mg (31.434 µg/L) > Ca (21.867 µg/L) > K (6.353 µg/L).

Keywords: Atikhisar Dam Lake, Alibey Dam Lake, Macro elements, Water quality

INTRODUCTION

Macro elements such as sodium (Na), magnesium (Mg), potassium (K), and calcium (Ca) are essential constituents of natural waters and play vital roles in maintaining aquatic ecosystem function and water quality (Köse et al., 2013; Çiçek et al., 2013; Tokatlı and Helvacıoğlu, 2020; Ali et al., 2025). They are crucial for biological processes in both aquatic organisms and humans, including osmoregulation, enzyme activity, and bone development. However, elevated concentrations of these elements—especially under the influence of anthropogenic inputs such as agricultural runoff, industrial discharges, and urbanization—may lead to ecological imbalances and potential health risks. For instance, excessive sodium or magnesium levels can affect water taste, soil structure, and even plant and aquatic organism health. Therefore, regular monitoring of macro element concentrations in freshwater bodies is essential for early detection of pollution, sustainable water management, and the protection of ecological and human health (Arslan et al., 2012; Tokatlı et al., 2020; Din et al., 2023; Tokatlı et al., 2024; Ustaoğlu et al., 2025).

Atikhisar Dam, located on Sarıçay Stream in the province of Çanakkale, was constructed between 1971 and 1975 primarily for irrigation and flood control. This earth-fill type dam has a body volume of approximately 1.99 million cubic meters and rises about 43 meters from the riverbed. Under normal water conditions, the reservoir has a surface area of 3.30 km² and a storage capacity of 40 hm³, supporting irrigation over nearly 5200 hectares of agricultural land (Anonymous, 2021a; Tokatlı et al., 2023). Similarly, Alibey Dam, situated on Alibey Stream in İstanbul, was built between 1975 and 1983 to supply drinking, utility, and industrial water.

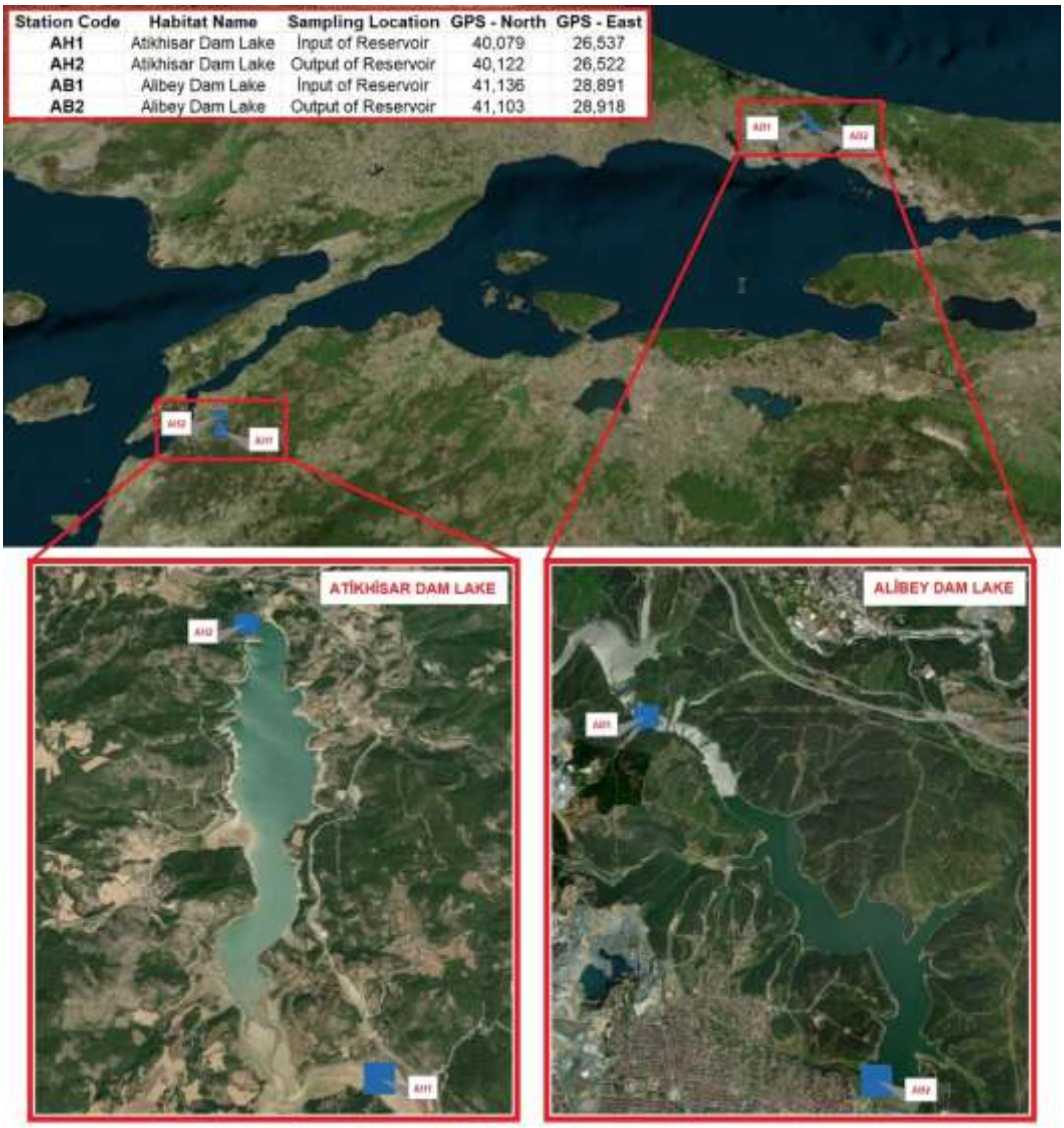
Also designed as an earth-fill dam, it has a body volume of about 1.93 million cubic meters and a height of 30 meters. The reservoir holds up to 66.80 hm^3 of water and spans a surface area of 4.66 km^2 at normal water levels, providing approximately 39 hm^3 of potable water annually for the city (Anonymous, 2021b; Varol and Tokatlı, 2024).

This study aims to assess the macro elemental composition of two strategically important reservoirs in the Marmara Region—Atikhisar and Alibey Dam Lakes—which serve as key sources of irrigation and drinking water. By focusing on the concentrations of sodium (Na), magnesium (Mg), potassium (K), and calcium (Ca), the research seeks to highlight the current status of these essential yet potentially harmful elements when present in excess. Considering the ecological significance of reservoirs and the increasing pressure from anthropogenic influences, the study underscores the necessity of regular monitoring to ensure sustainable water resource management and the protection of ecosystem and public health.

MATERIALS AND METHODS

Water samples were systematically collected from both the inlet and outlet zones of Atikhisar and Alibey Dam Lakes, comprising a total of four sampling stations, during the dry season at the end of summer in 2022. These specific locations were selected to represent potential spatial variations in water quality between the entry and exit points of each reservoir system. The geographical layout of the study area, along with the precise coordinates of the sampling stations, is illustrated in Figure 1 to provide a clear spatial reference for the sampling design and to enhance the reproducibility of the study.

For macro-elemental analysis, the pH of each one-liter water sample was adjusted to approximately 2.0 by the addition of 2 mL of concentrated nitric acid (HNO_3) to prevent precipitation and ensure element stabilization. Subsequently, the samples were filtered using cellulose nitrate membrane filters with a pore size of 0.45 µm to remove particulate matter. The filtrates were then diluted to a final volume of 50 mL with ultrapure water to standardize the analytical conditions. Concentrations of calcium (Ca), magnesium (Mg), sodium (Na), and potassium (K) were quantified using an inductively coupled plasma mass spectrometer (ICP–MS, Agilent 7700xx) at the internationally accredited laboratory of Trakya University (certified under TS EN ISO/IEC 17025). All measurements were conducted in triplicate, and the mean values were reported to ensure analytical precision and reproducibility (EPA, 2001).



Station Code	Habitat Name	Sampling Location	GPS - North	GPS - East
AH1	Atikhisar Dam Lake	İnput of Reservoir	40,079	26,537
AH2	Atikhisar Dam Lake	Output of Reservoir	40,122	26,522
AB1	Alibey Dam Lake	İnput of Reservoir	41,136	28,891
AB2	Alibey Dam Lake	Output of Reservoir	41,103	28,918

Figure 1. Study area and selected sampling locations

RESULTS AND DISCUSSION

The determined macro-elemental concentrations (Ca, Mg, Na, and K) at the designated sampling locations on both Atikhisar and Alibey Dam Lakes are presented in Figure 2. These values reflect the spatial distribution of the selected elements and provide insight into the ionic composition of the water bodies during the sampling period.

Notable spatial variations were observed between the two reservoirs. Sodium (Na) levels were markedly higher in Alibey Dam Lake, reaching a maximum of 128,219 µg/L at station AB1, whereas the lowest value (25,690 µg/L) was recorded at AH2 in Atikhisar Dam Lake. Similarly, calcium (Ca) concentrations were substantially higher at AH1 (33,837 µg/L), indicating possible geological or anthropogenic influences. Mean concentrations across all stations were calculated as 81,066 µg/L for Na, 31,434 µg/L for Mg, 6,353 µg/L for K, and 21,867 µg/L for Ca.

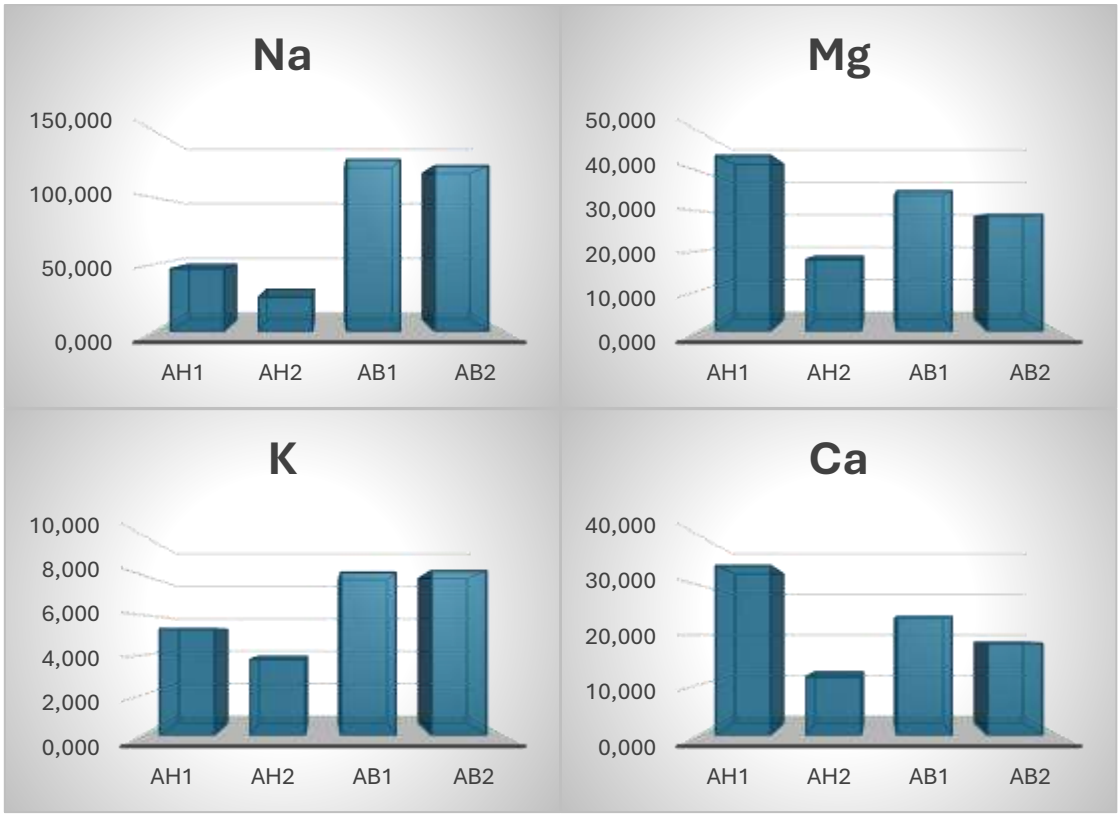


Figure 2. Spatial distributions of macro elements

These data suggest distinct hydrochemical characteristics between the two dam lakes, particularly with respect to the distribution of sodium and calcium. The markedly higher sodium concentrations observed in Alibey Dam Lake, compared to Atikhisar Dam Lake, may be indicative of differences in the geological composition of the catchment areas, such as the presence of sodium-rich sedimentary formations or evaporitic deposits (Hem, 1985; Gibbs, 1970). Moreover, anthropogenic factors such as agricultural runoff, domestic wastewater discharge, and land use practices—particularly irrigation with sodium-enriched water—can further contribute to elevated sodium levels in surface waters (Zhou et al., 2016). On the other hand, the relatively higher calcium content in Atikhisar Dam Lake may be attributed to carbonate-rich lithologies in the surrounding catchment or enhanced dissolution processes of calcareous materials, which are common in Mediterranean hydrological regimes (Meybeck, 1987). Additionally, inflow dynamics, including flow rate, residence time, and seasonal variability, can significantly influence the dilution or concentration of dissolved ions in reservoirs (Lerman et al., 2007). Overall, the observed elemental distribution reflects the combined influence of natural and anthropogenic processes, underscoring the importance of basin-specific evaluations in understanding freshwater chemistry.

CONCLUSIONS

In this study, surface water samples were collected from the inlet and outlet zones of Atikhisar and Alibey Dam Lakes to determine the concentrations of key macro elements (Na, Mg, K, and Ca) during the dry season of 2022. The elemental composition of these two important reservoirs, which supply water to the provinces of Çanakkale and İstanbul, was evaluated in order to assess potential ecological and water quality risks. The results revealed that Alibey Dam Lake exhibited notably higher average concentrations of all analysed elements compared to Atikhisar, suggesting greater exposure to anthropogenic inputs or distinct geochemical characteristics. Among the studied elements, sodium was found in the highest concentrations, followed by magnesium, calcium, and potassium. These findings underscore the need for regular monitoring of elemental loads in freshwater reservoirs, as elevated macroelement levels can be early indicators of ecological stress and potential threats to both aquatic ecosystems and human water use.

ACKNOWLEDGEMENTS

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BLACK CARBON CONTENT IN AIRBORNE PM2.5 DURING THE WINTER BURGAS, BULGARIA

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ABSTRACT

To study the black carbon (BC) concentration in outdoor airborne fine particles with an aerodynamic diameter of 2.5 microns ($PM_{2.5}$), samples were collected at Burgas State University (42°31'41.6"N 27°26'58.7"E) during the winter of 2023 (6 February – 5 March). The BC content was analysed using a sequential sampler coupled with a real-time Black Carbon analyzer, which measures the samples directly on the sampling filter. The analyzer is equipped with an optical module that measures the attenuation of a laser beam.

A total of 28 $PM_{2.5}$ samples were collected on 47 mm fiberglass filters in accordance with standard EN 12341:2023. The sampling duration was 24 hours per filter, with a flow rate of 2.3 $m^3 \cdot h^{-1}$, resulting in a total air volume of 55 m^3 passed through each filter. The black carbon concentration was recorded at 15-minute intervals. The detection limit of the method is 0.001 $\mu g \cdot m^{-3}$. The average BC concentration for the study period was 1.05 $\mu g \cdot m^{-3}$, with a maximum of 8.45 $\mu g \cdot m^{-3}$. Out of 2763 measurements, 726 (26.28%) were below the detection limit. The percentage content of BC in $PM_{2.5}$ ranged from 1.57% to 13.17%, with an average value of 5.18%. The highest BC concentration was recorded on 16 February, associated with a wind speed of up to 5.7 $m \cdot s^{-1}$ from the northeast-east and an atmospheric stability class of F (highly stable). This suggests a mobile emission source—intensive vehicle traffic near the monitoring point, indicating a local origin. The highest percentage of BC in $PM_{2.5}$ was observed on 28 February. That day was characterized by high wind speeds (up to 8.8 $m \cdot s^{-1}$) and neutral atmospheric stability (class D), suggesting long-range transport of pollutants. The south-west-west wind direction coincides with the urban area, where domestic heating is the primary emission source during the heating season. The largest number of measurements below the detection limit occurred on 25 February—37 out of 97 (38.14%) — a day with very high wind speeds (up to 8.8 $m \cdot s^{-1}$), indicating enhanced dispersion of atmospheric pollutants. The highest daytime BC concentration was recorded on 15 February, a day with moderate wind speed from the south and a very stable atmosphere (class F). The highest nighttime BC concentration was observed on the same date downwind of a large residential district. The number of days with higher average daytime BC concentrations was equal to the number of days with higher average nighttime concentrations (14 each out of 28 24-hour samples). The overall average BC concentration was identical for both day and night during the study period, at 1.04 $\mu g \cdot m^{-3}$.

Keywords: Black carbon, $PM_{2.5}$, Air pollution, Air quality, Meteorology

INTRODUCTION

Ambient air quality is a key factor for human health and environmental protection (Johnson et al., 2022; Davronbekovna, 2023). Efforts by governments, non-governmental organizations, industry representatives, and the public have contributed to improved air quality in recent years (Zhang et al., 2022). Nevertheless, air pollution remains the greatest environmental health risk, causing disease, reducing quality of life, and leading to preventable deaths (Fuller et al, 2022; EEA, 2023).

This is a particular challenge for temperate regions, which are characterized by distinct seasonal changes and associated meteorological patterns, as well as the extensive use of heating fuels during winter (Megaritis et al., 2014). On a global scale, tracking studies have shown that air masses arriving at rural sites from Eastern Europe contained approximately twice the amount of BC compared to those from Western Europe (Ahlberg et al., 2023).

In Burgas Municipality, residential heating stoves account for 50% of PM_{10} emissions during the heating season, followed by various transport sources (over 30%), industry (about 16%), and agriculture (0.4%) (Todorov et al., 2025; Burgas Municipality, 2021). Black carbon is a significant component of airborne particles, particularly those with an aerodynamic diameter of 2.5 microns ($PM_{2.5}$).

The present study was conducted to determine the concentrations of BC in $PM_{2.5}$ during the 2023 heating season in the city of Burgas, Bulgaria. The monitoring site was selected as a background location representative of the entire municipality, minimizing direct influence from any specific local emission source.

MATERIAL AND METHOD

The concentration of BC in outdoor airborne $PM_{2.5}$ was studied at Burgas State University (42°31'41.6"N 27°26'58.7"E) during the winter of 2023 (6 February – 5 March) (Figure 1). The nearest pollution source is the intensive vehicular traffic on the E73 highway and Prof. Yakim Yakimov Street. However, a natural belt of vegetation between the roads and the monitoring point screens the direct impact. The nearest industrial site is Kronoshpan Bulgaria Ltd, located 2 km southwest of the sampling point. Toplofikatsiya Burgas JSC is situated 4 km to the west, and Lukoil Neftochim Burgas JSC is 8 km in the same direction (Hristova et al., 2023; Naydenova et al., 2022).

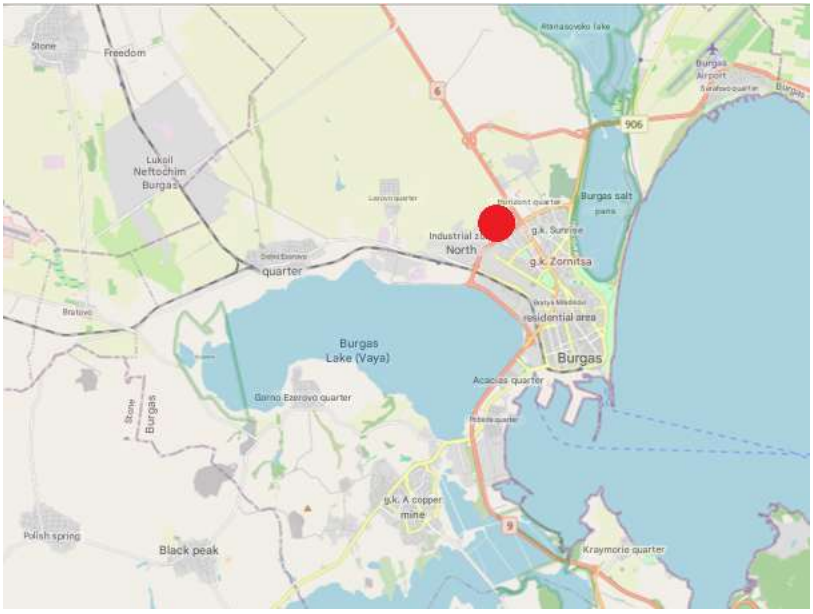


Figure 1. Location of the BC monitoring station on the territory of Burgas Municipality

Whatman® glass microfibre filters with a nominal pore size of 2 µm were used for the determination of $PM_{2.5}$ and BC mass concentrations (Zhang et al., 2022). Sample preparation involved conditioning the sampling filters in a controlled environment before gravimetric analysis (Wang et al., 2016). The filter mass was measured on three consecutive days to ensure

stability, after which they were loaded into filter holders and placed in the sampling equipment (Figure 2).

Figure 2. Pre-sampling filters

The Giano BC_1 is a sequential fine particle sampler with an integrated real-time BC analyzer, which enables the simultaneous determination of PM and BC mass concentrations directly on the filter during the sampling. The instrument can autonomously sample particulate matter across different size fractions ($PM_{2.5}$ and PM_{10}) for up to 21 days (Dunea et al., 2016). It is equipped with state-of-the-art data logging solutions, including RFID technology, local data download via USB, and remote data access and device control via a LAN connection.

The BC concentration is determined by an optical module that measures the attenuation (not reflection) of a laser beam (Yu et al., 2017). The Giano BC_1 is the first commercial solution to utilize a laser attenuation method (LAM) based on reflectometry, as opposed to the more common transmittance-based techniques. The concentration of black carbon is derived from the optical absorption coefficient, which is directly related to the light-absorbing properties of atmospheric particles (Cyrys et al., 2003).

Unlike other market analyzers that rely on transmittance, the Giano BC_1's patented technology is based on reflectometry (Heal et al., 2017). This innovative approach provides a linear correlation with the absorption coefficient used in the BC concentration calculation. A key advantage of this design is the seamless integration of the BC sensor into the sequential sampler, allowing for real-time, on-site monitoring of both PM and BC throughout the sampling operation (Figure 3).

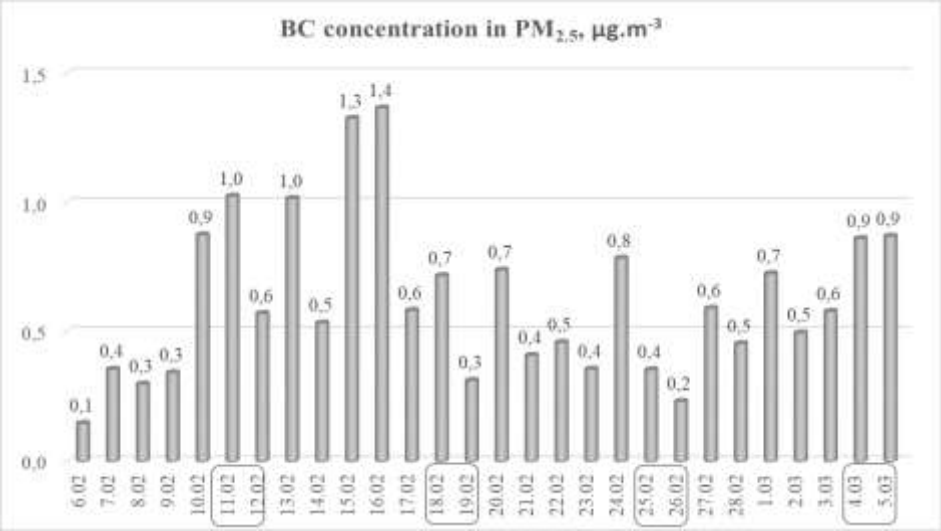
Figure 3. Giano BC_1 sampling device for determining the concentration of black carbon in $PM_{2.5}$

Following the aspiration of 55 m^3 of air over a 24-hour period (a measurement procedure conducted in accordance with standard BDS EN 12341:2023), the loaded filters were conditioned for 24 hours in a controlled environment (Figure 4) (Ciupek et al., 2024). The mass of each filter was then measured gravimetrically on three consecutive days to ensure stability. The PM mass concentration was calculated based on the mass gain of the filter and the exact volume of air that had passed through it. The black carbon concentration was determined at 15-minute intervals throughout each sampling period. For direct comparison with the gravimetric $PM_{2.5}$ data, the BC concentrations are reported as a 24-hour average mass concentration in $\mu g \cdot m^{-3}$.

Figure 4. Post-sampling filters

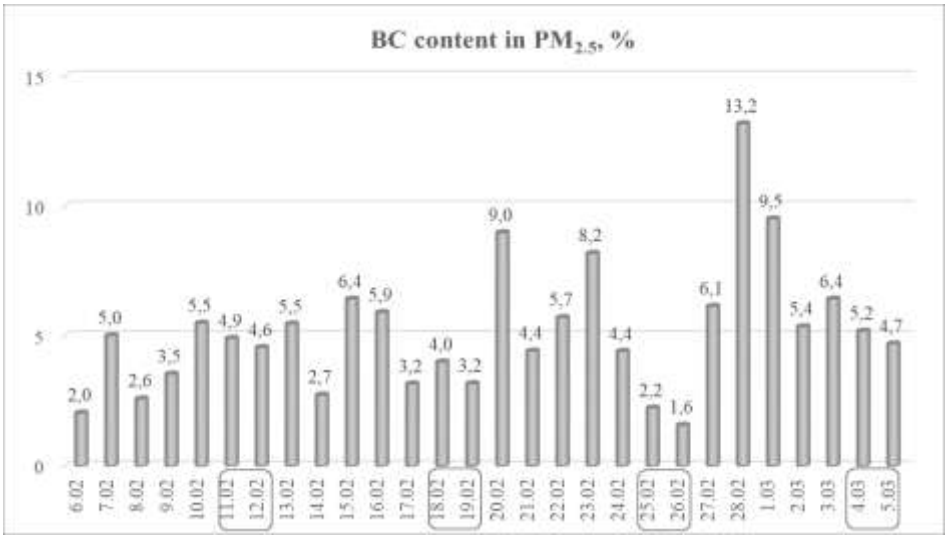
RESULTS AND DISCUSSION

The analysis of the average daily concentrations of BC in $PM_{2.5}$ (Graph 1) shows that the highest value was recorded on Thursday, 16 February (8.45 µg·m⁻³), and the lowest on Monday, 6 February (0.147 µg·m⁻³). The average concentration for the study period was 1.05 µg·m⁻³. The highest BC concentration on 16 February, associated with a wind speed of up to 5.7 m·s⁻¹ from the northeast-east and an atmospheric stability class of F (highly stable) (Graph 3). This suggests a mobile emission source—intensive vehicle traffic near the monitoring point, indicating a local origin.

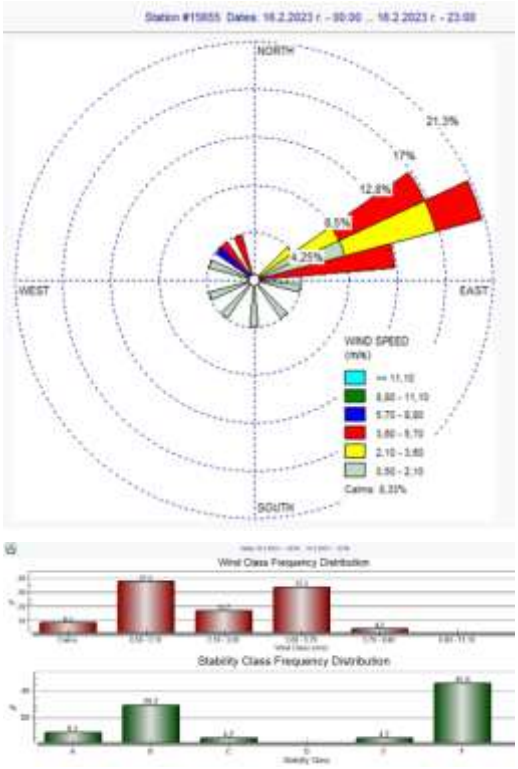


Graph 1. BC concentration in $PM_{2.5}$, µg.m^{-3}

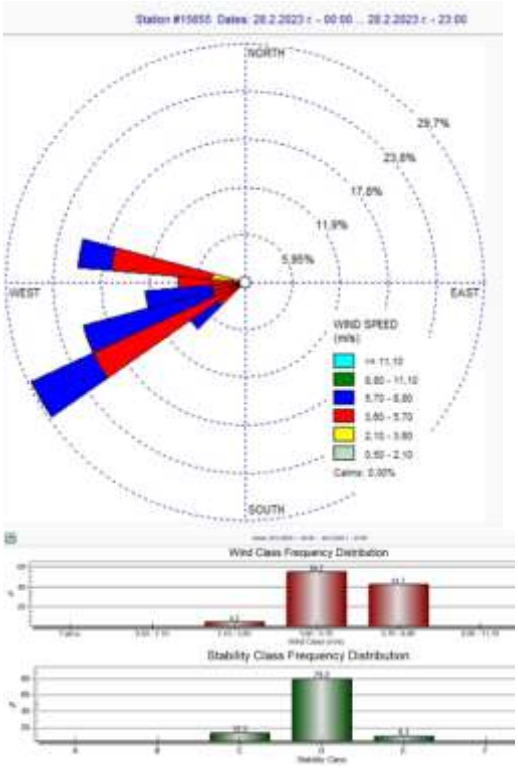
Graph 2 presents the percentage of BC in $PM_{2.5}$. The calculated values range from 1.6% to 13.2%. The lowest content is on Sunday, February 26 and the highest - on 28 February. That day was characterized by high wind speeds (up to 8.8 m·s⁻¹) and neutral atmospheric stability (class D), suggesting long-range transport of pollutants (Graph 4). The south-west-west wind direction coincides with the urban area, where domestic heating is the primary emission source during the heating season.



Graph 2. BC content in $PM_{2.5}$, %

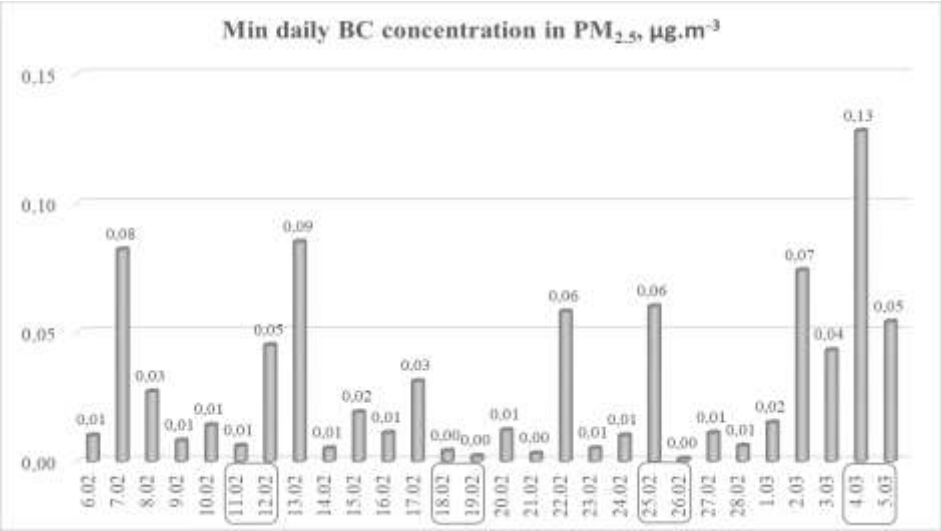


Graph 3. Meteorological data for 16.02.2023

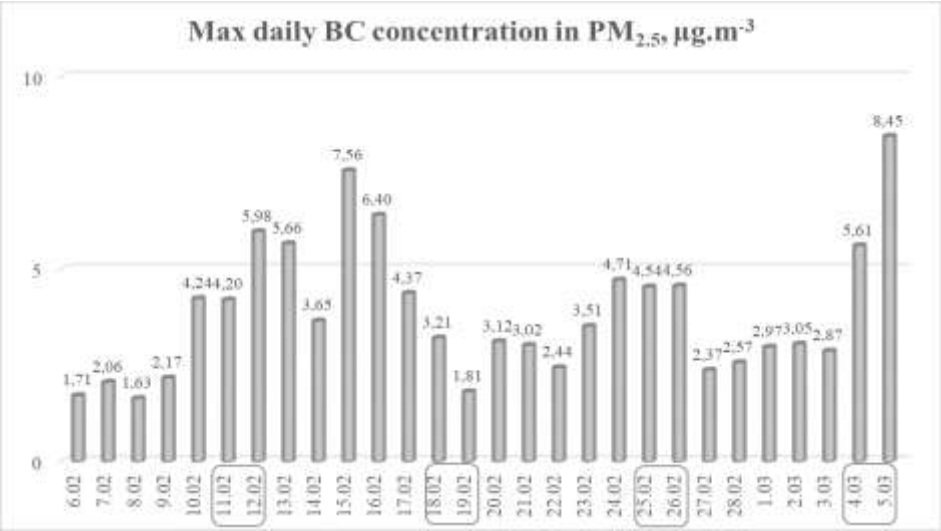


Graph 4. Meteorological data for 28.02.2023

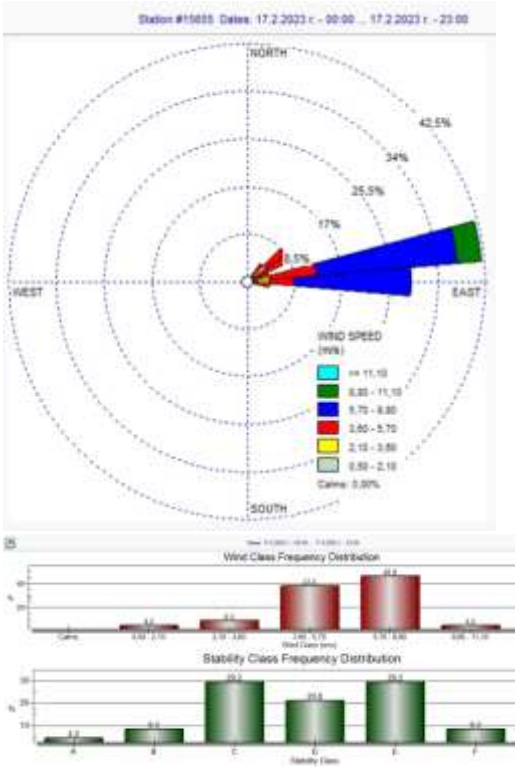
BC concentration data were recorded at 15-minute intervals, resulting in approximately 100 valid measurements per 24-hour period. Graphs 5 and 6 present the lowest non-zero and the highest recorded concentrations for each sampling day, respectively. The lowest minimum concentration (0.001 $\mu g \cdot m^{-3}$) was recorded on Sunday, 26 February, while the highest minimum concentration (0.128 $\mu g \cdot m^{-3}$) was observed on Saturday, 4 March. Conversely, the lowest maximum concentration (1.633 $\mu g \cdot m^{-3}$) was recorded on Wednesday, 8 February, and the highest maximum concentration (8.451 $\mu g \cdot m^{-3}$) was measured on Sunday, 5 March. The meteorological conditions for this day are characterized by low wind speeds (up to 3.6 $m \cdot s^{-1}$), without a clearly defined direction and a prevailing strong stable atmosphere category (F) (Graph 8).



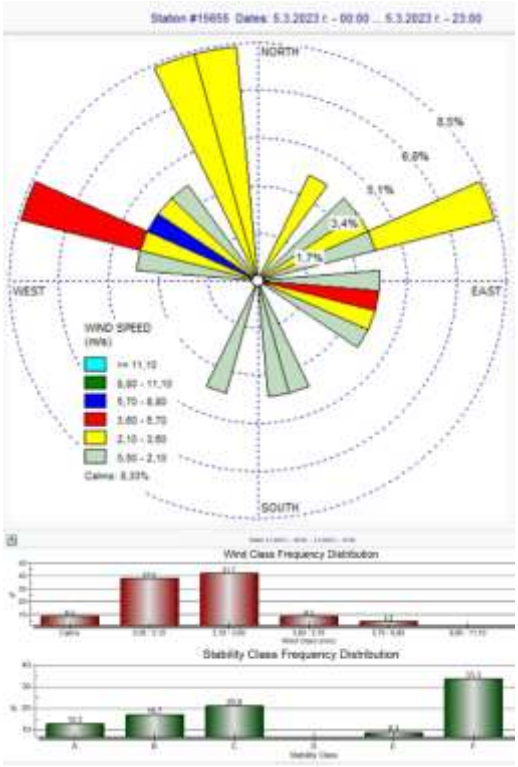
Graph 5. Min daily BC concentration $PM_{2.5}$, µg.m^{-3}



Graph 6. Max daily BC concentration in $PM_{2.5}$, µg.m^{-3}

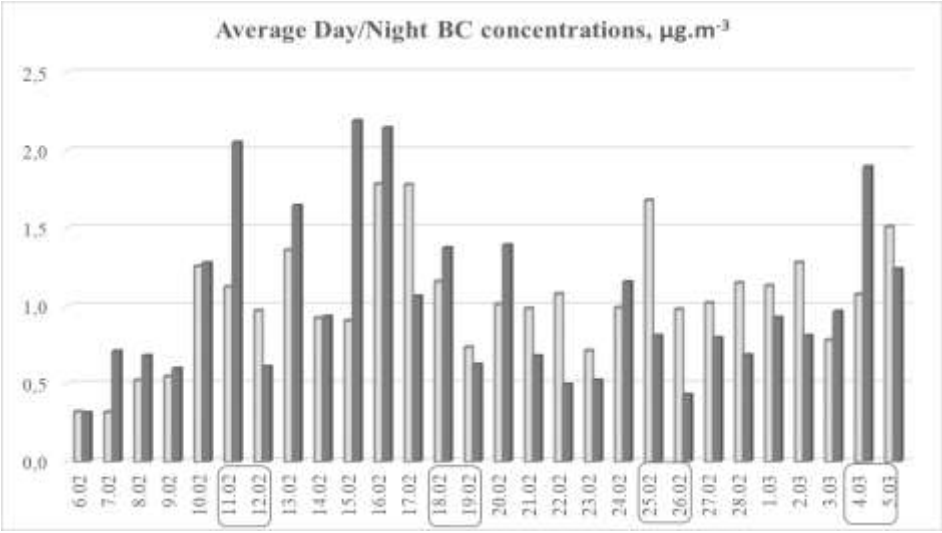


Graph 7. Meteorological data for 17.02.2023



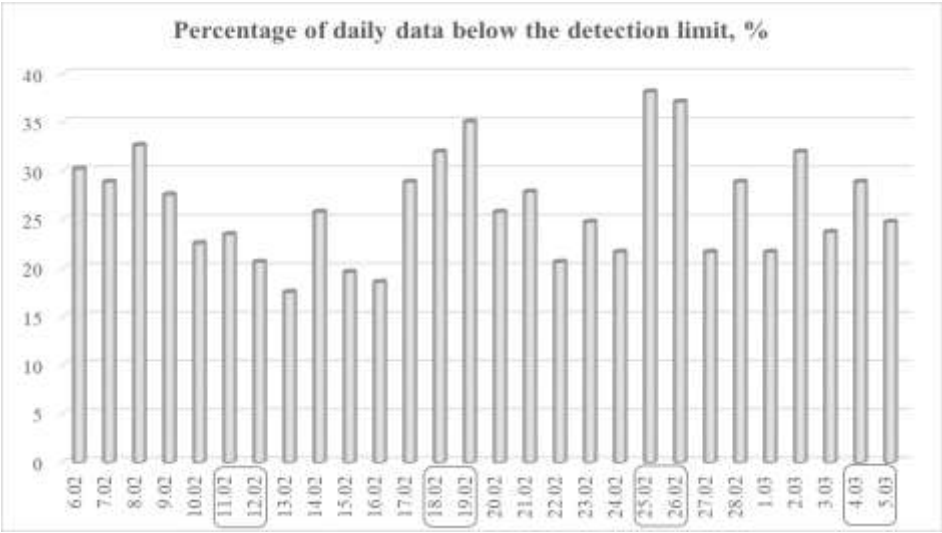
Graph 8. Meteorological data for 05.02.2023

The emission intensity of various urban pollution sources also exhibits diurnal variation (Jalava et al., 2015). Consequently, the data were analyzed by separating the light (daytime) and dark (nighttime) periods of the 24-hour cycle. At the beginning of the study period (6 February), sunrise occurred at 07:18 and sunset at 17:26, resulting in a day length of 10 hours and 7 minutes. On the final sampling day (5 March), sunrise was at 06:37 and sunset at 18:02, resulting in a day length of 11 hours and 25 minutes. The results of this diurnal analysis are presented in Graph 9. The greatest predominance of nighttime over daytime BC concentration was recorded on Wednesday, 15 February. On this day, the average nighttime concentration was 2.19 $\mu g \cdot m^{-3}$, compared to a daytime average of 0.91 $\mu g \cdot m^{-3}$. The strongest inverse relationship, where daytime concentrations exceeded nighttime values, was observed on Saturday, 25 February. On this day, the average daytime BC concentration was 1.68 $\mu g \cdot m^{-3}$, while the nighttime average was 0.81 $\mu g \cdot m^{-3}$. The highest daytime BC concentration was recorded on 17 February, a day with very high wind speed (up to 8.8 $m \cdot s^{-1}$) from the east and a neutral/stable atmosphere (equal classes C and E) (Graph 7). The highest nighttime BC concentration was observed on 5 February - low wind speeds (up to 3.6 $m \cdot s^{-1}$), without a clearly defined direction and a prevailing strong stable atmosphere category (F) (Graph 8).



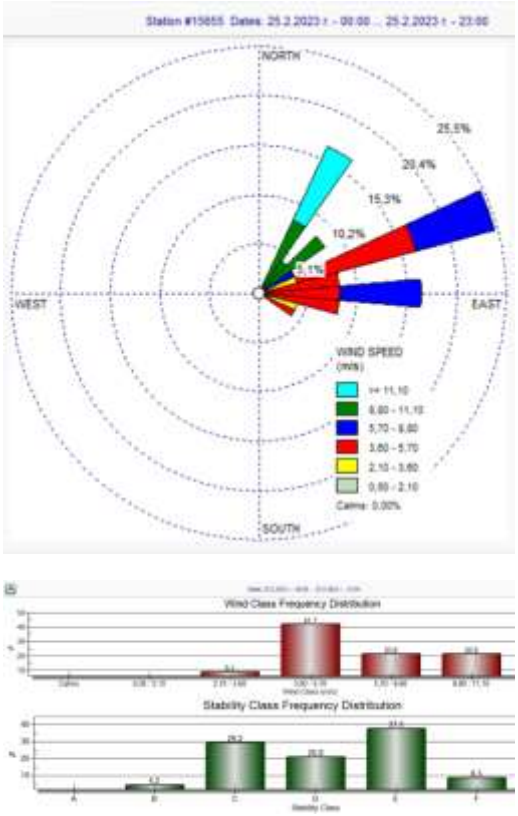
Graph 9. Average Day/Night BC concentrations, µg.m^{-3}

Given the fact that the main share of $PM_{2.5}$ emissions in the territory of Burgas Municipality is due to the burning of wood in residential stoves during the heating season, an unexpectedly large percentage of measured black carbon values below the detection limit was reported. The data by day is presented in Chart 10.



Graph 10. Percentage of daily data below the detection limit, %

The highest number of such cases was recorded on Saturday, February 25 (38.14%). On that day, the wind was very high and northeast-easterly, suggesting ideal conditions for the dispersion of atmospheric pollutants and the self-purification ability of the air in the coastal urban area (Graph 11).



Graph 11. Meteorological data for 25.02.2023

CONCLUSIONS

Analysis of the processed data reveals that even during the winter months, when the domestic stoves are the primary source of emissions, the meteorological conditions characteristic of coastal sea areas can contribute to the better dispersion of pollutants, thereby improving air quality and the quality of life for the population. However, local authorities continue to seek environmentally friendly solutions to mitigate air pollution in urban areas.

ACKNOWLEDGMENTS

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THE CORRELATION BETWEEN BLACK CARBON CONTENT IN AIRBORNE PM2.5, CONCENTRATION OF OTHER AIR POLLUTANTS AND METEOROLOGICAL CONDITIONS DURING THE WINTER – BURGAS, BULGARIA

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ABSTRACT

Emissions of gaseous air pollutants and particulate matter from anthropogenic and natural sources contribute to global climate change, which in turn leads to health and environmental risks. Air quality guidelines focus on fine particulate matter with an aerodynamic diameter of 2.5 µm ($PM_{2.5}$), nitrogen oxides (NO_x), sulfur dioxide (SO_2), and other pollutants to reduce air pollution. The Gothenburg Protocol recognizes black carbon (BC) as a major component of $PM_{2.5}$. However, air quality standards do not specifically target it, and it is not part of any climate protocol, revealing a significant gap in strategies. The data on the concentration of BC and both PM fractions – 2.5 and 10 microns were analysed by the scientific team on the territory of Burgas State University "Prof. Dr. Asen Zlatarov" (42°31'41.6"N 27°26'58.7"E). PM sampling was carried out according to the EN 12341:2023 standard, and BS was analysed by measuring the reflectivity of the laser beam. The meteorological data is from the Burgas Airport station, which provides high quality data representative of the studied territory and measures atmospheric pressure (hPa), air temperature and dew point (ºC), relative air humidity (%) and wind speed (m.s-1), taking average values for 24 hours, which is the duration of PM sampling. The concentrations of other atmospheric pollutants were recorded at the DOAS monitoring station, which operates on the principle of Differential Optical Absorption Spectroscopy and records the concentrations of ozone (O_3), nitrogen dioxide (NO_2), sulphur dioxide (SO_2), and benzene. The processing of the data from the correlation analysis between the concentrations of BC and PM shows a strong degree of correlation with $PM_{2.5}$ (0.69) and a moderate with PM_{10} (0.49). One of the reasons is that the 10-micron fraction also contains the 2.5-micron fraction, which in turn contains condensable particles (secondary aerosols) to a higher degree. This is also the reason why BC is analysed primarily in the 2.5-micron fraction. A positive moderate degree of correlation was recorded between the concentrations of BC and nitrogen dioxide (0.66), which is an indicator of high-temperature emission sources (industrial factories and vehicles during warm start). The negative moderate degree of correlation with ozone (- 0.61) shows probable absorption of secondary atmospheric pollutants obtained during chemical processes in the atmosphere, as this also applies to benzene, although the correlation with it is negative and very weak (- 0.06). No correlation with sulfur dioxide was recorded, which indicates that the emission sources do not use fuels containing sulfur (coal or diesel cars). A strong degree of correlation is recorded between aircraft and wind speed (-0.79). Its average value for the studied period is 5.06 m.s-1, which is the reason for the dispersion of local emission sources and does not show the impact of distant ones. Although the weak degree of correlation with air temperature (- 0.23) is characteristic of the reduction of the amounts of solid fuels used in residential stoves when temperatures rise. The correlations with dew point and relative humidity are very weak (- 0,15 and 0,14).

Keywords: Black carbon, PM, Air pollution, Air quality, Meteorology, Correlation

INTRODUCTION

The quality of the components of the environment is interconnected and also affects the living organisms that inhabit them (Deshmukh, 2024). From an agricultural point of view, not only the plants, preparations used and the quality of the soil are decisive (Suchkov et al., 2022|). Pollutants contained in the air can be transported and affect through dry precipitation (in the case of substances with a higher density than air) or through rainwater, thus changing the quality of the soil, the natural reservoirs used for irrigation, as well as directly affecting agricultural production in the growth phases (Borghi et al., 2023).

From this point of view, the study of the concentration of BC and its ability to retain other atmospheric pollutants, according to the size of its pores and the influence of meteorological parameters, is of utmost importance (Rumpel et al., 2006; Lagasio et al., 2025). If the structure of black carbon contains retained compounds such as carbon, sulfur or nitrogen oxide (D'Amico et al., 2025), falling into a humid environment (fog, rain or dry precipitation in a reservoir or humid soil), this would lead to a change in the acidic reaction of the environment (pH) (Abd et al., 2025).

The influence of meteorological parameters is also extremely important, because through them, preliminary measures can be taken to minimize the negative impact of atmospheric pollutants and lead to more sustainable eco- and agrarian systems (Girotti et al., 2025).

The present study was conducted to determine the concentrations of BC in $PM_{2.5}$ during the 2023 heating season in the city of Burgas, Bulgaria and the correlation between BC content in airborne PM2.5, concentration of other pollutants, and meteorological conditions. The monitoring site was selected as a background location representative of the entire municipality, minimizing direct influence from any specific local emission source.

MATERIAL AND METHOD

The correlation between BC content in airborn $PM_{2.5}$, concentration of other pollutants, and meteorological conditions was studied through measurements of BC and both fractions of fine dust particles on the territory of Burgas State University "Prof. Dr. Asen Zlatarov" (42°31'41.6"N 27°26'58.7"E), the DOAS - RIEW Stations (42°30'36.7"N 27°28'07.6"E) and the meteorological parameters from Burgas Airport (42°33'59.5"N 27°31'04.6"E). The location of the three stations is presented in Figure 1. The sampling period and data collection were organized during the winter of 2023 (6 February – 5 March).

The sampling station on the university's territory has two devices for determining the concentration of the two fractions of fine dust particles (2.5 and 10 microns), in accordance with standard EN 12341:2023 (Ciupek et al., 2024) and maximum permissible concentration according to Bulgarian and European legislation (Zaitseva et al., 2020) , and the concentration of black carbon, which has not yet permission level. The method for PM is standard gravimetric measurement for the determination of the PM_{10} or $PM_{2,5}$ mass concentration of suspended particulate matter. The concentration of black carbon is derived from the optical absorption coefficient, which is directly related to the light-absorbing properties of atmospheric particles (Cyrys et al., 2003).

The DOAS - RIEW station is owned by the Ministry of Environment and Water of the Republic of Bulgaria and measurements of atmospheric pollutant concentrations are carried out using an OPSIS device, operating on the principle of Differential Optical Absorption

Spectroscopy, a scientific technique for measuring atmospheric pollutants using light absorption (Baroncelli et al., 2025). Concentrations of ozone, sulfur dioxide, nitrogen dioxide, and benzene are measured. Each of these pollutants has a maximum allowable concentration limit for the atmospheric air of populated areas (Richelle, 2025).

The weather station at Burgas Airport uses Vaisala cells and records the values of atmospheric pressure, air temperature, dew point, relative humidity, wind speed and direction, visibility, cloud cover density at three levels and specific meteorological phenomena every 30 minutes (Díaz-Bedoya et al., 2025).

Figure 2 presents the three monitoring stations, and Table 1 presents the maximum permissible concentrations of the analyzed atmospheric pollutants.

Figure 2. Data stations

Table 1. Maximum permissible concentrations of atmospheric pollutants, according

	1 hour	8 hours	24 hours	1 year
$PM_{2.5}$	-	-	-	20 µg.m^{-3}
PM_{10}	-	-	50 µg.m^{-3}	40 µg.m^{-3}
Black carbon	-	-	-	-
Ozone	-	120 µg.m^{-3}	-	-
Sulfur dioxide	350 µg.m^{-3}	-	125 µg/m^{3}	-
Nitrogen dioxide	200 µg.m^{-3}	-	-	40 µg.m^{-3}
Benzene	-	-	-	5 µg.m^{-3}

RESULTS AND DISCUSSION

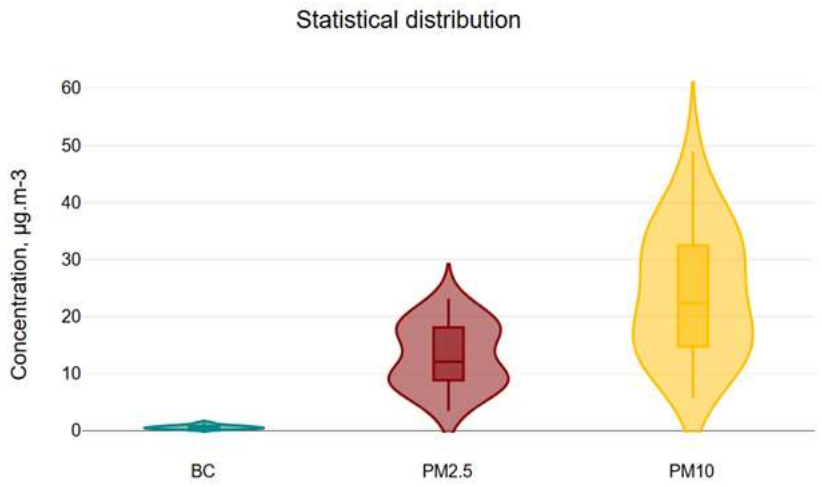
The data on the measured concentrations of atmospheric pollutants on the territory of the university are presented in Table 2. The concentrations of BC are in the range 0,147 - 1,376 µg.m^{-3} with averige value of 0,621 with standard deviation of 0,311. The $PM_{2.5}$ concentrations

are in the range 3,454 – 23,149 μg.m^{-3} with averige value of 13,066 with standard deviation of 5,537. For PM_{10} the range of mesurement concentrations is 5,784 – 48,926 μg.m^{-3} with averige value of 24,197 with standard deviation of 11,135. None of the measured values exceed the norm.

Table 2. Concentration of Black carbon, $PM_{2.5}$ and PM_{10} at the sampling point University

№	Date	BC [μg.m^{-3}]	$PM_{2.5}$ [μg.m^{-3}]	PM_{10} [μg.m^{-3}]
1	6.2.2023	0,147	7,211	10,636
2	7.2.2023	0,356	7,097	9,211
3	8.2.2023	0,299	11,574	15,450
4	9.2.2023	0,343	9,696	16,257
5	10.2.2023	0,876	15,940	30,531
6	11.2.2023	1,026	20,907	40,539
7	12.2.2023	0,572	12,544	21,549
8	13.2.2023	1,017	18,604	21,551
9	14.2.2023	0,535	19,574	29,602
10	15.2.2023	1,327	20,664	35,172
11	16.2.2023	1,367	23,149	38,377
12	17.2.2023	0,584	18,483	30,925
13	18.2.2023	0,718	17,937	33,068
14	19.2.2023	0,313	9,878	19,907
15	20.2.2023	0,74	8,241	14,151
16	21.2.2023	0,41	9,272	23,167
17	22.2.2023	0,46	8,060	17,490
18	23.2.2023	0,357	4,363	11,374
19	24.2.2023	0,786	17,816	48,926
20	25.2.2023	0,354	15,877	32,237
21	26.2.2023	0,232	14,786	40,186
22	27.2.2023	0,592	9,635	34,384
23	28.2.2023	0,455	3,454	5,785
24	1.3.2023	0,726	7,636	14,087
25	2.3.2023	0,497	9,272	14,680
26	3.3.2023	0,58	9,029	14,731
27	4.3.2023	0,862	16,604	25,390
28	5.3.2023	0,871	18,544	28,163

The most frequently reported concentrations for black carbon were in the range of 0.03 - 0.4 μg.m^{-3}, for $PM_{2.5}$ – 9 μg.m^{-3} and 18 μg.m^{-3}, and for PM_{10} – 15 μg.m^{-3} and 30 μg.m^{-3} (Graph 1).



Graph 1. Statistical distribution of BC, $PM_{2.5}$, PM_{10} concentrations, µg.m^{-3}

The data from the correlation analysis between Black carbon concentrations and the two particle fractions is presented in Table 3. A very strong correlation was reported between the small and large particle fractions, strong between BC and $PM_{2.5}$ and medium between BC and PM_{10}, indicating that BC is mainly contained in the small fraction.

Table 3. Correlation analysis between BC, $PM_{2.5}$ and PM_{10}

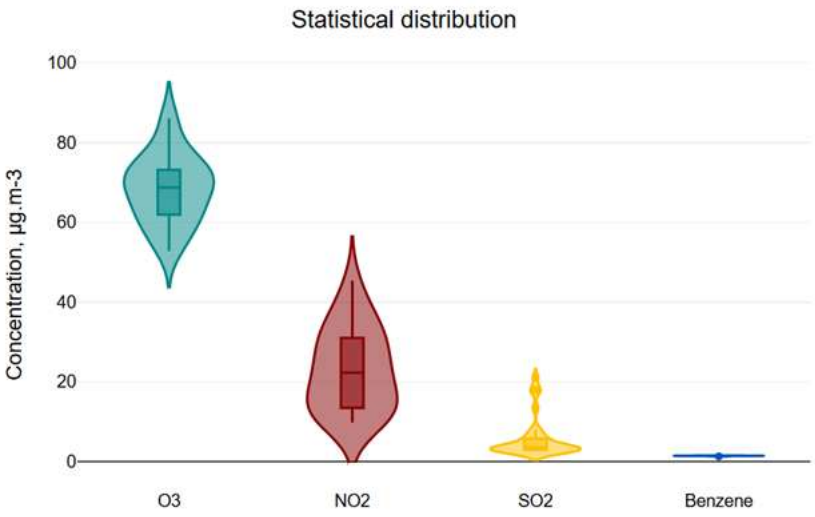
	BC [µg.m^{-3}]	$PM_{2.5}$ [µg.m^{-3}]	PM_{10} [µg.m^{-3}]
BC [µg.m^{-3}]	1		
$PM_{2.5}$ [µg.m^{-3}]	0,69	1	
PM_{10} [µg.m^{-3}]	0,49	0,82	1

The data on the measured concentrations of atmospheric pollutants at DOAS - RIEW station are presented in Table 4. The concentrations of O_3 are in the range 52,8 - 86,15 µg.m^{-3} with averige value of 68,41 with standard deviation of 8,54. The NO_2 concentrations are in the range 9,8 – 45,37 µg.m^{-3} with an averige value of 22,95 with standard deviation of 10,23. For SO_2 the range of mesurement concentrations is 2,65 – 21,02 µg.m^{-3} with averige value of 5,88 with standard deviation of 5,16. The benzene concentrations are in the range 1,29 - 1,51 µg.m^{-3} with averige value of 1,44 with standard deviation of 0,04. Again, none of the measured values exceed the norm.

Table 4. Concentration of O_3, NO_2, SO_2 and Benzene at the sampling point DOAS-RIEW

№	Date	O_3 [µg.m^{-3}]	NO_2 [µg.m^{-3}]	SO_2 [µg.m^{-3}]	Benzene [µg.m^{-3}]
1	6.2.2023	67,98	11,210	2,770	1,44
2	7.2.2023	70,79	13,510	2,990	1,49
3	8.2.2023	76,43	14,550	2,900	1,51
4	9.2.2023	86,15	12,390	2,870	1,47
5	10.2.2023	72,09	33,330	3,550	1,44
6	11.2.2023	61,84	38,180	3,580	1,46
7	12.2.2023	67,92	19,770	5,600	1,47
8	13.2.2023	66,12	30,660	3,760	1,43
9	14.2.2023	60,64	35,470	5,190	1,41
10	15.2.2023	60,66	32,550	3,260	1,41
11	16.2.2023	52,8	45,370	8,060	1,47
12	17.2.2023	63,25	40,440	17,880	1,36
13	18.2.2023	63,28	32,100	21,020	1,47
14	19.2.2023	72	24,610	17,900	1,44
15	20.2.2023	62	26,560	3,510	1,46
16	21.2.2023	68,42	28,260	13,410	1,4
17	22.2.2023	73,39	22,350	6,210	1,42
18	23.2.2023	76,78	9,800	2,820	1,42
19	24.2.2023	73,13	17,750	3,240	1,29
20	25.2.2023	75,1	22,250	3,610	1,47
21	26.2.2023	85,5	14,820	3,130	1,4
22	27.2.2023	72,2	19,540	3,110	1,45
23	28.2.2023	55,04	10,510	2,770	1,46
24	1.3.2023	70,15	10,030	2,650	1,45
25	2.3.2023	69,13	13,260	3,010	1,42
26	3.3.2023	79,31	12,850	2,880	1,41
27	4.3.2023	55,17	28,270	7,440	1,43
28	5.3.2023	58,17	22,310	5,440	1,48

The most frequently reported concentrations for ozone were at 70 µg.m^{-3}, for NO_2 – 14 µg.m$^{-3,}$ for SO_2 – 3 µg.m^{-3} and benzene – 1,4 µg.m^{-3} (Graph 2).



Graph 2. Statistical distribution of O_3, NO_2, SO_2 and Benzene concentrations, µg.m^{-3}

The data from the correlation analysis between Black carbon concentrations and O_3, NO_2, SO_2 and benzene is presented in Table 5. A moderate negative correlation was reported between BC and ozone. The likely reasons for this are : Absorption and Deposition (BC particles can absorb ozone and its precursors from the air, removing them from the atmosphere and leading to decreased ozone concentrations), Reduced Photolysis (BC absorbs solar radiation, which can reduce the photolysis (breaking down by light) of precursor molecules like NO_2, leading to lower ozone formation rates) and Vertical Stratification (At higher altitudes, BC aerosols in vertically stratified air masses can be negatively correlated with surface ozone due to downward mixing after the planetary boundary layer (PBL) develops). Correlation between BC and NO_2 is moderate again, which may be an indication of high-temperature emission processes. Thete is not correlation between BC and SO_2. The reason is the constantly low concentration of sulfur dioxide. It is the same with benzene.

Table 5. Correlation analysis between BC, O_3, NO_2, SO_2 and Benzene

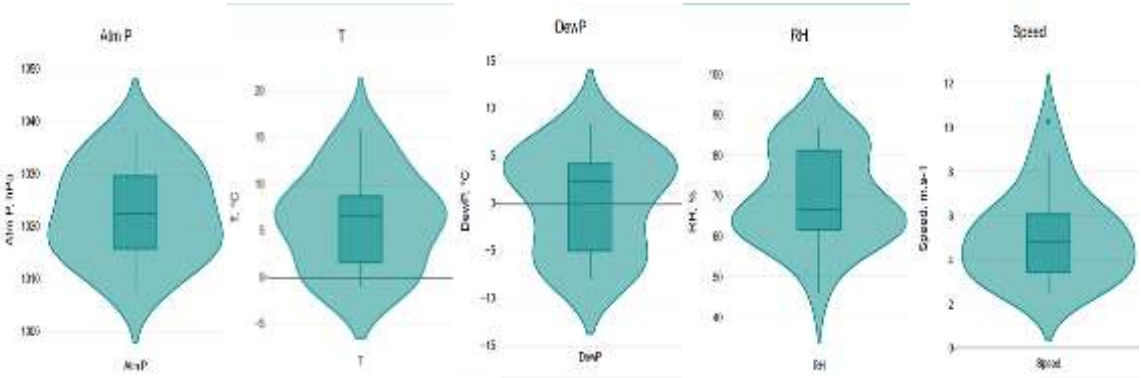
	BC [µg.m^{-3}]	O_3 [µg.m^{-3}]	NO_2 [µg.m^{-3}]	SO_2 [µg.m^{-3}]	Benzene [µg.m^{-3}]
BC [µg.m^{-3}]	1				
O_3 [µg.m^{-3}]	-0,61	1			
NO_2 [µg.m^{-3}]	0,66	-0,58	1		
SO_2 [µg.m^{-3}]	0,00	-0,24	0,49	1	
Benzene [µg.m^{-3}]	-0,06	-0,11	-0,09	-0,11	1

The meteorological data From Airport Burgas station (LBBG15655) are presented in Table 6. The values of atmospheric pressure are in the range 1007,77 - 1038,63 hPa with averige value of 1023,00 with standard deviation of 8,89. The air temperature is in the range -0,94 – 15,88°C with averige value of 6,15 with standard deviation of 5,08. For dew point the range of mesurement values is -7,94 – 8,44°C with averige value of 0,44 with standard deviation of 5,25. The relative humidity is in the range 45,88 - 86,63% with averige value of 69,20 with standard deviation of 11,19. The wind direction values are in the range 63,33 – 272,83 degree with averige value of 187,91 with standard deviation of 65,03. The last parameter, wind speed is in the range 2,46 – 10,27 $m.s^{-1}$ with averige value of 5,06 with standard deviation of 1,99.

Table 6. Data for Atmospheric pressure, Air temperature, Dew point, Relative humidity, Wind speed and direction from Meteorological station Airport Burgas (LBBG15655)

№	Date	Atm P [hPa]	T [°C]	DewP [°C]	RH [%]	Dir [Degree]	Speed [$m.s^{-1}$]
1	6.2.2023	1025,46	-0,15	-5,77	66,17	105,83	10,27
2	7.2.2023	1032,06	-0,73	-7,10	62,92	202,39	6,69
3	8.2.2023	1036,83	-0,94	-6,17	68,00	259,38	7,63
4	9.2.2023	1038,63	-0,75	-7,38	61,44	216,60	8,48
5	10.2.2023	1036,90	-0,92	-7,94	60,23	192,44	2,96
6	11.2.2023	1032,25	-0,58	-7,60	63,04	207,44	2,46
7	12.2.2023	1029,79	1,83	-4,73	63,08	212,77	4,10
8	13.2.2023	1035,06	1,33	-5,38	62,88	207,33	3,48
9	14.2.2023	1027,94	3,56	-3,06	64,08	271,91	4,21
10	15.2.2023	1029,81	4,31	-1,75	66,65	202,71	3,29
11	16.2.2023	1029,10	3,71	-1,58	71,04	198,44	3,08
12	17.2.2023	1023,63	8,33	0,06	59,25	258,30	5,25
13	18.2.2023	1018,81	10,15	1,85	57,23	256,04	5,27
14	19.2.2023	1015,50	14,52	4,40	53,25	265,00	5,48
15	20.2.2023	1019,75	7,94	3,17	73,44	216,52	4,71
16	21.2.2023	1013,52	12,52	-0,35	45,88	272,83	6,38
17	22.2.2023	1014,88	10,65	3,00	60,90	152,50	4,90
18	23.2.2023	1019,77	7,94	5,04	81,88	88,96	5,94
19	24.2.2023	1016,08	6,50	4,21	85,88	111,70	3,63
20	25.2.2023	1007,77	12,00	7,25	74,90	249,79	6,42
21	26.2.2023	1009,65	15,88	8,25	61,60	225,21	8,81
22	27.2.2023	1016,98	14,40	8,44	68,50	174,47	5,71
23	28.2.2023	1026,31	7,04	4,98	86,63	74,79	5,38
24	1.3.2023	1023,79	6,19	4,10	86,54	63,33	4,60
25	2.3.2023	1021,69	6,10	3,42	83,19	82,92	3,96
26	3.3.2023	1017,29	6,81	4,21	83,88	103,54	2,94
27	4.3.2023	1015,19	7,46	4,90	84,42	202,92	2,96
28	5.3.2023	1009,63	7,04	3,75	80,75	185,53	2,71

The most frequently reported values for Atmospheric pressure is 1018 hPa, for Air temperature – 7°C, for Dew point - 4 °C, for Relative humidity – 64% and for Wind speed – 4,5 $m.s^{-1}$ (Graph 3).



Graph 3. Statistical distribution of Atmospheric pressure, Air temperature, Dew point, Relative humidity and Wind speed

The data from the correlation analysis between Black carbon concentrations and Atmospheric pressure, Air temperature, Dew point, Relative humidity, Wind speed and direction is presented in Table 7. A strong negative degree of correlation is reported between BC and Wind speed. The likely reasons for this are : Pollutant Dispersion (wind acts as a natural cleaning mechanism, dispersing pollutants over a wider area and reducing their concentration in a specific location) and Reduced Accumulation (higher wind speeds prevent the accumulation of pollutants, such as black carbon, by moving them away from emission sources and diluting them into the atmosphere). The correlations between BC and the other meteorological parameters are weak or very weak.

Table 7. Correlation analysis between BC, AtmP, T, DewP, RH, Wwind dir and Wind speed

	BC [µg.m^{-3}]	Atm P [hPa]	T [°C]	DewP [°C]	RH [%]	Dir [Degree]	Speed [m.s^{-1}]
BC [µg.m^{-3}]	1						
Atm P [hPa]	0,23	1					
T [°C]	-0,23	-0,86	1				
DewP [°C]	-0,15	-0,86	0,87	1			
RH [%]	0,14	-0,20	-0,03	0,45	1		
Dir [Degree]	0,02	0,05	0,10	-0,27	-0,73	1	
Speed [m.s^{-1}]	-0,79	-0,02	0,09	-0,04	-0,30	0,10	1

CONCLUSIONS

The results obtained from the analysis of air quality data and meteorological parameters during the winter period show that the maritime climate, characterized by its strong winds, leads to an improvement in air quality, as well as through the absorption of ozone in its carbon. Decisions related to the high correlation with $PM_{2.5}$ and nitrogen dioxide can be a reduction in emissions from residential stoves and motor vehicles. Such measures have already been successfully undertaken and implemented by the Municipality of Burgas.

ACKNOWLEDGMENTS

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SUSTAINABILITY ASSESSMENT OF OPEN AND CLOSED SYSTEMS IN MICROALGAE CULTIVATION: A REVIEW

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ABSTRACT

Microalgae are attracting interest in many sectors, particularly agriculture, energy, environment, food, medicine, and cosmetics, due to their high biomass production capacity and ability to grow in various environments. The environmental, economic, and social sustainability of products derived from microalgae is one of the most discussed topics today. The cultivation system for microalgae is the most important factor influencing their long-term viability. Microalgae are typically grown in two systems: open ponds and closed systems, also known as photobioreactors. Open systems, like open raceway ponds, natural lakes, and lagoons, have direct contact with the atmosphere and are generally simpler. In contrast, photobioreactors are closed systems that produce microalgae under controlled conditions. In this study, these two fundamental systems commonly used in microalgae cultivation were compared in terms of sustainability. Open-system microalgae production offers greater economic benefits; it is less environmentally sustainable. The primary causes of this are the high risk of contamination and evaporation resulting from these systems' contact with the environment. Although product yield is low in these systems, more resilient species can typically be cultivated. Because closed systems produce microalgae under more controlled conditions and have a higher biomass yield, they have been found to be more beneficial for environmental sustainability. However, because of setup capital and operating costs, microalgae production in these systems is more costly. To summarize, both open and closed systems for microalgae cultivation have benefits and drawbacks in terms of environmental and economic sustainability. As a result, rather than selecting a single system, working with hybrid systems and focusing on operational conditions to local needs and resources should be viewed as the key to microalgae sustainability.

Keywords: Microalgae, cultivation system, photobioreactors, economic sustainability, environmental impacts, social sustainability

INTRODUCTION

Today, increasing energy demand, depletion of resources, and global environmental issues are driving growing interest in renewable and sustainable resources. In this context, microalgae have become one of the most promising sources for sustainable production systems due to their high biomass yield, rapid growth, ability to utilize CO_2, and capacity to synthesize various valuable products (Brooke et al., 2025). The sustainability of the system in which microalgae are grown has a direct impact on the sustainability of the final product (Ugya et al., 2025). Microalgae are grown in both open and closed environments. The advantages and disadvantages of each system in terms of sustainability are different. In the study, open and

closed systems were compared, and it was determined which system was better suited for microalgae cultivation under what conditions.

MICROALGAE

Microalgae are microscopic organisms with photosynthetic capabilities. They can multiply rapidly in various environments (Singh & Saxena, 2015). Their high biomass yields, ability to use atmospheric carbon dioxide, and ability to synthesize a variety of valuable products have made them an important biomass source in the biotechnology, food, energy, and health industries. Microalgae can produce a variety of products, including biofuels from lipids, food supplements from proteins, pigments, and antioxidants. They also have important roles in environmental applications like wastewater treatment and carbon capture (Chisti, 2020).

MICROALGAE CULTIVATION SYSTEMS

Microalgae production is typically divided into two types: open and closed systems. The type of system used depends on the product, economic resources, environmental conditions, and technical capabilities.

Open systems are simple systems in which microalgae are grown in an open environment. Shallow ponds are the most commonly used type of open system. These systems are typically installed outside, have an open top, and are capable of absorbing carbon dioxide from the air (Costa et al., 2019). The main advantage of these systems is that they are simple and inexpensive to set up and operate. The use of natural light and ventilation lowers the energy consumption of these systems. The primary disadvantage of open systems is that they are completely exposed to the outside world. This can make microalgae susceptible to contamination from bacteria, fungi, or unwanted algae species. Furthermore, environmental factors such as temperature, light, and wind cannot be controlled in these systems. This lowers production efficiency. Growth rates are low, and product quality varies in these systems (Zuccaro et al., 2020).

Closed systems, also known as photobioreactors, are systems for cultivating microalgae in a controlled environment. These systems are made up of tubes, pipes, and transparent panels like glass or plastic (Zuccaro et al., 2020). These systems get their light and air from the outside. The most significant advantage of closed systems is that production conditions are entirely controllable. Light, temperature, and pH levels can be kept constant. This translates to higher yields and higher-quality products. The risk of contamination is extremely low. Closed systems allow for greater microalgae production per unit area. The primary disadvantage of closed systems is their high installation and operating costs (Jerney & Spilling, 2020). Additionally, technical knowledge and experience are required for the system to function.

SUSTAINABILITY OF MICROALGAE CULTIVATION SYSTEMS

Microalgae cultivation systems' sustainability is not a one-dimensional issue; it needs to be assessed alongside resource consumption, environmental effects, expenses, and societal advantages.

Energy and water consumption are important factors for microalgae cultivation systems when evaluating environmental sustainability. The carbon footprint of closed systems is increased by the high energy requirements for lighting, CO_2 injection, and continuous mixing. Even though open ponds use less energy, their lower yields may have a greater environmental impact per unit of product (Gurreri et al., 2023). Water consumption is a major issue for both systems. In this sense, one notable strategy that could reduce costs and environmental impacts is the use of wastewater for microalgae cultivation.

Nutrient inputs used in microalgae cultivation are another factor that impacts sustainability. Industrial fertilizers are commonly used to supply nutrients like nitrogen and phosphorus. This imposes additional environmental costs in terms of fossil fuel consumption and greenhouse gas emissions. However, in recent years, the use of waste streams as nutrient sources, particularly urban and industrial wastewater, has provided circular economy-compatible solutions (Branco-Vieira et al., 2020). This promotes wastewater treatment while also minimizing the environmental impact of nutrient inputs.

Economically, the method of utilizing microalgal biomass is critical. The use of microalgae biomass for biofuel production is economically limited due to high production costs. Applications aimed at high-value-added products, on the other hand, appear to be more profitable. Microalgae derivatives used in cosmetics, pharmaceuticals, and food additives provide a higher revenue potential as well as a shorter return on investment (Acién et al., 2017; Pérez-López et al., 2018).

Microalgae production, on the other hand, can provide both environmental and economic benefits, as well as social benefits. The establishment of local production facilities has the potential to create jobs in rural areas while also contributing to regional development (Josa & Garfí, 2023).

CONCLUSIONS

Consideration of the environmental, economic, and social aspects of microalgae cultivation systems' sustainability reveals notable variations amongst them. Although open systems are inexpensive and simple to set up, they have poor environmental efficiency; closed systems, on the other hand, are more controllable and effective in terms of the environment, but their high energy consumption limits their economic viability. On a social level, both systems provide opportunities for local production and employment; however, hybrid solutions seem to be more sustainable.

The development of hybrid systems, integration of renewable energy, and more efficient use of waste streams can all improve the sustainability of microalgae technologies.

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SYNTHESIS AND EVALUATION OF THE ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF AN ESTER DERIVATIVE OF MALONIC ACID

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ABSTRACT

In this study we were interested in the synthesis and evaluation of the antimicrobial and antioxidant activities of a highly reactive malonic ester derivative, the bispentachlorophenyl ester of malonic acid, with the aim of employing it in the preparation of heterocyclic mesoionic compounds containing a pyrimidine ring. Due to its its high reactivit, this malonic ester undergoes rapid condensation with amine derivatives under mild conditions to produce pyrimidinium betaines, and the reaction proceeds within minutes.

Microbiological assays performed using the disk diffusion method demonstrated that this malonic ester exhibits significant antimicrobial activity against the target germs: *Bacillus subtilus, Salmonella enterica, Mucor ramannianus* and *Candida albicans* in a concentration-dependent manner. Furthermore, the assessment of its radical-scavenging capacity through the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay revealed that the compound reduces the DPPH free radical only slowly, most likely due to its limited solubility in organic solvents.

Keywords: Malonic esters derivatives , Bispentachlorophenyl ester of malonic acid, Synthesis,

INTRODUCTION

Bacteria have been reported as the cause of potentially life-threatening infections worldwide (Berber et al., 2003; Mitscher et al., 1999). Therefore, the development of new antimicrobial drugs is undoubtedly crucial, and ongoing intensive research is focused on the design of new agents (Narasimhan et al., 2007). Free radicals are major products of lipid oxidation and have been implicated in more than a hundred diseases (Thomas, 1995). Antioxidants may therefore play a crucial role in disease prevention due to their ability to scavenge reactive free radicals (Gülçin et al., 2004).

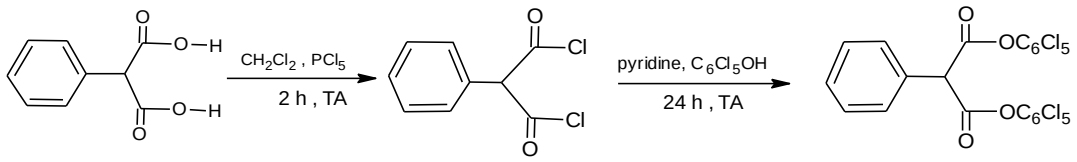
Malonic ester derivatives are widely used in organic chemistry as intermediates for the synthesis of diverse compounds. Organochlorine derivatives, in particular, represent a class of organic compounds with broad applications in agriculture, industry, medicine, and domestic use (Manoranjan et al.. 2020). They exhibit remarkabe biological activities and distinctive chemical properties, attributable to the presence of chlorine in their molecular structure—an essential element of antibacterial and antifungal agents (Trudgill et al., 1971, Monde et al., 1998). Consequently, these compounds are extensively applied as pesticides, insecticides, and fungicides in agriculture (Adelodun et al., 2020).

In this study, we focused on the synthesis of a highly reactive chlorinated malonic ester derivative, dipentachlorophenyl phenylmalonate, and on the evaluation of its antimicrobial activity using the disc diffusion method **(**Balouiri et al., 2016). This compound is widely employed in organic synthesis due to the strong electron-withdrawing effect of the chlorophenyl groups **(**Dvortsak et al., 1976, Kappe et al., 1971). The presence of ten chlorine atoms in its structure is expected to enable under very mild conditions and within a shorter time with other reagents (Dvortsak et al., 1976, Malki et al. 2014), The study also examined the antioxidant potential of this ester using the DPPH assay, with the aim of exploring its potential valorization as an antioxidant

MATERIAL AND METHOD

Chemical part

The compound investigated in our study, dipentachlorophenyl phenylmalonate 2, can be prepared according to the procedure reported by (Huhn et al.,1976), by the action of phosphorus pentachloride on phenylmalonic acid **1** (Scheme 1).



1 **2**

Scheme1. Synthesis of dipentachlorophenyl phenylmalonate **2**

Biological part

Antimicrobial activity

The antibacterial and antifungal activities of the synthesized malonic ester (2) were evaluated using the agar diffusion method, also known as the disc diffusion assay (Balouiri et al., 2016).

The microbial strains tested included:
Bacteria (Gram-): *Salmonella enterica (S.e),* (Gram+): *Bacillus subtilus (B.s)*ATCC 6633
Fungi: *Fusarium oxysporum lini (F.o.l*), *Mucor romannianus (M.r)*NRRL 1829*Fusarium culmorum (F.c)*
Yeasts: *Candida albicans (C.a)* IPA 200.

The tested microorganisms were obtained from the Laboratory of Microbial Systems Biology (LBSM), École Normale Supérieure, Kouba, Algiers, Algeria. The culture medium used was ISP2 [International Streptomyces Project], as recommended by Shirling and Gottlieb (1966). Its composition was as follows: 4 g glucose, 4 g yeast extract, 10 g malt extract, 10 g agar, and 1 L distilled water. The pH of the medium was adjusted to 7.2.
The diameters of the inhibition zones surrounding the discs containing the test samples were measured; a positive antimicrobial activity was indicated by a clear zone around the disc corresponding to the inhibition of microbial growth (Gülçin et al., 2008).

Gentamicin, fluconazole, and amphotericin B were used as reference antibiotic and antifungal agents, respectively.

Antioxidant activity

The antioxidant activity of a compound corresponds to its ability to resist oxidation. In this context, the antioxidant activity of the synthesized malonic ester **2** was evaluated *in vitro* using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay **(**Blois, 1958; Brand-Williams, 1995), with slight modifications. The principle of this method is based on the measurement of the radical scavenging ability of DPPH in methanol solution. The addition of an antioxidant to the DPPH solution leads to its decolorization, which is directly proportional to the antioxidant capacity of the tested compound, thus indicating the reduction of the radical to diphenylpicrylhydrazine (DPPHH). This decolorization can be monitored spectrophotometrically by measuring the decrease in absorbance at 517 nm. Solutions of test compounds of increasing concentrations were prepared, the absorbance relative to the negative control containing only the DPPH solution is measured by spectrophotometry at 517 nm .The positive control was represented by a solution of a standard antioxidant, butylated hydroxytoluene (BHT), whose absorbance was measured under the same conditions as the samples. All tests were performed in triplicate. The percentage of DPPH inhibition (I%) was calculated according to the following formula (Harififar et al., 2007; Malki et al., 2019).

I%= [(Abs contrôle – Abs test)/ Abs contrôle] x 100

Abs : Absorbance at 517 nm

RESULTS AND DISCUSSION

Dipentachlorophenyl phenymalonate **2** was synthesized as described in scheme 1. It was obtained in the form of a white solid with good yield. This compound was characterized through various analytical methods and its structure was confirmed (Malki et al., 2011) . The physical and spectroscopic characteristics of ester **2** are summarized in Table 1. The presence of two chlorophenyl groups in its structure makes the carbonyl groups highly reactive toward nucleophilic agents, including amidine derivatives (Malki et al., 2015).

Table 1. Physical and spectroscopic characteristics of malonic ester **2**

Compound	Aspect	T_{fus} (°C)	Yield (%)	λ_{max} (nm) (ε) (Dioxanne	IR (KBr) $\bar{\upsilon}$ (cm^{-1})
Dipentachlorophenyl phenylmalonate **2**	White solid	226-230	87	303 (5050)	1815 (ester) 1360 ,1390 (penta-chlorophényle

Antimicrobial activity

Antimicrobial substances are defined as compounds used either to destroy microorganisms or to inhibit their growth, including antibiotics as well as other antibacterial and antifungal agents.

In the present study, malonic ester **2** was tested *in vitro* to evaluate its antibacterial and antifungal activities using the disc diffusion method (Balouiri, 2016)**.** The activity against the tested microorganisms was assessed based on the presence or absence of inhibition zones. The diameters of the inhibition zones for bacteria, fungi, and yeasts are presented in Tables 2 and 3, respectively.

Table 2. Zones of inhibition of bacteria obtained with the synthesized malonic ester **2**

Concentration (µg/disque)	Inhibition zone diameters * (mm)	
	Gram + bacteria	Gram- bacteria
	B.s	*S.e*
20	12	7
60	16	14

* The diameter of the discs is included in the zone of inhibition.
− Absence of Inhibitory activity
B.s: Bacillus subtilus, *S.e: Salmonella enterica*

Table 3**.** Zones of inhibition of fungi and yeasts obtained with the synthesized malonic ester.

Concentration (µg/disque)	Inhibition zone diameters * (mm)			
	Fungi			Yeasts
	F.c	*F.o.l*	*M.r*	*C.a*
20 µg	−	−	8	8
60 µg	−	−	10	8

* The diameter of the discs is included in the zone of inhibition.
− Absence of inhibitory activity
F. o.l : Fusarium oxysporum lini, *M.r : Mucor romannianus,*

F.c : *Fusarium culmorum* *C.a : Candida albicans*

The results presented in Table 2 demonstrate that malonic ester **2** displays a pronounced antibacterial activity against the Gram-positive bacterium ***(B.s)***with inhibition zones ranging from 12 to 16 mm, while exhibiting comparatively lower activity against the Gram-negative strain *Salmonella enterica (S.e).* These findings indicate that both Gram-positive and Gram-negative bacteria are susceptible to this compound.

Regarding antifungal activity (Table 3), the highest inhibition zone was observed against the filamentous fungus *Mucor ramannianus (M.r),* followed by a relatively weak effect against the yeast *Candida albicans (Ca)*. No activity was detected against the fungi *Fusarium culmorum (F.c) and Fusarium oxysporum lini (F.o.l).*

Furthermore, it was observed that this activity increases proportionally with the concentration of the tested ester. The presence of ten chlorine atoms, structural elements frequently associated with antibacterial and antifungal properties, within the ester structure is probably responsible for its antimicrobial activity (Naumann, 2000**).** Figure 1 illustrates the antibiogram of the malonic ester **2** tested against the Gram-positive bacteria *Bacillus subtilus (B.s)* and the fungus *Mucor romannianus (M.r).*

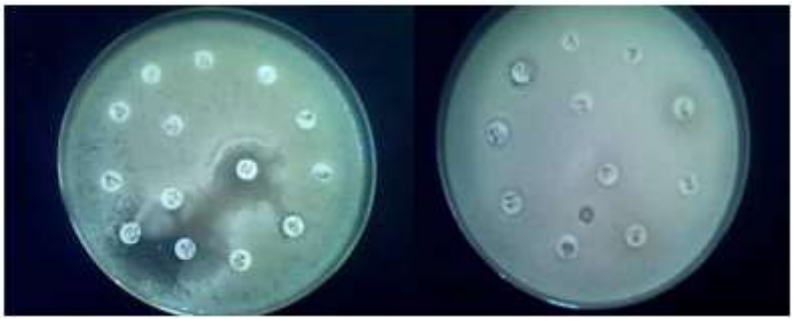


Figure 1. Antibiogram of the malonic ester **2** tested against the Gram-positive bacteria *Bacillus subtilus* (left) and the fungus *Mucor ramannianus* (right).

Antioxidant Activity

The *in vitro* antioxidant potential of the synthesized malonic ester **2** was assessed using the DPPH radical scavenging assay. This radical was one of the first free radicals employed to investigate the structure–antioxidant activity relationship of phenolic compounds (Olszowy-Tomczyk, 2021).

The reduction kinetics of DPPH of the tested ester, Dipentachlorophenyl phenylmalonate **2** at a concentration of 25 µg/mL and the reference antioxidant BHT were monitored over time until reaching the effective time value (TE50), corresponding to a 50% reduction in the DPPH activity (absorbance) in the reaction medium (Małgorzata, 2019) . The obtained kinetic profile revealed a time-dependent antiradical activity. The longer the reaction time, the higher the percentage of DPPH inhibition. Figures 2a and 2b illustrate this effect in the case of malonic ester **2** and the standard antioxidant BHT.

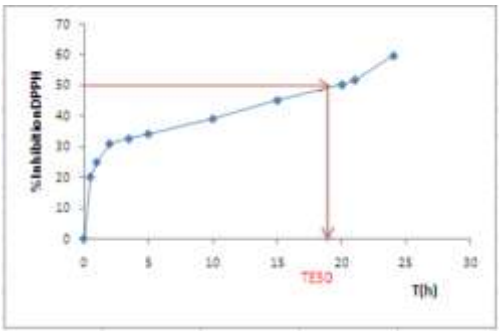


Figure 2a**.** %of DPPH inhibition over time by malonic ester **2**

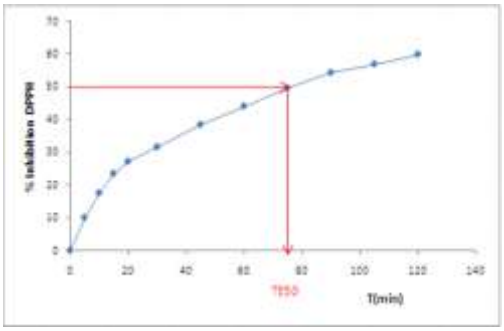


Figure 2b**.** % of DPPH inhibition over time by BHT

From the curve plotting the relationship between the percentage of DPPH inhibition (I% DPPH) and reaction time, the effective time (TE50) required to reduce 50% of DPPH was determined by graphical interpolation. It was observed that ester **2** reduced the DPPH free radical very slowly. At, t = 3 h, the % inhibition of the DPPH radical is only 33% and the is of the order of 19,2h, which is considerably slower compared to BHT, whose TE50 was about 75 min. The TE50 values, expressing the effective time required for trapping and reducing 50% of DPPH in methanolic solution, are summarized in Table 4.

Table 4. Effective time (TE50) values of malonic ester **2** and BHT.

Compound	TE50
Malonic ester **2**	19.2 h
BHT	75min

As shown in Table 4, malonic ester **2** exhibits antioxidant activity; however, its efficiency was markedly lower than that of the standard antioxidant BHT. The presence of a hydrogen atom adjacent to two carbonyl groups in malonic ester **2** may account for its DPPH radical scavenging activity. In the presence of the DPPH free radical, this hydrogen atom is transferred to the radical, which is subsequently converted into a stable DPPHH molecule (Molyneux, 2004; Sanchez-Moreno, 1998). This process leads to a decrease in both the free radical concentration and the absorbance over the course of the reaction, until the hydrogen-donor antioxidant capacity is exhausted (Villano et al., 2007).

Regarding the molecular structures, the high efficiency of BHT is primarily attributed to the presence of hydroxyl groups in its structure. In contrast, the absence of such groups in the structure of the ester and its poor solubility in organic solvents, are likely responsible for its comparatively weak antioxidant potential. It has been demonstrated that molecules such as ascorbic acid, butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) display strong radical scavenging activity owing to the hydroxyl groups in their structures, which which readily donate hydrogen atoms to DPPH radicals (Bondet et al., 1997)**.**

CONCLUSION

In this study, we reported a simple synthetic route to obtain a highly reactive malonic ester derivative, dipentachlorophenyl phenylmalonate in good yield. This ester exhibits notable antibacterial and antifungal activities in a concentration-dependent manner. In addition, it demonstrates a modest antioxidant effect against the DPPH free radical, which can be attributed to its limited solubility in organic solvents.Therefore, further investigations are needed to determine the optimal conditions for its potential applications in the agri-food and pharmaceutical–medical fields.

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ASSESSMENT OF THE IMPACT OF SURFACE WATER TABLE IRRIGATION ON THE PHYSICOCHEMICAL PROPERTIES OF SOILS IN OUED M'ZAB (ALGERIA)

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ABSTRACT

This study focuses on evaluating the quality of surface water table in the Oued M'Zab region to determine its suitability for agricultural use. The adopted methodology is based on spatial analysis of water salinity and sodicity, as well as classification according to irrigation standards. The results indicate that the groundwater exhibits poor quality for irrigation, with significant risks of soil salinization. This calls for strict irrigation management to promote salt leaching. Additionally, the alkalinization risk ranges from low to moderate, with potential sodium toxicity for sensitive crops.

Keywords: Risk, Irrigation, Surface water table, Soils, Oued M'Zab,

INTRODUCTION

Agriculture represents the largest consumer of water resources. These resources, depending on the regions from which they originate and their possible contact with sources of pollution, display highly diverse characteristics (Bouaroudj, 2012). Consequently, the quality of irrigation water varies according to the nature and the concentration of dissolved salts. An increase in salt content is responsible for several problems affecting both soil and plants. The most frequently encountered issues are associated with salinity, sodicity, specific ion toxicity, as well as other problems linked to excess nitrogen and bicarbonates (Daddi Bouhoun, 1997).

In southern Algeria, particularly in the M'Zab Valley, the climatic water deficit is such that resorting to irrigation constitutes a vital necessity for agricultural production. Groundwater represents the only resource available to meet the needs of irrigation and various domestic uses, which highlights the importance of thoroughly assessing the quality of these waters and protecting them against any risk of contamination (Taleb Bahmed, 2008).

The aim of this study is to evaluate the quality of irrigation water from the surface water table of the M'Zab Valley through the determination of electrical conductivity (E.C) and the Sodium Adsorption Ratio (S.A.R), with a view to their use in agriculture.

MATERIAL AND METHOD

Our study was carried out in the M'Zab Valley, located 600 km south of the capital Algiers. Administratively, it belongs to the province of Ghardaïa (Benhedid, 2015; Achour et *al.*, 2016).

The M'Zab Basin covers an area of approximately 5,000 km², with poorly defined boundaries in its eastern part. The Oued M'Zab flows roughly from west to east over 320 km, from the Botma-Rouila region at an altitude of 750 m to the Sebkhet Safioune at 107 m, with a relatively steep slope between Ghardaïa and Bounoura (Dubief, 1953). Its width ranges from 1.50 to 2.35 km upstream and gradually decreases downstream to reach 0.55 km south of the town of El Atteuf (Achour, 2014).

The main water resources in this area are of groundwater origin. They are contained in two types of aquifers: the shallow Quaternary alluvial aquifer and the deep Continental Intercalaire aquifer (Bensaha et al., 2011; Benhedid, 2015).

In order to properly assess the quality of water from the surface water table of the Oued M'Zab for irrigation purposes, our study was conducted at four stations corresponding to the municipalities of Daya Bendahoua, Ghardaïa, Bounoura, and El Atteuf, distributed along the Oued M'Zab from upstream to downstream (Fig. 1).

A total of forty-five traditional wells, distributed from upstream to downstream of the Oued M'Zab with a variable number per study station, were sampled for physico-chemical analyses. This allowed for the classification of these waters according to the irrigation standards established by the Riverside Laboratory and modified by Durand (1983). The number of sampling sites in the stations of Daya Bendahoua, Ghardaïa, Bounoura, and El Atteuf was 5, 29, 6, and 5 respectively (Fig. 1).

Water samples were collected following the standards of Rodier et *al.* (2009), during the high-water period from February to March 2013. The parameters studied were electrical conductivity (E.C.) and the Sodium Adsorption Ratio (S.A.R). Electrical conductivity was measured using a conductimeter (L.F. 538 type). The S.A.R was calculated from the concentrations (in meq/L) of Na^{+}, Ca^{2+}, and Mg^{2+} in the water according to the following formula:

$$\mathrm{S.A.R.} = \frac{\mathrm{Na^{+}}}{\sqrt{(\mathrm{Ca^{++}} + \mathrm{Mg^{++}})/2}}$$

Sodium (Na^{+}) was determined using a flame emission spectrophotometer, while calcium (Ca^{2+}) and magnesium (Mg^{2+}) were analyzed by titrimetry.

To determine the spatial distribution of the risks of soil salinization and alkalinization due to the waters of the shallow aquifer, the results obtained were represented in the form of maps generated using Surfer 11 software, based on a Google Earth satellite image (2013).

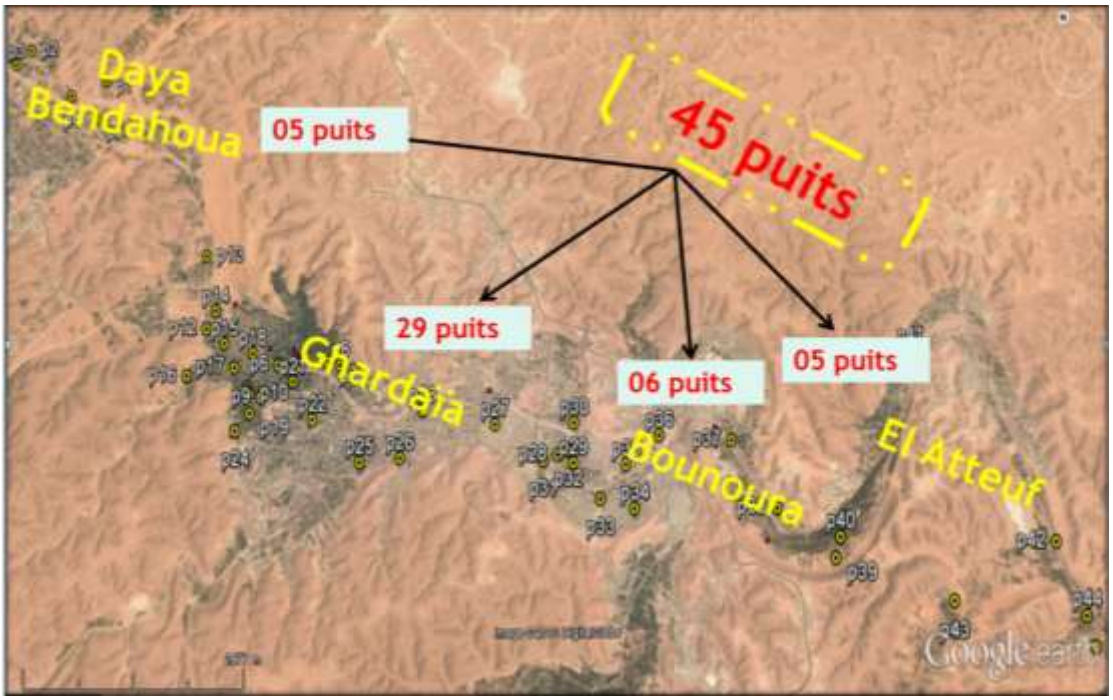

Figure 1. Location of the surface water table wells in Oued M'Zab

RESULTS AND DISCUSSION

Water Salinity

The classification of water salinity proposed by the Riverside Laboratory and modified by Durand (1983) reveals the presence of three classes (Fig. 2 and Table 1):

Class C3 (750 < E.C < 2250 µS/cm): This class includes only four samples, representing 9% of all analyzed water points. These correspond to wells P5 in Daya Bendahoua, P12 and P33 in Ghardaïa, and P37 in the Bounoura station. Such waters are unsuitable for soils with restricted drainage. Even with adequate drainage, special salinity control practices may be necessary, and only salt-tolerant crops can be cultivated.

Class C4 (2250 < E.C < 5000 µS/cm): This class comprises the majority of wells, representing 62%, which indicates that most waters from this aquifer exhibit very high salinity. These waters are generally unsuitable, except in highly permeable soils with good drainage, combined with excess irrigation to ensure strong leaching of salts from the soil. Crops grown under these conditions must be highly salt-tolerant.

Class C5 (5000 < E.C < 20000 µS/cm): This class corresponds to most of the wells located in the El Atteuf station (80%) and to 50% of those in Bounoura, while it represents only 29% of all wells overall. In general, the surface water table of Oued M'Zab are characterized by very high salinity: 62% of the wells, excessive salinity: 29%, and high salinity: 9%.

The spatial variation of water E.C shows an increase from upstream to downstream, following the direction of water flow. Salt accumulation in the aquifer is favored by irrigation leaching, wastewater discharge, and the geological layers crossed by the wells. In addition, excessive pumping of irrigation water from certain wells can lead to salt concentration, while reduced pumping or non-use of wells during winter allows for a decrease in water salinity due to dilution from rainfall.

According to Daddi Bouhoun (1997) and Couture (2004), high water E.C may cause an excess of soluble salts in the soil, thereby reducing water absorption by plants (osmotic pressure phenomenon), and consequently, reducing plant growth depending on crop tolerance to salinity.

Table 1. Salinity classes of the surface water table of Oued M'Zab

Salinity / Stations	C3 High salinity		C4 Very high salinity		C5 Excessive salinity	
	Number of wells	%	Number of wells	%	Number of wells	%
Daya Bendahoua	1	20	3	60	1	20
Ghardaïa	2	7	22	76	5	17
Bounoura	1	17	2	33	3	50
El Atteuf	-	-	1	20	4	80
Total	4	9	28	62	13	29

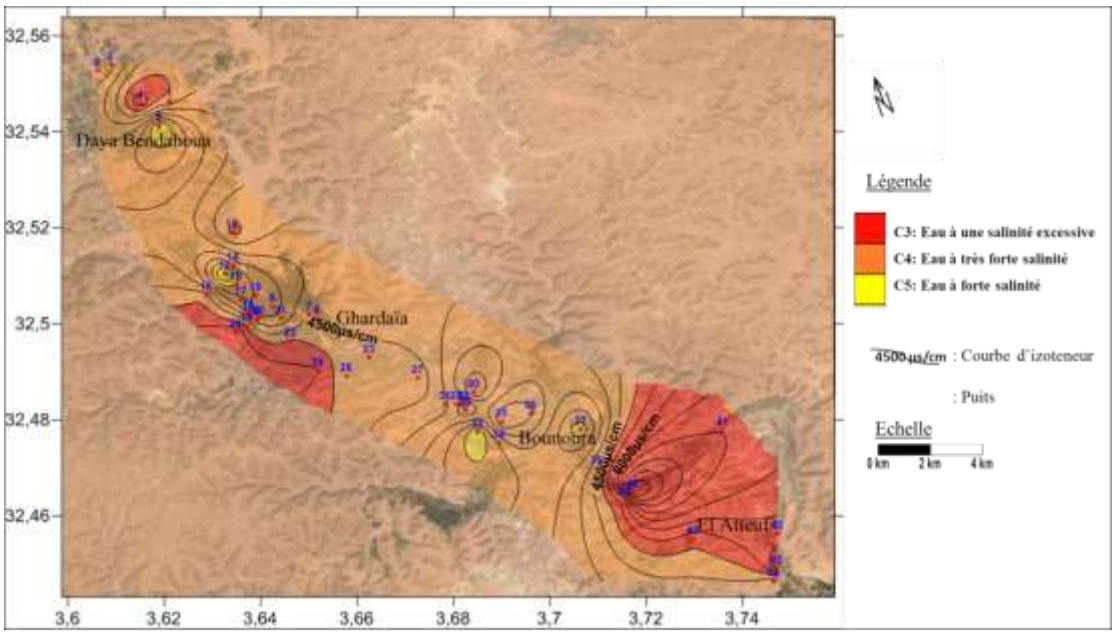

Figure 2. Map of soil salinization risk in the Oued M'Zab

Water Sodicity

The Riverside classification makes it possible to determine the risk of soil alkalinization by class, based on the S.A.R and E.C values of the waters, using the Riverside diagram (Fig. 4, 5, 6). This classification reveals the presence of four classes (Table 2; Fig. 3) (Durand, 1983):

Class S1: This class represents only 24.5% of all water points. These waters can be used for the irrigation of almost all soils with little risk of alkalinization.

Class S2: The majority of wells belong to this class, representing 62.5%, particularly the waters of the El Atteuf wells. The risk of soil alkalinization is significant in fine-textured soils with high cation exchange capacity. However, this water is suitable for coarse-textured soils or organic soils with good permeability.

Class S3: This class corresponds to waters that may induce a hazardous alkalinity in most soils. Their use requires proper drainage, strong leaching, and the addition of organic matter. It represents 11% of the wells.
Class S4: The risk of alkalinization is very high. Such waters can be used for irrigation only if their low salinity allows for the addition of soluble calcium or if the soil already contains sufficient amounts. This class corresponds only to well P40, representing 2%.

Table 2. Sodicity classes of the surface water table of Oued M'Zab

Stations / Sodicity	Daya Bendahoua		Ghardaïa		Bounoura		El Atteuf		Total	
	Nb	%	Nb	%	Nb	%	Nb	%	Nb	%
S1	2	40	8	28	1	17	-	-	11	24,5
S2	2	40	17	58	4	66	5	100	21	62,5
S3	1	20	4	14	-	-	-	-	5	11
S4	-	-	-	-	1	17	-	-	1	2

Nb : Number of wells

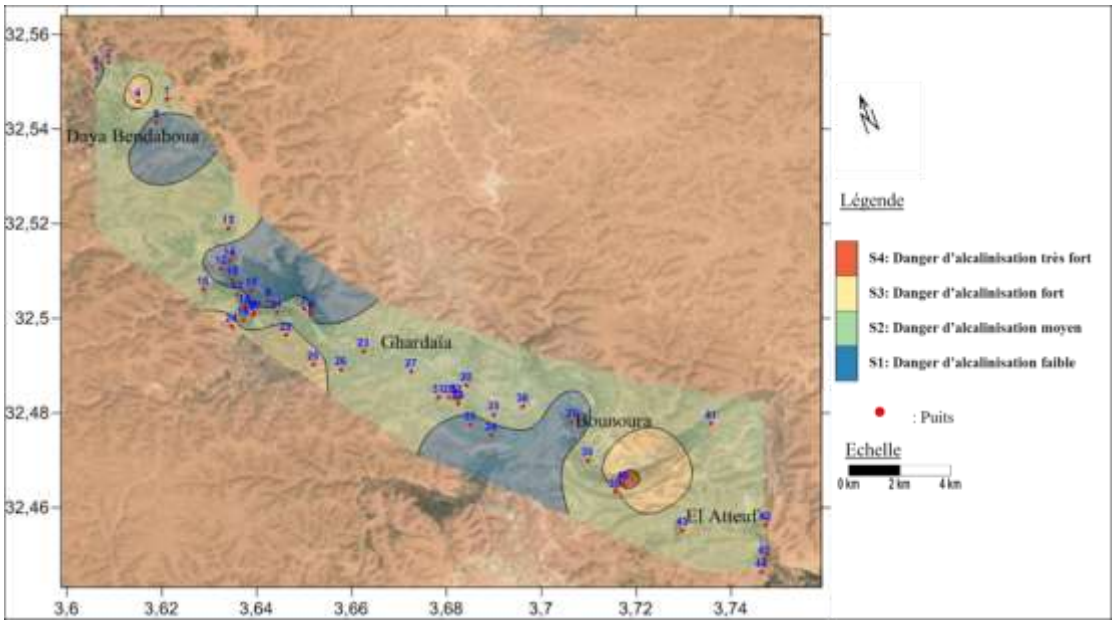

Figure 3. Map of soil alkalinization risk in the Oued M'Zab

Classification of Irrigation Waters

The Riverside diagrams, modified by Durand (1983) (Fig. 4, 5, 6), together with the determination of alkalinization risk, allow for the classification of irrigation waters based on the measured parameters of E.C and S.A.R This approach enabled us to identify six classes of water in the M'Zab Valley, as follows (Table 3):

Class C3-S1: Waters with high salinity and low alkalinization risk. This class includes only four wells, representing 9% of the total.

Class C4-S1: Waters with very high salinity and low alkalinization risk, representing 15.5% of the analyzed water points.

Class C4-S2: Waters with very high salinity and medium alkalinization risk. This class represents 47% of the wells, including 55% of those in Ghardaïa.

Class C5-S2: Waters with excessive salinity and medium alkalinization risk, accounting for 15.5%, and representing 80% of the wells in El Atteuf.

Class C5-S3: Waters with excessive salinity and high alkalinization risk, accounting for 11% of all wells, including 20% in Daya Bendahoua and 14% in Ghardaïa.

Class C5-S4: This class corresponds only to well P40 and represents 2% of the wells. These waters exhibit excessive salinity and very high alkalinization risk.

Table 3. Water classes of the surface water table of the Oued M'Zab

Stations / Water classes	Daya Bendahoua		Ghardaïa		Bounoura		El Atteuf		Total	
	Nb	%	N°	%	N°	%	N°	%	N°	%
C3-S1	1	20	2	7	1	17	-	-	4	9
C4-S1	1	20	6	21	-	-	-	-	7	15,5
C4-S2	2	40	16	55	2	33	1	20	21	47
C5-S2	-	-	1	3	2	33	4	80	7	15,5
C5-S3	1	20	4	14	-	-	-	-	5	11
C5-S4	-	-	-	-	1	17	-	-	1	2

Nb : Number of wells

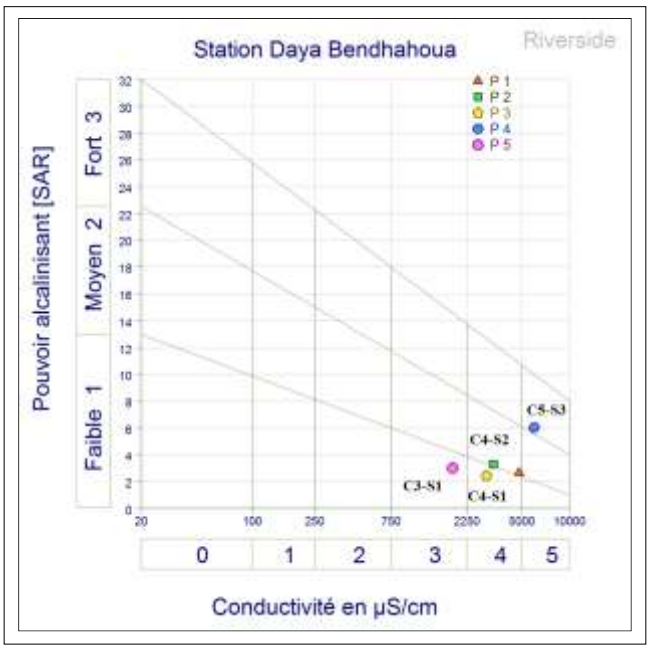

Figure 4. Irrigation water quality in the Daya Bendahoua station as a function of E.C and S.A.R

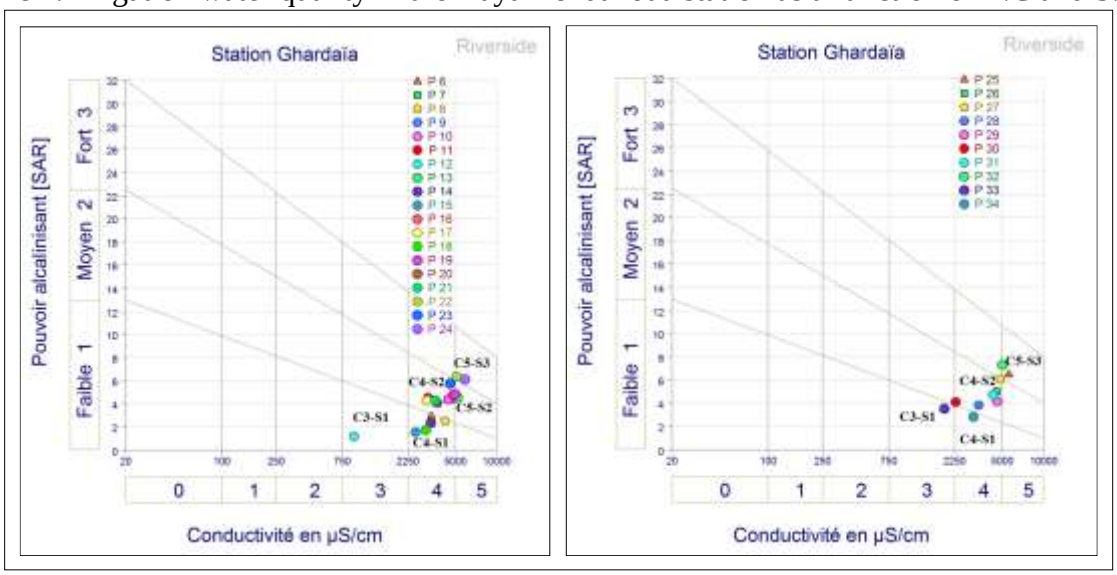

Figure 5. Irrigation water quality in the Ghardaïa station as a function of E.C and S.A.R

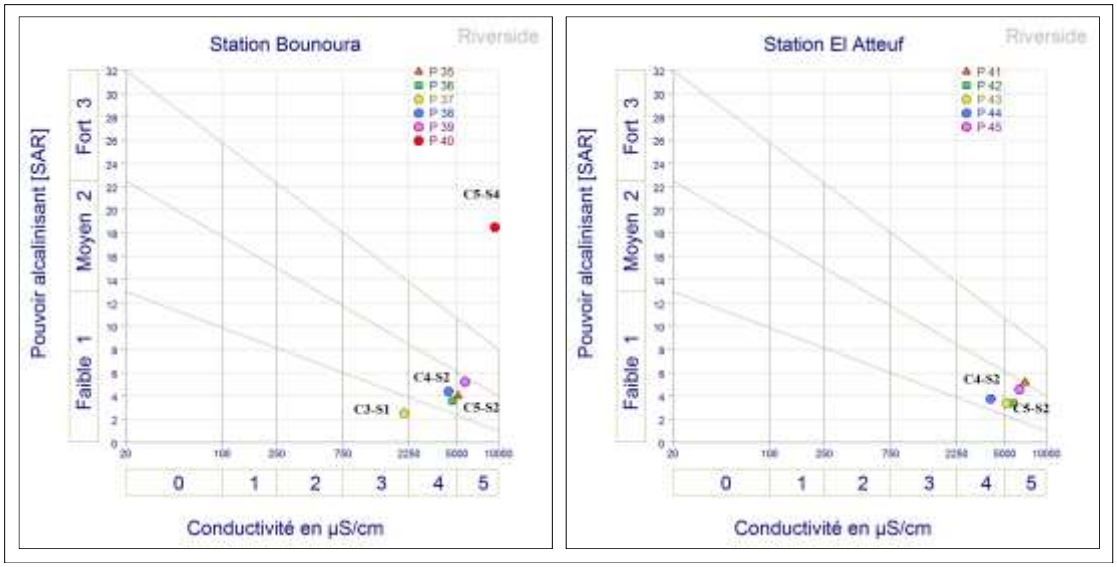

Figure 6. Irrigation water quality in the Bounoura and El Atteuf stations as a function of E.C and S.A.R

CONCLUSIONS

The study of the quality of the surface water table of the Oued M'Zab, with reference to irrigation water standards, revealed that these waters are highly saline. This situation reflects both the effect of lithology and the agro-urban anthropogenic impact, which contribute to the increase in the concentrations of certain elements.

The agricultural classification of these waters indicates that they are of poor quality for irrigation, particularly downstream in Bounoura and El Atteuf. In general, the waters present a risk of soil salinization, which requires careful irrigation management to ensure adequate salt leaching and thereby avoid salt stress for crops. The alkalinization risk is moderate, with a possible risk of sodium toxicity for sensitive crops.

Our study highlights the degradation of groundwater resources due to agricultural and urban activities in the M'Zab Valley. It is necessary to optimize the use of these groundwater resources and to raise farmers' awareness in order to improve agricultural practices. Further research is essential in the region

to monitor water quality and assess agricultural and environmental impacts. Additional management measures are also needed with a view to safeguarding the ecosystem in support of sustainable development in the M'Zab Valley.

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RESEARCH ON CONVENTIONAL WHEAT CULTIVATION TECHNOLOGY, INCLUDING SOIL TILLAGE AND PHASED FERTILIZATION, APPLIED TO THREE WHEAT VARIETIES UNDER NON-IRRIGATED CONDITIONS, IN THE PEDOCLIMATIC CONDITIONS OF GĂLBINAȘI, BUZĂU COUNTY, DURING THE 2023–2024 AGRICULTURAL YEAR

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ABSTRACT

The present study aimed to evaluate the agronomic behavior and qualitative performance of three winter wheat varieties (Katarina, Biharia, Apache) cultivated under rainfed conditions in the pedoclimatic context of Gălbinași, Buzău County, Romania, during the 2023–2024 agricultural year. The experiment was carried out on a 45 ha experimental field and applied a conventional cultivation technology including soil tillage and phased fertilization, tested in three variants: control (no additional fertilization), two-phase fertilization, and three-phase fertilization. Data collection included plant density at emergence and harvest, thousand-kernel weight, number of grains per spike, test weight, yield per hectare, and grain quality indices (moisture, protein content, wet gluten content). Statistical analysis (ANOVA and linear regression) demonstrated the significant influence of fertilization on plant density and kernel weight ($p < 0.05$), with a moderate correlation between fertilization level and yield ($R^2 = 0.58$). The variety Katarina recorded the highest stability and yield potential (7711.4 kg/ha with three-phase fertilization), followed by Biharia and Apache. Grain quality was superior for Katarina and Biharia regarding protein and gluten content, while Apache showed lower values under low fertilization. The results highlight the potential of phased nitrogen fertilization to mitigate drought stress effects and improve both yield and grain quality under the restrictive climatic conditions of southeastern Romania. The study provides valuable recommendations for optimizing winter wheat cultivation technologies in areas with similar pedoclimatic conditions.

Keywords: soil tillage, fertilization, wheat, drought, climate change

INTRODUCTION

Agriculture is one of the oldest human occupations, emerging with the transition from nomadism to sedentary living (Guș & Tianu, 1991). Throughout the evolution of human societies, agriculture has diversified and improved, being strongly influenced by technological and social progress. A defining moment was the invention of the plough, evolving from simple wooden beams to modern ploughs with mechanical traction and multiple moldboards (Panaitescu, 2016; Panaitescu & Niță, 2011). The expansion of mechanization, the use of chemical fertilizers, and the introduction of genetic engineering have significantly increased yields but have also generated negative environmental impacts (Budoi & Penescu, 1996; Cakpo et al., 2025).

In Romania, studies by the National Research and Development Institute for Soil Science, Agrochemistry and Environmental Protection have highlighted the degradation of soil quality through

erosion, acidification, salinization, compaction, and chemical pollution (Patriche et al., 2023a; Patriche et al., 2023b; Stănilă, 2023). Globally, the intensification of conventional agricultural practices has contributed to biodiversity loss, water pollution, and contamination of agri-food products (Gagiu et al., 2023; Gagiu et al., 2025). Therefore, adapting these practices is essential to protect ecosystems and human health (Budoi & Penescu, 1996; Calistru et al., 2024).

In recent decades, climate change has become a major challenge for global agriculture. The global average temperature has increased by approximately 1.7°C compared to the pre-industrial period and is projected to rise by up to 2.7°C in the coming decades (Bandoc & Prăvălie, 2015; Moise, 2023; European Environment Agency, 2023). In temperate continental regions, such as Muntenia, these changes translate into prolonged droughts, uneven precipitation distribution, heatwaves, torrential rains, hail, and extreme winds (Niță et al., 2024; European Environment Agency, 2023). The Gălbinași commune, Buzău County, frequently faces such phenomena, requiring the adaptation of agricultural technologies to the new conditions (Gagiu et al., 2025).

Winter wheat (Triticum aestivum L.) remains one of the key crops in Romania due to its economic value and its ability to adapt to local soil and climate conditions (Panaitescu, 2016; Popescu et al., 2023). The choice of suitable variety, the application of adapted cultivation technologies, and soil conservation are key factors in achieving stable and high-quality yields (Guș & Tianu, 1991; Cakpo et al., 2025).

The aim of this study is to analyze the behavior and suitability of three winter wheat varieties grown under non-irrigated conditions in Gălbinași, Buzău County, during the 2023–2024 agricultural year, characterized by extreme climatic conditions. The applied cultivation technology was conventional, including soil tillage (Cakpo et al., 2025), and phased fertilization varied depending on variety and treatment (Calistru et al., 2024). For each variety, three experimental variants were tested: control (no additional phased fertilization), two-stage phased fertilization, and three-stage phased fertilization.

The specific objectives of the study are: to compare the agronomic behavior of the three wheat varieties under identical technological and climatic conditions; to analyze the performance of the varieties under conventional systems in terms of agronomic efficiency and conservation of soil and water resources, within the context of sustainable agriculture; to formulate technical recommendations on variety selection and cultivation technology adapted to the specific conditions of Gălbinași, with the possibility of extending the results to areas with similar pedoclimatic conditions; to reduce production costs and achieve high and superior quality yields.

MATERIAL AND METHOD

The study was conducted during the 2023–2024 agricultural year on a 45-hectare experimental plot belonging to Denagro Martrans company, located in Gălbinași, Buzău County, in a region representative of large-scale farming in southeastern Romania. The area's climate is excessively continental, characterized by hot, dry summers with maximum temperatures reaching 39°C, and cold winters with frequent blizzards and temperatures dropping below -20°C. Annual precipitation is low and unevenly distributed, while the average annual temperature is around 11.5°C. The soils in the experimental area are classified as cambic chernozems, with a loamy texture in the upper horizon and loamy-silty texture in intermediate horizons, developed on calcareous loess deposits, and with a physical structure suitable for conventional agricultural practices.

The biological material consisted of three winter wheat (Triticum aestivum L.) varieties: Apache, Katarina, and Biharia. Apache is an early, awnless variety with medium height, excellent lodging resistance, high tillering and winter hardiness, and good tolerance to foliar diseases,

particularly rusts and fusarium. Katarina, also early and awnless, has strong emergence vigor, good winter hardiness, high yield potential, and resistance to major diseases. Biharia is a semi-late variety, with short stature and short awns, notable for its drought, lodging, and disease resistance, as well as for its very good tillering capacity.

The cultivation technology followed conventional system principles, including primary ploughing, soil loosening and leveling, followed by sowing at a 12.5 cm row spacing with the same seeding rate for all experimental variants. The experiment comprised nine variants, three for each variety, organized as follows: a control variant (without additional phased fertilization, to highlight the biological potential and the effect of the previous crop), a variant with two-stage phased fertilization, and a variant with three-stage phased fertilization. Plant protection treatments (herbicides, fungicides, and insecticides) were uniformly applied across all variants, ensuring that the observed differences could be attributed exclusively to the genotypes and fertilization regime.

To evaluate the agro-productive performance of the three varieties, observations and measurements were carried out throughout the growing season. These included plant density at emergence and harvest (expressed as plants/m²), thousand kernel weight (TKW), determined by weighing a standardized sample of mature grains, number of grains per spike, determined by manual harvest and counting, hectoliter weight expressed in kg/hl according to SR 13574:2010 standard, and average yield per hectare, calculated based on the weight of grains relative to the cultivated area. Additionally, grain quality parameters (moisture, protein, and gluten content) were analyzed using appropriate laboratory equipment.

To analyze differences among the experimental variants and assess the influence of fertilization under the water stress conditions characteristic of the study year, the data were subjected to statistical analysis. Analysis of variance (ANOVA) was used to test the significance of differences between treatments, and linear regression models were applied to evaluate the relationship between fertilization level, yield, and lack of precipitation. The significance level was set at $p \leq 0.05$. Data processing was performed using SPSS statistical software (version 26) and Microsoft Excel.

RESULTS AND DISCUSSION

In the experiment, soil preparation for the winter wheat crop followed agrotechnical principles aimed at maximizing the productive potential of the crop. Winter wheat performs best when preceded by early-harvest species that leave the soil rich in nitrogen and other essential nutrients, thereby enabling timely execution of primary soil work. In all nine experimental variants, the preceding crop was pea, a legume highly valued agronomically due to its ability to fix atmospheric nitrogen and leave the soil weed-free, enriched with plant residues that, once incorporated, increase organic matter content and stimulate soil microbiological activity.

The experiment was conducted on a total area of 45 hectares, equally divided among the three tested wheat varieties: Apache, Katarina, and Biharia. Each variety was cultivated on 15 hectares, further split into three experimental variants of 5 hectares each: control (without additional fertilization), two-stage phased fertilization, and three-stage phased fertilization.

For the control variant, the crop relied solely on residual nitrogen and nutrients left in the soil after the pea crop, to assess the biological potential of the varieties without the influence of additional fertilization. In the fertilized variants, the first application coincided with sowing in the first decade of October, with 300 kg/ha of Duofertil Eurocereal 34 (N, P, S) from Timac, a product designed to mobilize and protect nutrients and stimulate root development thanks to the Mappa Duo and XCK complex. The second application, applied to both the two- and three-stage fertilization variants, took place on March 18, using Sulfammo 25 Mappa Duo at a rate of 150 kg/ha, a solid fertilizer with 25%

nitrogen and 31% sulfur trioxide. The three-stage fertilization variant also received an additional foliar treatment on April 28, at flag leaf emergence, applying 30 l/ha of In-Folen (19% nitrogen, 5% magnesium oxide, 10% sulfur trioxide), aimed at enhancing enzymatic activity and optimizing grain quality parameters.

Soil preparation included deep ploughing at approximately 25 cm depth in the second decade of August, using a Lemken EurOpal 5 plough, with the purpose of turning over the furrow, incorporating plant residues, and ensuring good soil aeration and loosening. The ploughing depth was adjusted according to the soil's moisture level and the amount of plant residue.

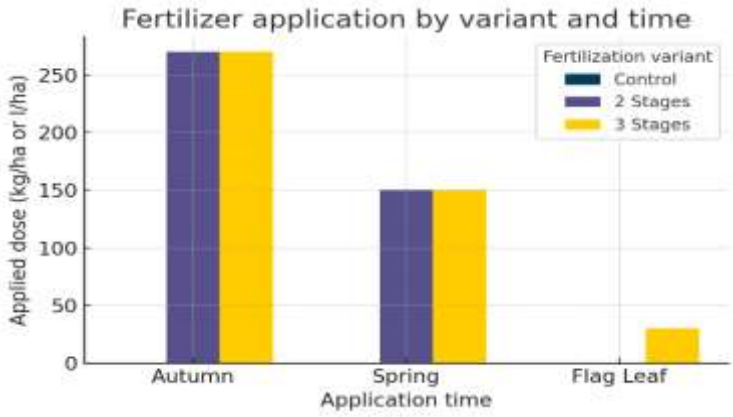

Figure 1 – Fertilization scheme of the three winter wheat varieties across experimental variants

The climatic conditions recorded during the growing season were characterized by positive temperature anomalies and a significant precipitation deficit, which increased the importance of the applied technology and fertilization regime. The climatic data recorded in the experimental area are presented in figure 2. Monthly average temperatures were significantly higher than the multiannual normal, with notable differences in December 2023 and January 2024, where temperatures exceeded the normal values by approximately 5°C.

Regarding precipitation, the total amount accumulated during the 2023–2024 agricultural year was 311.4 mm, which is 114.4 mm below the multiannual average (425.8 mm). Severe water deficits were recorded especially during the active growing months (March–June), with a total of 116.7 mm compared to the estimated requirement of 280–320 mm for optimal wheat development at this stage. Only January and April recorded values above the normal average, but the surplus was insignificant.

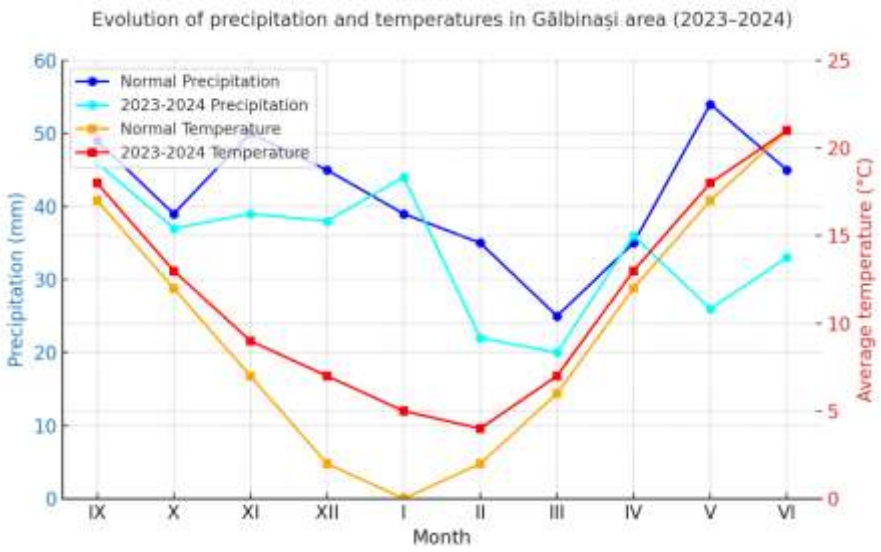

Figure 2 – Evolution of precipitation and temperatures in the study area, Gălbinași locality, during the 2023–2024 agricultural year

Seedbed preparation was carried out on October 4, 2023, with a single pass of the Vaderstad TopDown 400 cultivator, which broke down the plough furrows, loosened, and leveled the soil to facilitate optimal seed-to-soil contact. The seeds used came from certified seed lots (C1 biological category), with a physical purity of 99.80% for Biharia and Katarina and 98.70% for Apache, and a germination capacity of 97%. The thousand kernel weight (TKW) was 42 g for Biharia and Katarina, and 40 g for Apache.

Before sowing, the seeds were mandatorily treated with Austral Plus (500 ml/100 kg seed), a broad-spectrum insecto-fungicide. Sowing took place on October 6, 2023, at a row spacing of 12.5 cm and a sowing depth of 4 cm, with a seeding rate of 390 germinable seeds/m², corresponding to approximately 210 kg seed/ha.

Weed control was achieved through integrated preventive methods and chemical treatments. The main weeds identified were *Sinapis arvensis, Cirsium arvense, Centaurea cyanus, Chenopodium album, Galium aparine, Capsella bursa-pastoris* (dicotyledonous species), and *Avena fatua, Poa annua* (monocotyledonous species). Control of monocotyledonous weeds was achieved with Starane Super (0.4 l/ha), applied on April 24 together with insecto-fungicides. Dicotyledonous weeds were controlled with Biathlon 4D (0.07 kg/ha) and adjuvant Dash HC (1 l/ha), applied at the 6–8 leaf stage on May 11.

Plant protection against diseases was carried out in two stages: the first treatment with Evolus (1 l/ha) on April 24, and the second with Falcon Pro (0.9 l/ha) on May 11, providing preventive, curative, and eradicative protection. Pest control was initially ensured by seed treatment, followed by two chemical treatments: Lamdex Extra (0.3 kg/ha, April 24) and Delmetros 100SC (0.15 l/ha, May 11).

Harvesting was performed on June 28, 2024, with a Deutz Fahr C7206 combine equipped with a cereal header. The grain moisture at harvest ranged between 11.5% and 12.6%. After threshing, the straw was baled, and the grain was transported and stored in designated facilities.

The yield results showed a clear influence of the fertilization regime and variety used. The average yields obtained were: for Katarina, 7,711.4 kg/ha (three fertilizations), 6,425.1 kg/ha (two fertilizations), and 5,689.6 kg/ha (control); for Biharia, 6,543.6 kg/ha (three fertilizations), 5,689.6

kg/ha (two fertilizations), and 4,615.4 kg/ha (control); for Apache, 6,108.7 kg/ha (three fertilizations), 4,848.4 kg/ha (two fertilizations), and 3,365.4 kg/ha (control). These results confirm the benefits of split nitrogen application, especially under challenging climatic conditions and significant water deficits.

Plant density at emergence was determined using standardized methods by counting plants per linear meter over 10 rows with three repetitions for each experimental variant at the first true leaf stage. The average values show a positive influence of fertilization on final crop density.

For Katarina, the average plant density for the three-fertilization variant was 308 plants/m² (min. 27, max. 34 plants), for two fertilizations 302 plants/m² (min. 27, max. 33 plants), and for the control 288 plants/m² (min. 26, max. 34 plants). For Biharia, the values were 305 plants/m² (three fertilizations), 300 plants/m² (two fertilizations), and 283 plants/m² (control), with individual ranges between 26 and 34 plants/m². For Apache, densities were 300 plants/m² (three fertilizations), 299 plants/m² (two fertilizations), and 276 plants/m² (control), ranging from 26 to 33 plants/m².

The results show a clear tendency for increased plant density as fertilization intensity increased across all three varieties. This is attributed to improved germination vigor and seedling emergence, resulting from the additional nutrient (mainly nitrogen) supply applied at sowing. The data also indicate good emergence uniformity, with consistent values between repetitions, highlighting seed quality and effective seedbed preparation.

Spike density at harvest was determined by counting spikes per linear meter over 10 rows, with three repetitions for each variety and fertilization variant. The average values showed a clear influence of fertilization on spike density, demonstrating the positive effect of nitrogen supplementation on tillering capacity and plant survival up to harvest.

For Katarina, spike density was 567 spikes/m² (three fertilizations), 531 spikes/m² (two fertilizations), and 508 spikes/m² (control). For Biharia, values were 532 spikes/m² (three fertilizations), 508 spikes/m² (two fertilizations), and 491 spikes/m² (control). For Apache, values were 509 spikes/m² (three fertilizations), 480 spikes/m² (two fertilizations), and 474 spikes/m² (control). The observed minimum–maximum ranges per row were 44–62 spikes for Katarina, 42–61 spikes for Biharia, and 40–62 spikes for Apache, confirming moderate variability but with a clear tendency for increased spike density with increased fertilization.

These results support the hypothesis that split fertilizer application leads to higher final plant densities at harvest, due to reduced plant mortality and stimulated secondary tillering during the growing season.

To validate the significance of the differences between experimental variants for spike density at harvest, raw data (10 repetitions per variant) were subjected to analysis of variance (ANOVA). The ANOVA test showed extremely significant differences between varieties and fertilization variants ($F(2,87) = 12.28$; $p < 0.0001$). These data confirm that the fertilization regime had a strong impact on spike density at harvest, mainly by stimulating secondary tillering and reducing plant mortality under the severe water deficit conditions recorded in the 2023–2024 agricultural year. A directly proportional relationship between increased fertilization intensity and spike density was consistently observed across all three studied varieties.

The results confirm the hypothesis that split nitrogen application, adapted to wheat phenological stages, optimizes crop architecture and contributes to yield stability under challenging climatic conditions.

To provide a detailed representation of experimental data variability, spike density at harvest was also illustrated using a boxplot diagram (Figure 3). This enabled visualization of the full distribution of the 10 repetitions for each variety and fertilization variant. The boxplot highlights medians, interquartile ranges, and potential outliers, providing a comprehensive picture of result consistency. A clear trend of higher average density for Katarina, followed by Biharia and Apache, was observed, in line with the previously calculated averages. Slightly higher variability for Biharia was noted, possibly due to greater sensitivity to water stress conditions.

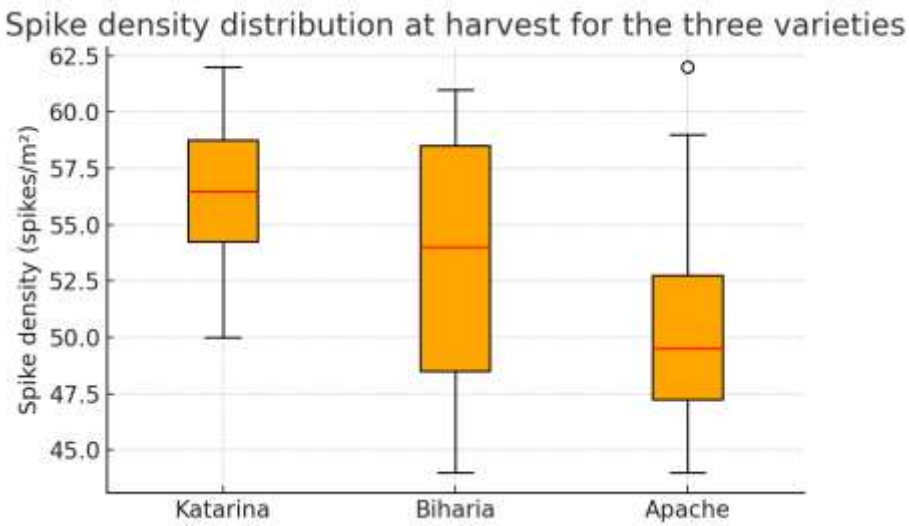

Figure 3 – Boxplot of spike density distribution at harvest for the three wheat varieties

To better understand the relationship between fertilization level and final spike density at harvest, the average data obtained for the three varieties were subjected to linear regression analysis. Fertilization was coded ordinally (0 = control, 1 = two fertilizations, 2 = three fertilizations). The regression model revealed a positive relationship between increasing fertilization intensity and final spike density. The resulting regression line indicated a clear trend of increased crop density with additional fertilizer application. The coefficient of determination ($R^2 = 0.45$) suggests that approximately 45% of the variation in spike density at harvest can be attributed to differences in the applied fertilization regimes.

This result supports the conclusions of the ANOVA test and confirms the hypothesis that phased fertilization, adapted to the phenological requirements of the crop, significantly contributes to reducing plant mortality and stimulating secondary tillering, thereby optimizing crop architecture under water stress conditions.

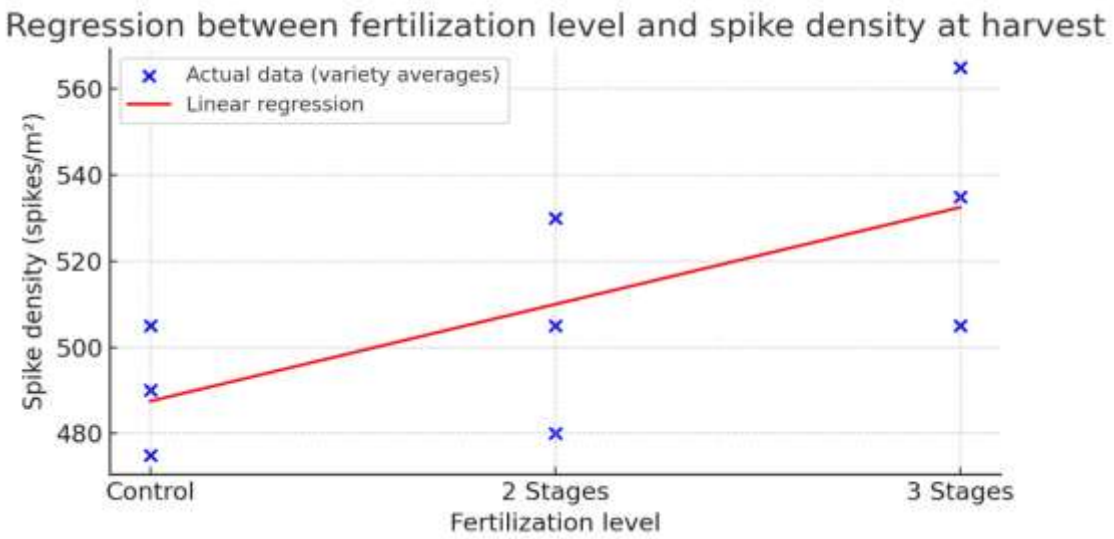

Figure 4 – Regression between fertilization level and spike density at harvest

The determination of the thousand kernel weight (TKW) was carried out by repeated weighing of samples taken from all experimental variants, in order to identify the influence of the fertilization regime on this essential quality indicator. The results showed a clear tendency for TKW to increase with the intensification of fertilization for all three varieties studied. For Katarina, the average values were 44 g (three fertilizations), 42 g (two fertilizations), and 40 g (control). For Biharia, values were 44 g (three fertilizations), 40 g (two fertilizations), and 35 g (control). For Apache, the values were 43 g, 39 g, and 31 g, respectively.

To validate the statistical significance of the differences, raw data were subjected to ANOVA testing. The analysis revealed significant differences between varieties and variants ($F(2,60) = 7.35$; $p = 0.0014$). These results demonstrate the strong influence of split fertilizer application on thousand kernel weight, particularly under the difficult pedoclimatic conditions of the 2023–2024 agricultural year, characterized by precipitation deficit and water stress. It can be concluded that judicious application of nitrogen fertilization, aligned with crop phenological stages, optimizes the grain filling process and contributes to achieving superior grain quality.

To provide a detailed characterization of data variability from the determination of thousand kernel weight (TKW), a visual analysis was performed using a boxplot (Figure 5). This representation highlighted the distribution of values obtained for the three studied varieties: Katarina, Biharia, and Apache. The boxplot reveals clear differences between the three genotypes, with Katarina showing the lowest internal variability, which may indicate superior stability of this trait under unfavorable pedoclimatic conditions. In contrast, Biharia and Apache showed greater dispersion of values, suggesting a stronger reaction to abiotic stress or interaction with the applied fertilization technology.

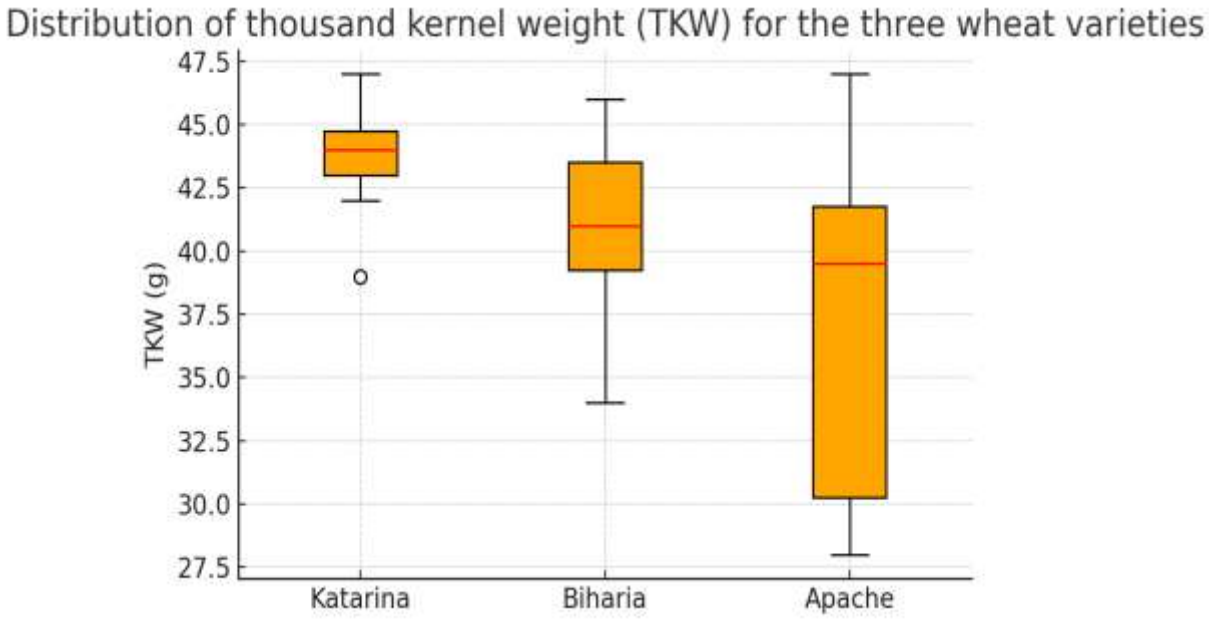

Figure 5 – Boxplot of thousand kernel weight (TKW) distribution for the three wheat varieties

To quantify the relationship between fertilization level and thousand kernel weight (TKW), a linear regression model was applied to the average values obtained for the three varieties and the three fertilization regimes. The results indicated a positive relationship between increased fertilization intensity and higher TKW values. The regression analysis produced a coefficient of determination (R^2) of 0.69, meaning that approximately 69% of the variation in thousand kernel weight can be explained by the applied fertilization level. This demonstrates a strong and predictable influence of the nutritional regime on grain quality. The results are fully consistent with those highlighted by the ANOVA test and the boxplot analysis, further supporting the hypothesis that phased fertilization, adapted to the specific requirements of the crop, plays a decisive role in improving the quality parameters of the wheat harvest.

The yield per hectare was determined based on the harvest obtained for each variety and experimental variant. The results showed a clear trend of yield increase with the intensification of the fertilization regime.

For the Katarina variety, yields of 7,711.4 kg/ha (three fertilizations), 6,425.1 kg/ha (two fertilizations), and 5,689.6 kg/ha (control) were recorded. For Biharia, yields were 6,543.6 kg/ha (three fertilizations), 5,689.6 kg/ha (two fertilizations), and 4,615.4 kg/ha (control). For Apache, values of 6,108.7 kg/ha, 4,848.4 kg/ha, and 3,365.4 kg/ha were recorded for the same variants.

To test the significance of the differences between varieties and fertilization variants, an analysis of variance (ANOVA) was applied. Although notable value differences were observed, the statistical test indicated a lack of statistical significance ($F(2,6) = 1.96$; $p = 0.22$). This is explained by the absence of multiple biological replicates for each variant, as the data were obtained from a single experimental plot per treatment. Therefore, these results must be interpreted primarily descriptively, without rigorous statistical validation. Nevertheless, the clear trend of increased yield with increasing fertilization level is evident and correlates with the other agro-productive data previously analyzed (spike density at harvest, number of grains per spike, thousand kernel weight), confirming the

effectiveness of phased fertilization even under the difficult climatic conditions of the 2023–2024 agricultural year.

To highlight the trend of yield increase with fertilization level, the average data obtained for the three varieties were analyzed using a linear regression model. The results indicated a positive relationship between the application of phased fertilization and yield values per hectare. The coefficient of determination ($R^2 = 0.58$) showed that approximately 58% of the yield variation can be explained by the administered fertilization level. This suggests a significant influence of the fertilization regime on crop performance, even under the challenging climatic conditions of the 2023–2024 agricultural year.

It is important to note that this analysis is indicative, as it was based on data obtained from a single experimental plot for each treatment. The absence of multiple biological replicates limits the full statistical validity of the model. Nevertheless, the trend illustrated supports the descriptive observations and is consistent with the results of the other agro-productive analyses conducted in this study.

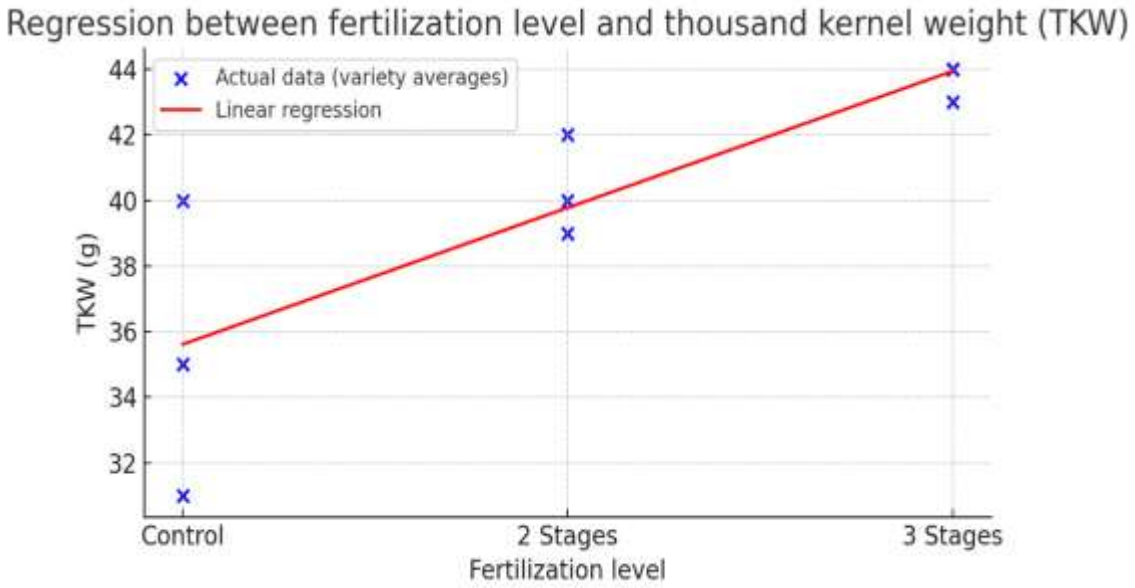

Figure 6 – Regression graph between fertilization level and thousand kernel weight (TKW)

The grain quality of wheat was evaluated based on three essential parameters: protein content, wet gluten content, and hectoliter weight (HW). These indicators directly reflect the influence of the applied technological regime and climatic conditions on the baking characteristics of the harvest.

Protein content varied both between varieties and between fertilization variants. Katarina showed the most stable and highest values, ranging between 12.5% and 13.8%, demonstrating superior protein accumulation even under moderate drought conditions. Apache showed a slight decrease in protein content in the control variant (below 12%), suggesting greater sensitivity to the lack of nutritional input. Biharia presented greater variability, with values ranging from 12.1% to 14.3%, indicating better fertilizer use efficiency but also a stronger reaction to fluctuations in moisture and temperature during grain filling.

Wet gluten content, an essential indicator of baking quality, followed a similar pattern to protein content. Katarina was in the superior category, with values between 27.5% and 28.8%, showing stable gluten quality under water stress. Biharia had high values, between 28.6% and 32.3%,

but with greater variation between variants, suggesting a complex interaction between nitrogen supply and climatic conditions. In contrast, Apache showed a reduction in gluten content, especially in the control variant (23.7%–24.5%), confirming the negative impact of nutrient deficiency on gluten protein quality.

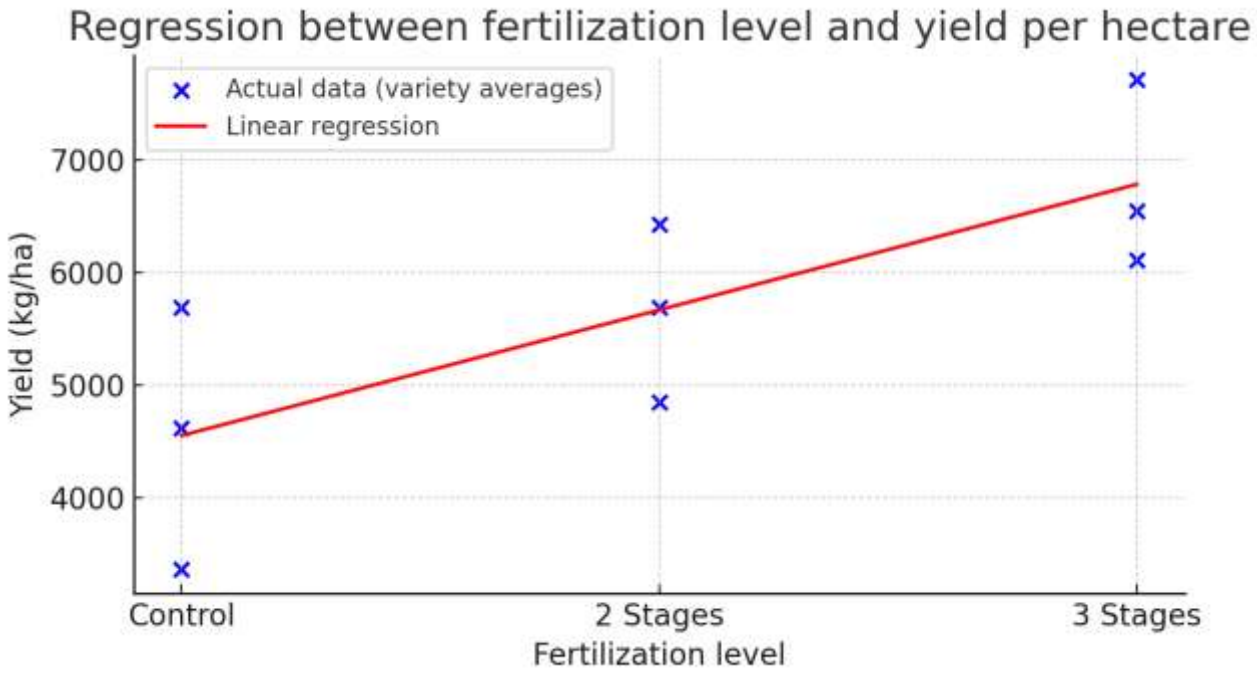

Figure 7 – Regression graph between fertilization level and yield per hectare

Hectoliter weight (HW), an indicator of grain filling and commercial quality, recorded values above the minimum threshold (76 kg/hl) for all varieties, with the best results for Katarina (79–81 kg/hl). For Biharia, values ranged between 76–79 kg/hl, while for Apache, the control variant slightly dropped below 76 kg/hl, suggesting incomplete grain filling and greater sensitivity to nutrient and moisture deficiencies.

To provide a synthetic and comparative overview of the performance of the three varieties regarding grain quality, the maximum values obtained for protein content, wet gluten content, and hectoliter weight (HW) were graphically represented (Figure 8).

The graph highlights the superiority of Katarina in terms of stability and high values of hectoliter weight (81 kg/hl) and protein content (13.8%), demonstrating excellent adaptability to moderate drought conditions and the applied fertilization technology. Biharia recorded the highest values for wet gluten content (32.3%), indicating excellent baking quality but showed greater variability in the other parameters. Apache had the lowest maximum values, especially for hectoliter weight (76 kg/hl) and gluten (24.5%), which can be explained by its higher sensitivity to the lack of additional fertilization.

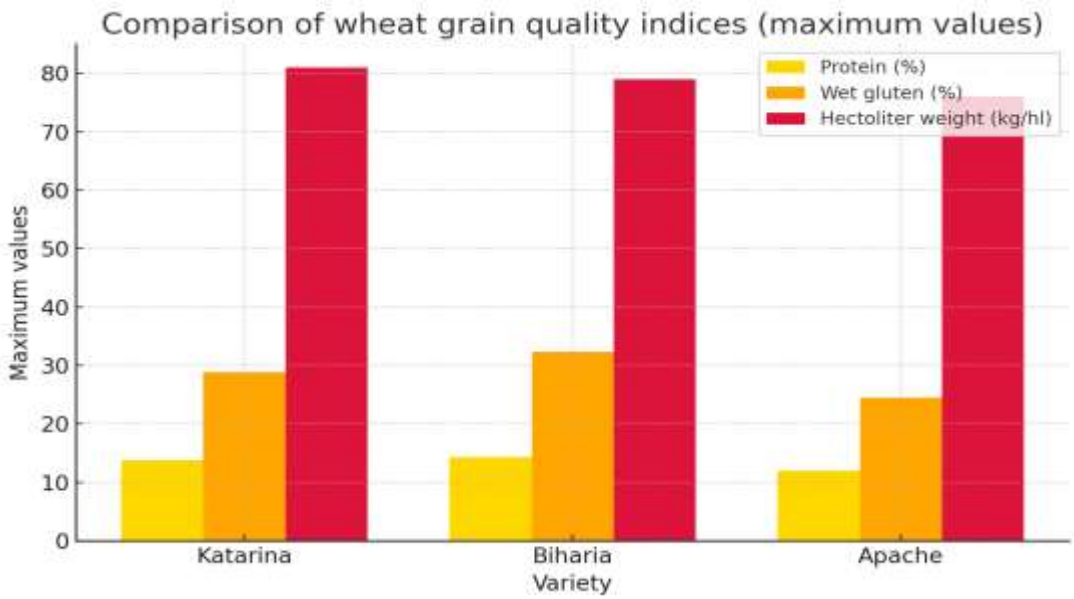

Figure 8 – Graph of the main grain quality indices: protein, wet gluten, and hectoliter weight

CONCLUSIONS

The research conducted at Denagro Martrans, in Gălbinași, Buzău County, during the 2023–2024 agricultural year, aimed to evaluate the agronomic and quality performance of three winter wheat varieties (Katarina, Biharia, Apache) grown under rainfed conditions, in restrictive climatic conditions characterized by severe precipitation deficits and elevated average temperatures.

The results demonstrated a significant influence of the fertilization regime on most of the analyzed parameters. Regarding spike density at harvest, phased fertilization applied in two and three stages led to a significant increase of this indicator, statistically confirmed by ANOVA analysis ($p < 0.0001$). Additional nitrogen application promoted both secondary tillering and reduced plant mortality during the vegetation cycle, resulting in a more uniform and efficient crop architecture.

The thousand kernel weight (TKW), an essential indicator for grain quality, was strongly influenced by fertilization, as shown by the statistical analysis ($p = 0.0014$) and the strong correlation obtained by regression analysis ($R^2 = 0.69$). Split fertilization contributed to improved grain filling and the achievement of higher quality yields, despite unfavorable pedoclimatic conditions.

Although the statistical analysis of yield per hectare did not show significant differences ($p = 0.22$), descriptive data indicated a clear trend of yield increase with increasing fertilization levels. Linear regression showed a positive relationship between fertilization level and yield ($R^2 = 0.58$). Katarina recorded the highest yields, reaching 7,711.4 kg/ha with three fertilizations, followed by Biharia and Apache, highlighting the importance of judicious fertilizer application in maximizing genetic potential.

Grain quality analysis revealed specific differences between varieties. Katarina stood out for its superior stability in protein content (12.5–13.8%) and high hectoliter weight (79–81 kg/hl). Biharia had a high wet gluten content (28.6–32.3%), indicating superior baking quality, but with slightly higher internal variability. Apache, although characterized by good drought tolerance, recorded the lowest values for all quality parameters in the control variant, confirming its higher sensitivity to the absence of additional fertilization.

In conclusion, the study confirmed that adaptation and intensification of phased fertilization can partially compensate for the negative effects of water stress on winter wheat crops, contributing both to yield improvement and grain quality enhancement. These results provide a solid basis for technological recommendations for farmers practicing agriculture under pedoclimatic conditions similar to those of the Gălbinași area and the southeastern regions of Romania.

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NUTRACEUTICAL POTENTIAL OF ACHILLEA MILLEFOLIUM. HEALTH BENEFITS, DERMATOLOGICAL USE AND SAFETY

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ABSTRACT

Achillea millefolium L. (Asteraceae), commonly referred to as yarrow, is a medicinal plant with extensive ethnopharmacological use and increasing scientific interest due to its diverse phytochemical profile and broad-spectrum bioactivity. This review evaluates the current literature on the phytoconstituents, pharmacological properties, cosmetic applications, nutraceutical potential, toxicological considerations, and commercial viability of A. millefolium, with emphasis on its integration into contemporary dermatological formulations.The plant is characterized by a rich composition of bioactive compounds, including flavonoids such as apigenin, luteolin, quercetin, phenolic acids (chlorogenic acid , caffeic acid), sesquiterpene lactones (achillin, millefolide), and volatile constituents (camphor, borneol, linalool,cineole). These metabolites exhibit significant antioxidant, anti-inflammatory, antimicrobial, and cytoprotective activities, as demonstrated in both in vitro and in vivo models. In cosmetic science, A. millefolium extracts have shown efficacy in mitigating oxidative stress, modulating inflammatory pathways, enhancing wound healing, and inhibiting melanogenesis via tyrosinase suppression, thereby supporting their use in anti-aging and depigmenting formulations.In the nutraceutical domain, A. millefolium demonstrates therapeutic potential in gastrointestinal modulation, hepatoprotection, and glycemic control. Preclinical studies suggest its utility in managing metabolic syndrome and inflammatory conditions, although clinical trials remain limited. Toxicological assessments conducted by regulatory bodies, including the Cosmetic Ingredient Review (CIR) and European Medicines Agency (EMA), affirm its safety within defined concentration thresholds, with caution advised for individuals with Asteraceae sensitivities and during pregnancy.The plant's favorable regulatory status, sustainable cultivation profile, and multifunctional bioactivity underscore its commercial relevance. Future research should prioritize phytochemical standardization, advanced delivery systems, and translational studies to substantiate its efficacy and optimize its application in integrated cosmetic-nutraceutical formulations. This review consolidates the scientific foundation for A. millefolium as a promising candidate in evidence-based natural product development.

Keywords :Achillea millefolium, , phytochemicals, nutraceuticals, dermatology,safety assessment,

INTRODUCTION

Achillea millefolium L., commonly referred to as yarrow, is a perennial species within the Asteraceae family, renowned for its longstanding role in traditional healing systems across diverse cultures. Historical records trace its medicinal use back over 3,000 years, with applications documented in ancient European, Asian, and Indigenous North American practices (Bashir et al., 2022). Traditionally, yarrow has been employed to treat wounds, gastrointestinal ailments, and inflammatory conditions (Strzępek-Gomółka et al., 2021). The genus name "*Achillea*" is derived from the legendary Greek warrior Achilles, who, according to myth, used the herb to tend battlefield injuries-underscoring its historical association with trauma care and anti-inflammatory effects (Singh et al., 2025). In Chinese herbal medicine, yarrow is valued for its ability to stimulate circulation, while European folk remedies favor its use in teas and poultices for digestive and dermal support (Jangjoo et al., 2023). Ethnobotanical documentation from Native American communities highlights its analgesic and antiseptic properties, particularly in treating minor wounds, insect stings, and bruises (Kachura, 2018).The resurgence of interest in plant-based therapeutics has propelled *A. millefolium* from a folkloric remedy to a scientifically endorsed botanical, driven by consumer preference for natural alternatives and growing pharmacological evidence supporting its efficacy (Barda et al., 2021). Yarrow is now widely incorporated into both topical skincare products and internal wellness supplements, owing to its antioxidant, antimicrobial, anti-inflammatory, and skin-regenerating effects (Gaweł-Bęben et al., 2020). These qualities make it a versatile ingredient in formulations ranging from anti-aging cosmetics to herbal infusions within the nutraceutical sector. Regulatory endorsements from institutions such as the European Medicines Agency (EMA) and the Cosmetic Ingredient Review (CIR) panel further validate its safety and functional potential (EMA, 2016; Becker et al., 2016).Phytochemically, *A. millefolium* is rich in bioactive constituents, including flavonoids like luteolin and apigenin, phenolic acids such as chlorogenic and rosmarinic acid, terpenes, sesquiterpene lactones, and essential oils (Konarska et al., 2025; Mihaylova, 2015). These compounds are responsible for its therapeutic actions, including modulation of inflammatory pathways, reduction of oxidative stress, and promotion of tissue regeneration (Farasati Far et al., 2023). Emerging clinical evidence supports its use in managing conditions such as dysmenorrhea, irritable bowel syndrome, and metabolic dysfunctions (Daneshvar-Ghahfarokhi et al., 2025; Moradi et al., 2023).Recent innovations in extraction and delivery technologies have enhanced the efficiency and stability of yarrow-derived compounds.Techniquessuchas ultrasound-assisted extraction (UAE),microwave-assisted extraction (MAE), and supercritical fluid extraction have improved phytochemical yield and integrity (Trendafilova& Todorova, 2008). Additionally, encapsulation strategies—including nanoliposomes, chitosan-based nanocarriers, and complex coacervation—have optimized bioavailability and targeted delivery, particularly in dermatological and pharmaceutical contexts (Nateghi et al., 2022; Székely-Szentmiklósi et al., 2024).This review seeks to consolidate contemporary research on Achillea millefolium, bridging its ethnobotanical legacy with modern pharmacological findings. It will examine the plant's chemical composition, mechanisms of action, therapeutic relevance, safety profile, commercial applications, and avenues for future investigation.

METHODOLOGY

This review was conducted using a structured, multi-phase approach to synthesize current scientific literature on *Achillea millefolium* L. and its applications in nutraceutical and cosmetic science. Articles published between 2015 and 2025 were retrieved from databases including PubMed, Scopus, Web of Science, and ScienceDirect using keywords such as "*Achillea millefolium*," "yarrow," "phytochemistry," "cosmetic applications," "nutraceuticals," "mechanism of action," and "clinical trials." Inclusion criteria focused on studies that provided experimental, clinical, or ethnobotanical data relevant to the plant's bioactive compounds, pharmacological mechanisms, safety profile, and formulation technologies.Data extraction emphasized phytochemical composition, validated bioactivities, and technological advancements in extraction and delivery systems. Studies were categorized by application domain (nutraceutical, cosmetic, pharmacological) and evaluated for methodological rigor, sample size, and reproducibility. Ethnobotanical sources and regulatory documents were also reviewed to contextualize traditional uses and safety standards. Qualitative synthesis was used to identify trends, gaps, and future research directions.

RESULTS AND DISCUSSION

The literature review revealed consistent evidence supporting the multifunctional bioactivity of *Achillea millefolium*. Across selected studies, the plant demonstrated potent antioxidant activity validated by TEAC, FRAP, and CUPRAC assays, with phenolic acids and flavonoids identified as key contributors. Anti-inflammatory effects were confirmed through both in vitro and in vivo models, showing inhibition of COX enzymes and downregulation of NF-κB and MAPK pathways.Cosmetic applications were supported by studies showing tyrosinase and elastase inhibition, enhanced fibroblast activity, and improved skin elasticity. Hydro glycolic and ethanolic extracts were found effective in anti-aging formulations, with SPF values and collagen-regulating properties documented in UVA-induced photoaging models. Clinical trials reported significant improvements in wound healing, menstrual pain, and gastrointestinal symptoms, aligning with traditional uses.Advanced extraction methods such as supercritical fluid extraction and microwave-assisted extraction improved phytochemical yield and stability.Encapsulation technologies-including nanoliposomes and PEG-based hydrogels-enhanced bioavailability and controlled release. Safety assessments confirmed low cytotoxicity and minimal adverse reactions, with regulatory recognition from EMA and CosIng databases.

Phytochemical Profile and Mechanism of Action

Achillea millefolium L. exhibits a complex and therapeutically significant phytochemical profile, especially within its aerial parts such as leaves and flowers, which contain a diverse array of bioactive compounds including essential oils, flavonoids, phenolic acids, sesquiterpene lactones, guaianolides, triterpenoids, and phytosterols (Konarska et al., 2023; Michalak et al., 2025). The essential oil fraction, typically obtained via hydro distillation, is rich in monoterpenes like β-pinene, sabinene, 1,8-cineole, camphor, linalool, and borneol, alongside sesquiterpenes such as chamazulene

and caryophyllene, which contribute to the plant's antimicrobial, anti-inflammatory, and antispasmodic properties (Gaweł-Bęben et al., 2020; Székely-Szentmiklósi et al., 2024). Polar extracts derived from hydroalcoholic and hydroglycolic solvents contain flavonoids including apigenin, luteolin, quercetin, and rutin—primarily in glycosylated forms—that exhibit potent antioxidant activity through reactive oxygen species scavenging and enhancement of endogenous enzymatic defenses (Villalva et al., 2022; Yeltay et al., 2025). Additionally, phenolic acids such as chlorogenic, caffeic, rosmarinic and dicaffeoylquinic acids reinforce the plant's antioxidant and antimicrobial profile while supporting hepatoprotective and cytoprotective functions (Ivanović et al., 2022; Tripon et al., 2024). Lipophilic constituents like achillin and leucodin, classified as sesquiterpene lactones and guaianolides, are known to inhibit inflammatory pathways including NF-κB and MAPK and modulate cytokine production, enhancing their relevance in both systemic and topical applications (Farasati et al., 2023). Furthermore, triterpenoids and phytosterols such as β-sitosterol and taraxasterol derivatives contribute to skin barrier reinforcement, anti-aging effects, and inflammation modulation, making them valuable ingredients in modern cosmetic formulations (Michalak et al., 2025). The diverse bioactivities of Achillea millefolium stem from the synergistic interactions among its rich array of phytochemicals, including phenolic acids, flavonoids, sesquiterpene lactones, triterpenes, lignans, and essential oils. These compounds act on multiple molecular targets, contributing to the plant's antioxidant, anti-inflammatory, antimicrobial, antispasmodic, and skin-regenerative effects, which collectively support its integration into nutraceutical and cosmetic formulations.Antioxidant properties of *A. millefolium* have been extensively validated through assays such as Trolox Equivalent Antioxidant Capacity (TEAC), Ferric Reducing Antioxidant Power (FRAP), and Cupric Ion Reducing Antioxidant Capacity (CUPRAC). These confirm that phenolic acids like chlorogenic acid and flavonoids such as luteolin and apigenin effectively neutralize reactive oxygen species (ROS), thereby mitigating oxidative stress at the cellular level (Raudone et al., 2024; Marengo et al., 2025). This antioxidant activity is particularly relevant in dermatological applications, where ROS-induced damage contributes to photoaging, collagen degradation, and loss of skin elasticity.The anti-inflammatory effects of *A. millefolium* are mediated through inhibition of cyclooxygenase (COX) enzymes, attenuation of pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), and downregulation of nuclear factor kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) signaling pathways (Saadat et al., 2024; Ali et al., 2017). These mechanisms underpin its efficacy in managing inflammatory skin conditions, menstrual pain, and systemic inflammatory disorders, as demonstrated in both experimental and clinical studies.

In addition to its anti-inflammatory and antioxidant roles, *A. millefolium* exhibits targeted enzymatic inhibition relevant to cosmetic applications. Its extracts have been shown to inhibit tyrosinase activity, a key enzyme in melanin biosynthesis, offering potential skin-lightening effects in formulations designed to treat hyperpigmentation and uneven skin tone (Raudone et al., 2024; Marengo et al., 2025). The modulation of melanogenesis is further supported by its influence on oxidative stress-related signaling pathways, including c-Jun N-terminal kinase (JNK) and extracellular signal-regulated kinase (ERK), which are implicated in pigmentation and cellular apoptosis.The plant's antispasmodic properties are primarily linked to its inhibitory effects on calcium channels, which regulate smooth muscle contraction. By modulating intracellular calcium influx, *A.*

*millefolium*induces tracheal and gastrointestinal muscle relaxation, offering therapeutic potential in conditions such as dysmenorrhea and irritable bowel syndrome (Ali et al., 2017; Barda et al., 2021). These effects are complemented by its gastroprotective actions, including mucosal healing and reduction of inflammation in the gastrointestinal tract.Moreover, *A. millefolium* demonstrates broad-spectrum antimicrobial activity against pathogens such as Helicobacter pylori, Staphylococcus aureus, and various fungal species. Essential oils and phenolic compounds disrupt microbial cell membranes and inhibit biofilm formation, enhancing its utility in topical formulations for acne, wound care, and oral hygiene (Ali et al., 2017; Barda et al., 2021). These properties are particularly valuable in cosmetic products aimed at maintaining skin barrier integrity and preventing microbial colonization.Collectively, these mechanisms highlight the therapeutic and cosmetic relevance of Achillea millefolium, providing a scientifically grounded rationale for its use in formulations designed to combat oxidative stress, inflammation, and microbial infections

Nutraceutical Application

Achillea millefolium L., commonly known as yarrow, has demonstrated extensive nutraceutical potential supported by both traditional use and contemporary clinical research. Its gastrointestinal benefits are well established, with aqueous and alcoholic extracts showing antispasmodic, prokinetic, and antiulcer activities that contribute to improved recovery in conditions such as gastritis and irritable bowel syndrome (Salehi et al., 2020; Jangjoo et al., 2023). These effects are linked to its ability to reduce pro-inflammatory mediators, confirming its traditional role in soothing digestive inflammation (Ali et al., 2017; Akbar, 2020). Beyond digestive health, *A. millefolium* exhibits antidiabetic properties, including reductions in blood glucose, lipid profiles, and liver enzyme levels in diabetic models, largely attributed to its antioxidant-rich polyphenolic compounds that mitigate oxidative stress (Jangjoo et al., 2023). Its systemic effects extend into oncological applications, where in vitro studies have shown cytotoxic activity against breast, prostate, and gastric cancer cell lines through mechanisms involving apoptosis and cell cycle arrest, mediated by flavonoids and sesquiterpene lactones (Konarska et al., 2023; Barda et al., 2021). Some evidence suggests synergistic effects when combined with conventional chemotherapeutic agents. Neuromodulator and immune functions have also been observed, with clinical trials indicating anxiolytic and cognitive-enhancing effects, particularly when *A. millefolium* is used in combination with botanicals such as *Boswellia carterii* and *Zingiber officinale* (Salehi et al., 2020). These effects may be mediated through GABAergic modulation and antioxidant signaling pathways, while its immunomodulatory activity supports enhanced immune function via anti-inflammatory mechanisms (Barda et al., 2021; Farasati Far et al., 2023). In the domain of reproductive health, clinical trials have confirmed its efficacy in alleviating menstrual pain and dysmenorrhea, reinforcing its spasmolytic and anti-inflammatory properties as a natural alternative for managing primary dysmenorrhea (Raudone et al., 2024; Barda et al., 2021). Altogether, these findings highlight *A. millefolium* as a multifunctional botanical with validated applications in nutraceutical formulations targeting gastrointestinal, metabolic, oncological, neurological, immunological, and reproductive health

Cosmetic Applications and Technological Advancements of *Achillea millefolium*

Achillea millefolium has emerged as a valuable botanical in cosmetic science, largely due to its potent antioxidant, anti-inflammatory, and regenerative effects that support skin renewal, wound healing, and microbial defense. Research indicates that its extracts promote fibroblast activity and stimulate collagen synthesis-key processes for preserving skin elasticity and minimizing the appearance of wrinkles (Akbar, 2020). Hydro glycolic formulations containing yarrow have shown measurable improvements in skin elasticity, collagen regeneration, and photoprotection, alongside inhibition of tyrosinase, a key enzyme in melanin synthesis (Gaweł-Bęben et al., 2020). Its ability to inhibit elastase and tyrosinase at low concentrations further supports its use in anti-aging and pigmentation-targeted treatments (Marengo et al., 2025).Experimental models simulating UVA-induced photoaging reveal that yarrow extracts help regulate collagen turnover and reduce cell death in dermal fibroblasts. Essential oil constituents such as linalyl acetate have been shown to influence melanogenesis through modulation of JNK and ERK signaling pathways (Marengo et al., 2025). Antioxidant evaluations using TEAC, FRAP, and CUPRAC assays demonstrate efficacy comparable to kojic acid, and ethanolic extracts have been successfully incorporated into peel-off gel masks for topical use (Strzępek-Gomółka et al., 2021). Variability in phytochemical composition across different morphotypes and growing conditions highlights the importance of standardized cultivation and sourcing practices (Raudone et al., 2024).In clinical contexts, *A. millefolium* has shown promise in wound care, exhibiting antimicrobial, anti-inflammatory, and tissue-repairing properties. These effects have been observed in applications such as episiotomy wound treatment, where reductions in pain, swelling, and erythema were reported (Salehi et al., 2020; Ali et al., 2017; Hajhashemi et al., 2018). The underlying mechanisms involve modulation of cytokine activity and enhancement of cellular repair processes (Barda et al., 2021; Ali et al., 2017). Its antioxidant capacity also contributes to mitigating oxidative stress, further supporting skin recovery. Ethnobotanical traditions that used yarrow for skin inflammation and wound healing are now substantiated by clinical evidence, including reduced skin reactions in patients undergoing radiotherapy (Jangjoo et al., 2023). Toxicological assessments affirm its safety for topical use (Ali et al., 2017; Marengo et al., 2025).

Recognized by the European Pharmacopoeia as a standardized herbal material, *A. millefolium* is widely formulated into creams, gels, and ointments for skin regeneration (Konarska et al., 2023). Its skin-conditioning and antimicrobial properties are linked to its ability to inhibit tyrosinase and neutralize reactive oxygen species, offering protection against environmental stressors such as UV radiation and pollution (Marengo et al., 2025; Konarska et al., 2023; Strzępek-Gomółka et al., 2021; Raudone et al., 2024). Its inclusion in the EU CosIng database further validates its regulatory acceptance and widespread use in moisturizers, anti-inflammatory products, and wound care formulations (Marengo et al., 2025).Recent innovations in extraction and delivery systems have significantly enhanced the stability and efficacy of yarrow-derived bio actives. Supercritical anti-solvent fractionation has proven effective in selectively concentrating key phenolic constituents of *Achillea millefolium*, including luteolin-7-O-glucoside and apigenin-7-O-glucoside. This method has been associated with a marked increase in antioxidant capacity and a higher concentration of anti-

inflammatory compounds such as camphor and borneol (Villalva et al., 2019; Villalva et al., 2022). Advances in nano emulsion technology have further enhanced the antimicrobial efficacy of yarrow extracts, improving droplet uniformity and colloidal stability. Gas chromatography–mass spectrometry (GC-MS) has identified a diverse profile of 51 volatile compounds, while zeta potential measurements confirm the structural integrity of the emulsions (Abu Safe et al., 2023). Additionally, hydro glycolic extraction using polyethylene glycol (PEG)-based systems has demonstrated strong preservation of polyphenols like caffeoylquinic acid and axillarin. These extracts exhibit notable sun protection factor (SPF) values and effective tyrosinase inhibition, making them highly suitable for incorporation into cosmetic formulations (Gaweł-Bęben et al., 2020).These advanced techniques facilitate better retention of volatile oils and polyphenolic compounds, improving topical delivery and bioavailability. Controlled-release formulations-such as ethosomal gels and PEG-based hydrogels-ensure that active ingredient concentrations remain within safe and effective ranges, minimizing adverse effects while maximizing therapeutic benefits (Andleeb et al., 2021; Czech et al., 2023).

Safety Profile: Preclinical and Clinical Evidence

Extensive safety assessments of *Achillea millefolium* have been conducted across multiple research platforms, including cellular assays, animal toxicology studies, and human dermatological trials. Laboratory-based in vitro analyses consistently reveal that yarrow extracts, when used at conventional concentrations, exhibit minimal cytotoxicity and do not trigger mutagenic or chromosomal damage. Investigations involving human lymphocytes and rodent bone marrow cells have further substantiated its non-genotoxic nature, supporting its safe incorporation into both topical and ingestible health products (Becker et al., 2016; Ali et al. 2017).Clinical dermatology studies reinforce its favorable safety profile. Cosmetic formulations containing less than 0.04% *A. millefolium* have undergone Human Repeated Insult Patch Testing (HRIPT), demonstrating negligible irritation or allergic reactions—even among individuals with sensitive skin conditions such as eczema or atopic dermatitis (Farasati Far, Behzad, & Khalili, 2023; Becker et al., 2016). These outcomes are further validated by advanced tissue-engineered models, including three-dimensional human corneal constructs, which confirm the extract's compatibility with delicate epithelial tissues (Strzępek-Gomółka, Gaweł-Bęben, & Kukula-Koch, 2021).In vivo toxicological studies using rodent models have established a high safety margin, with oral LD50 values surpassing 2000 mg/kg. This suggests a low likelihood of systemic toxicity under standard therapeutic or cosmetic use (Ali et al., 2017; Marengo et al., 2025). These findings support its use in nutraceuticals aimed at enhancing systemic health, particularly due to its antioxidant, anti-inflammatory, and hepatoprotective properties.

Adverse Reactions and Usage Precautions

While *A. millefolium* is generally well tolerated, certain constituents require cautious handling. Thujone, a monoterpene present in its essential oil, has shown neurotoxic and carcinogenic effects in high-dose animal studies, necessitating strict regulation of its concentration in oral supplements and aromatherapeutic products (Becker et al., 2016; Raudone et al., 2024). Additionally, compounds such as linalool and α-peroxyachifolid have demonstrated sensitization potential in dermal models,

although adverse reactions are rare at the low concentrations typically used in cosmetic applications (Becker et al., 2016; Strzępek-Gomółka et al., 2021).Occupational exposure to dried yarrow-particularly in agricultural or manufacturing settings-has been linked to allergic contact dermatitis, rhinitis, and respiratory symptoms including asthma. These cases underscore the need for protective measures and post-market surveillance to safeguard workers handling raw plant materials (Strzępek-Gomółka et al., 2021; Marengo et al., 2025).Regulatory frameworks, such as those outlined in the EU Cosmetic Ingredient Database (CosIng), recommend conservative usage thresholds: typically, below 0.04% in leave-on products and 0.03% in rinse-off formulations. These limits are based on toxicological evaluations and ensure consumer safety while preserving the therapeutic benefits of *A. millefolium* in skin care applications (Farasati Far et al., 2023; Becker et al., 2016).

Extraction, Formulation, and Quality Control

The extraction method plays a crucial role in determining the phytochemical composition and biological efficacy of *Achillea millefolium* products. Techniques such as supercritical fluid extraction and supercritical anti-solvent fractionation have been shown to preserve volatile compounds and concentrate bioactive constituents, thereby enhancing antioxidant, anti-inflammatory, and antimicrobial properties relevant to both nutraceutical and cosmetic applications (Salehi et al., 2020; Strzępek-Gomółka et al., 2021; Villalva et al., 2022). Hydro glycolic extraction, using water and polyethylene glycol, has also been optimized to yield stable formulations rich in chlorogenic acid and flavonoids, which exhibit strong tyrosinase-inhibitory and antioxidative activities suitable for skin care products (Gaweł-Bęben et al., 2020; Jangjoo et al., 2023a). These advanced extraction methods contribute to improved product performance and ensure batch-to-batch consistency, which is essential for regulatory compliance and consumer safety (Konarska et al., 2023). researchers have developed encapsulation systems such as nano emulsions, liposomes, and polymeric nanoparticles to enhance the stability and bioavailability of *A. millefolium*'s active compounds. These delivery platforms protect sensitive phytochemicals from degradation, improve dermal absorption, and enable controlled release for topical and oral applications (Salehi et al., 2020; Zanfirescu et al., 2020). Such innovations are particularly effective in addressing the volatility and hydrophobicity of certain compounds, thereby expanding the potential for high-performance cosmeceuticals and nutraceuticals (Villalva et al., 2022). Given the natural variability in plant morphology, cultivation conditions, and seasonal factors, standardization of *A. millefolium* extracts is essential. Analytical techniques such as high-performance liquid chromatography (HPLC) and metabolomic profiling are employed to quantify key biomarkers including chlorogenic acid, apigenin, and sesquiterpene lactones, ensuring consistency and reproducibility across batches (Strzępek-Gomółka et al., 2021)

Table 1: Major Phytochemical Classes in *Achillea millefolium* and Their Biological Functions

Phytochemical Class	Representative Compounds	Extraction Method	Key Biological Activities
Essential Oils	β-pinene, sabinene, 1,8-cineole, camphor, linalool, borneol	Hydrodistillation	Antimicrobial, anti-inflammatory, spasmolytic, skin penetration enhancer
Sesquiterpenes	Chamazulene, caryophyllene	Hydrodistillation	Antioxidant, anti-inflammatory, soothing effects
Flavonoids	Apigenin, luteolin, quercetin, rutin	Hydroalcoholic, hydroglycolic extraction	ROS scavenging, anti-aging, UV protection, collagen synthesis stimulation
Phenolic Acids	Chlorogenic, caffeic, rosmarinic, dicaffeoylquinic acids	Hydroalcoholic, PEG-based hydroglycolic	Antioxidant, antimicrobial, hepatoprotective, anti-inflammatory
Sesquiterpene Lactones	Achillin, leucodin	Lipophilic solvent extraction	NF-κB and MAPK inhibition, cytokine modulation, cytotoxicity
Triterpenoids	Taraxasterol derivatives	Ethanol-based extraction	Skin barrier repair, anti-aging, emollient, anti-inflammatory
Phytosterols	β-sitosterol	Ethanol-based extraction	Anti-inflammatory, skin regeneration, barrier enhancement

These quality control measures are critical for guaranteeing clinical efficacy and safety in final products.The therapeutic potential of *Achillea millefolium*, encompassing its antioxidant, antimicrobial, wound-healing, and cytotoxic activities, has been substantiated through both experimental research and clinical evaluations, reinforcing its suitability for formulations aimed at treating skin inflammation, combating oxidative stress, and promoting tissue regeneration (Jangjoo et al., 2023; Konarska et al., 2023; Salehi et al., 2020; Strzępek-Gomółka et al., 2021).Traditional preparations such as oil macerates and alcoholic extracts have long been used for treating wounds and sunburn, and modern cosmetic products now incorporate enriched extracts or purified compounds to meet growing consumer demand for natural, multifunctional skin care solutions.Further investigation using human subjects and advanced 3D skin models is advised to validate the long-term safety of *Achillea millefolium* and refine its formulation approaches, despite its overall favorable safety profile (Strzępek-Gomółka et al., 2021). All the findings are shown in table 1.

CONCLUSION

Achillea millefolium exemplifies the successful evolution of a traditional medicinal plant into a scientifically validated ingredient for modern nutraceutical and cosmetic applications. Its rich phytochemical composition-including flavonoids, phenolic acids, terpenes, and essential oils-confers a broad spectrum of bioactivities such as antioxidants, anti-inflammatory, antimicrobial, wound-healing, and cytotoxic effects (Garcia-Oliveira et al., 2021; Jangjoo et al., 2023; Konarska et al., 2023). These properties support its use in managing gastrointestinal disorders, metabolic imbalances, dysmenorrhea, and skin conditions ranging from inflammation to aging (Salehi et al., 2020; Strzępek-Gomółka et al., 2021; Barda et al., 2021).Technological advancements have significantly enhanced the therapeutic potential of *A. millefolium*. Supercritical anti-solvent fractionation and nanoencapsulation techniques have improved the stability, bioavailability, and targeted delivery of its bioactive compounds, including luteolin-7-O-glucoside, camphor, and borneol (Villalva et al., 2022; Abu Safe et al., 2023). PEG-based hydroglycolic systems have preserved key polyphenols like caffeoylquinic acid and axillarin, boosting SPF and tyrosinase inhibition in topical formulations (Gaweł-Bęben et al., 2020). These innovations have expanded its commercial viability, particularly in cosmeceuticals targeting skin regeneration, pigmentation control, and protection against environmental stressors (Strzępek-Gomółka et al., 2021; Marengo et al., 2025).Despite its favorable safety profile, further validation through human clinical trials and advanced 3D skin models is recommended to confirm long-term tolerability and optimize formulation strategies (Becker et al., 2016; Strzępek-Gomółka et al., 2021). Regulatory harmonization remains essential, especially for international distribution, with *A. millefolium* already listed in the EU CosIng database and widely used in consumer products (Marengo et al., 2025; European Medicines Agency, 2020). Safety assessments indicate low toxicity at standard concentrations, though certain constituents like thujone and hydroquinone warrant careful formulation oversight (Becker et al., 2016).The literature from the last decade consistently reinforces *A. millefolium*'s multifunctional applications. Its antioxidant and antimicrobial properties support its inclusion in skin care products aimed at preventing oxidative damage and microbial infections, while its cytotoxic and immunomodulatory effects suggest potential roles in cancer chemoprevention and immune support (Jangjoo et al., 2023; Tripon et al., 2024; Ali et al., 2017). Comparative Phyto profiling studies further highlight the importance of morphotype and organ-specific extract selection to optimize therapeutic outcomes (Raudone et al., 2024).Looking ahead, future research should focus on dose-finding clinical trials, mechanistic studies, and long-term safety evaluations across diverse populations. Mapping chemotypes and morphotypes data to pharmacological activities will be crucial for standardizing extract quality and maximizing efficacy (Raudone et al., 2024; Salehi et al., 2020). Additionally, exploring novel formulations tailored to specific consumer needs-such as anti-aging, skin repair, or metabolic support-will expand its market potential (Jangjoo et al., 2023).In conclusion, *Achillea millefolium* stands out as a versatile botanical resource with scientifically supported bioactivities that align with both traditional use and modern therapeutic demands. Its integration into nutraceuticals and cosmetics reflects a growing preference for natural, multifunctional ingredients. Continued interdisciplinary research and regulatory

refinement will be key to unlocking its full potential in health and wellness innovation (Barda et al., 2021).

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RESEARCH ON CONVENTIONAL WHEAT CULTIVATION TECHNOLOGY, INCLUDING SOIL TILLAGE AND PHASED FERTILIZATION, APPLIED TO THREE WHEAT VARIETIES UNDER NON-IRRIGATED CONDITIONS, IN THE PEDOCLIMATIC CONDITIONS OF GĂLBINAȘI, BUZĂU COUNTY, DURING THE 2023–2024 AGRICULTURAL YEAR

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ABSTRACT

The present study aimed to evaluate the agronomic behavior and qualitative performance of three winter wheat varieties (Katarina, Biharia, Apache) cultivated under rainfed conditions in the pedoclimatic context of Gălbinași, Buzău County, Romania, during the 2023–2024 agricultural year. The experiment was carried out on a 45 ha experimental field and applied a conventional cultivation technology including soil tillage and phased fertilization, tested in three variants: control (no additional fertilization), two-phase fertilization, and three-phase fertilization. Data collection included plant density at emergence and harvest, thousand-kernel weight, number of grains per spike, test weight, yield per hectare, and grain quality indices (moisture, protein content, wet gluten content). Statistical analysis (ANOVA and linear regression) demonstrated the significant influence of fertilization on plant density and kernel weight ($p < 0.05$), with a moderate correlation between fertilization level and yield ($R^2 = 0.58$). The variety Katarina recorded the highest stability and yield potential (7711.4 kg/ha with three-phase fertilization), followed by Biharia and Apache. Grain quality was superior for Katarina and Biharia regarding protein and gluten content, while Apache showed lower values under low fertilization. The results highlight the potential of phased nitrogen fertilization to mitigate drought stress effects and improve both yield and grain quality under the restrictive climatic conditions of southeastern Romania. The study provides valuable recommendations for optimizing winter wheat cultivation technologies in areas with similar pedoclimatic conditions.

Keywords: soil tillage, fertilization, wheat, drought, climate change

INTRODUCTION

Agriculture is one of the oldest human occupations, emerging with the transition from nomadism to sedentary living (Guș & Tianu, 1991). Throughout the evolution of human societies, agriculture has diversified and improved, being strongly influenced by technological and social progress. A defining moment was the invention of the plough, evolving from simple wooden beams

to modern ploughs with mechanical traction and multiple moldboards (Panaitescu, 2016; Panaitescu & Niță, 2011). The expansion of mechanization, the use of chemical fertilizers, and the introduction of genetic engineering have significantly increased yields but have also generated negative environmental impacts (Budoi & Penescu, 1996; Cakpo et al., 2025).

In Romania, studies by the National Research and Development Institute for Soil Science, Agrochemistry and Environmental Protection have highlighted the degradation of soil quality through erosion, acidification, salinization, compaction, and chemical pollution (Patriche et al., 2023a; Patriche et al., 2023b; Stănilă, 2023). Globally, the intensification of conventional agricultural practices has contributed to biodiversity loss, water pollution, and contamination of agri-food products (Gagiu et al., 2023; Gagiu et al., 2025). Therefore, adapting these practices is essential to protect ecosystems and human health (Budoi & Penescu, 1996; Calistru et al., 2024).

In recent decades, climate change has become a major challenge for global agriculture. The global average temperature has increased by approximately 1.7°C compared to the pre-industrial period and is projected to rise by up to 2.7°C in the coming decades (Bandoc & Prăvălie, 2015; Moise, 2023; European Environment Agency, 2023). In temperate continental regions, such as Muntenia, these changes translate into prolonged droughts, uneven precipitation distribution, heatwaves, torrential rains, hail, and extreme winds (Niță et al., 2024; European Environment Agency, 2023). The Gălbinași commune, Buzău County, frequently faces such phenomena, requiring the adaptation of agricultural technologies to the new conditions (Gagiu et al., 2025).

Winter wheat (Triticum aestivum L.) remains one of the key crops in Romania due to its economic value and its ability to adapt to local soil and climate conditions (Panaitescu, 2016; Popescu et al., 2023). The choice of suitable variety, the application of adapted cultivation technologies, and soil conservation are key factors in achieving stable and high-quality yields (Guș & Tianu, 1991; Cakpo et al., 2025).

The aim of this study is to analyze the behavior and suitability of three winter wheat varieties grown under non-irrigated conditions in Gălbinași, Buzău County, during the 2023–2024 agricultural year, characterized by extreme climatic conditions. The applied cultivation technology was conventional, including soil tillage (Cakpo et al., 2025), and phased fertilization varied depending on variety and treatment (Calistru et al., 2024). For each variety, three experimental variants were tested: control (no additional phased fertilization), two-stage phased fertilization, and three-stage phased fertilization.

The specific objectives of the study are: to compare the agronomic behavior of the three wheat varieties under identical technological and climatic conditions; to analyze the performance of the varieties under conventional systems in terms of agronomic efficiency and conservation of soil and water resources, within the context of sustainable agriculture; to formulate technical recommendations on variety selection and cultivation technology adapted to the specific conditions of Gălbinași, with the possibility of extending the results to areas with similar pedoclimatic conditions; to reduce production costs and achieve high and superior quality yields.

MATERIAL AND METHOD

The study was conducted during the 2023–2024 agricultural year on a 45-hectare experimental plot belonging to Denagro Martrans company, located in Gălbinași, Buzău County, in a region representative of large-scale farming in southeastern Romania. The area's climate is excessively continental, characterized by hot, dry summers with maximum temperatures reaching 39°C, and cold winters with frequent blizzards and temperatures dropping below -20°C. Annual precipitation is low and unevenly distributed, while the average annual temperature is around 11.5°C. The soils in the

experimental area are classified as cambic chernozems, with a loamy texture in the upper horizon and loamy-silty texture in intermediate horizons, developed on calcareous loess deposits, and with a physical structure suitable for conventional agricultural practices.

The biological material consisted of three winter wheat (Triticum aestivum L.) varieties: Apache, Katarina, and Biharia. Apache is an early, awnless variety with medium height, excellent lodging resistance, high tillering and winter hardiness, and good tolerance to foliar diseases, particularly rusts and fusarium. Katarina, also early and awnless, has strong emergence vigor, good winter hardiness, high yield potential, and resistance to major diseases. Biharia is a semi-late variety, with short stature and short awns, notable for its drought, lodging, and disease resistance, as well as for its very good tillering capacity.

The cultivation technology followed conventional system principles, including primary ploughing, soil loosening and leveling, followed by sowing at a 12.5 cm row spacing with the same seeding rate for all experimental variants. The experiment comprised nine variants, three for each variety, organized as follows: a control variant (without additional phased fertilization, to highlight the biological potential and the effect of the previous crop), a variant with two-stage phased fertilization, and a variant with three-stage phased fertilization. Plant protection treatments (herbicides, fungicides, and insecticides) were uniformly applied across all variants, ensuring that the observed differences could be attributed exclusively to the genotypes and fertilization regime.

To evaluate the agro-productive performance of the three varieties, observations and measurements were carried out throughout the growing season. These included plant density at emergence and harvest (expressed as plants/m²), thousand kernel weight (TKW), determined by weighing a standardized sample of mature grains, number of grains per spike, determined by manual harvest and counting, hectoliter weight expressed in kg/hl according to SR 13574:2010 standard, and average yield per hectare, calculated based on the weight of grains relative to the cultivated area. Additionally, grain quality parameters (moisture, protein, and gluten content) were analyzed using appropriate laboratory equipment.

To analyze differences among the experimental variants and assess the influence of fertilization under the water stress conditions characteristic of the study year, the data were subjected to statistical analysis. Analysis of variance (ANOVA) was used to test the significance of differences between treatments, and linear regression models were applied to evaluate the relationship between fertilization level, yield, and lack of precipitation. The significance level was set at $p \leq 0.05$. Data processing was performed using SPSS statistical software (version 26) and Microsoft Excel.

RESULTS AND DISCUSSION

In the experiment, soil preparation for the winter wheat crop followed agrotechnical principles aimed at maximizing the productive potential of the crop. Winter wheat performs best when preceded by early-harvest species that leave the soil rich in nitrogen and other essential nutrients, thereby enabling timely execution of primary soil work. In all nine experimental variants, the preceding crop was pea, a legume highly valued agronomically due to its ability to fix atmospheric nitrogen and leave the soil weed-free, enriched with plant residues that, once incorporated, increase organic matter content and stimulate soil microbiological activity.

The experiment was conducted on a total area of 45 hectares, equally divided among the three tested wheat varieties: Apache, Katarina, and Biharia. Each variety was cultivated on 15 hectares, further split into three experimental variants of 5 hectares each: control (without additional fertilization), two-stage phased fertilization, and three-stage phased fertilization.

For the control variant, the crop relied solely on residual nitrogen and nutrients left in the soil after the pea crop, to assess the biological potential of the varieties without the influence of additional fertilization. In the fertilized variants, the first application coincided with sowing in the first decade of October, with 300 kg/ha of Duofertil Eurocereal 34 (N, P, S) from Timac, a product designed to mobilize and protect nutrients and stimulate root development thanks to the Mappa Duo and XCK complex. The second application, applied to both the two- and three-stage fertilization variants, took place on March 18, using Sulfammo 25 Mappa Duo at a rate of 150 kg/ha, a solid fertilizer with 25% nitrogen and 31% sulfur trioxide. The three-stage fertilization variant also received an additional foliar treatment on April 28, at flag leaf emergence, applying 30 l/ha of In-Folen (19% nitrogen, 5% magnesium oxide, 10% sulfur trioxide), aimed at enhancing enzymatic activity and optimizing grain quality parameters.

Soil preparation included deep ploughing at approximately 25 cm depth in the second decade of August, using a Lemken EurOpal 5 plough, with the purpose of turning over the furrow, incorporating plant residues, and ensuring good soil aeration and loosening. The ploughing depth was adjusted according to the soil's moisture level and the amount of plant residue.

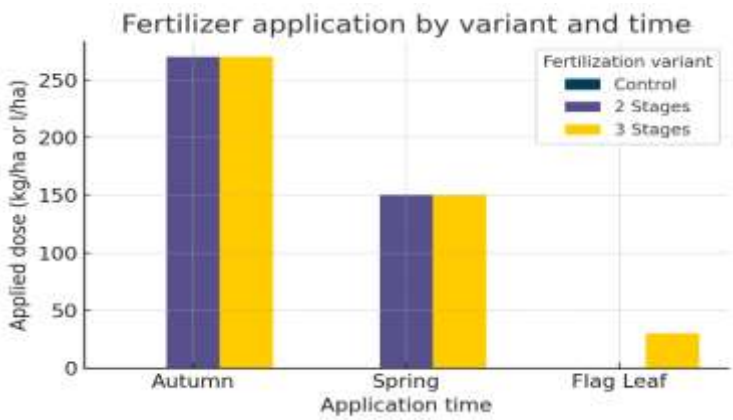

Figure 1 – Fertilization scheme of the three winter wheat varieties across experimental variants

The climatic conditions recorded during the growing season were characterized by positive temperature anomalies and a significant precipitation deficit, which increased the importance of the applied technology and fertilization regime. The climatic data recorded in the experimental area are presented in figure 2. Monthly average temperatures were significantly higher than the multiannual normal, with notable differences in December 2023 and January 2024, where temperatures exceeded the normal values by approximately 5°C.

Regarding precipitation, the total amount accumulated during the 2023–2024 agricultural year was 311.4 mm, which is 114.4 mm below the multiannual average (425.8 mm). Severe water deficits were recorded especially during the active growing months (March–June), with a total of 116.7 mm compared to the estimated requirement of 280–320 mm for optimal wheat development at this stage. Only January and April recorded values above the normal average, but the surplus was insignificant.

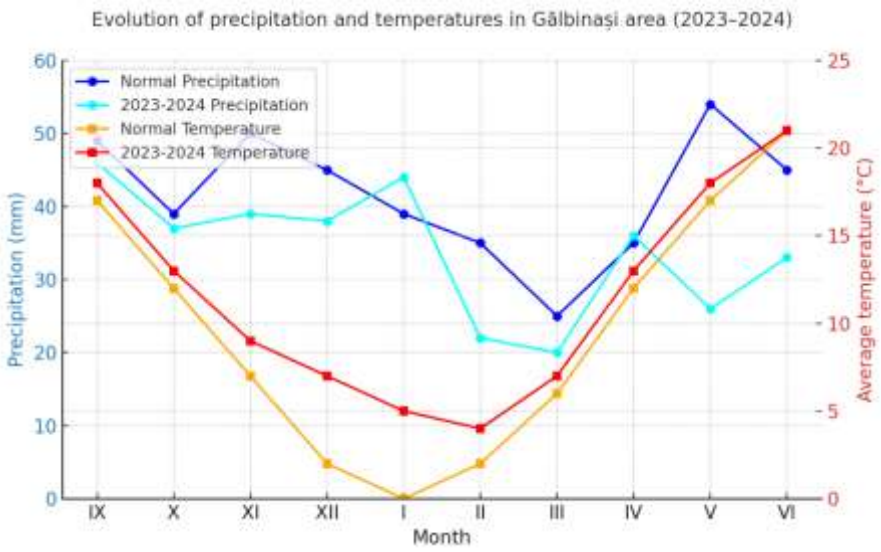

Figure 2 – Evolution of precipitation and temperatures in the study area, Gălbinași locality, during the 2023–2024 agricultural year

Seedbed preparation was carried out on October 4, 2023, with a single pass of the Vaderstad TopDown 400 cultivator, which broke down the plough furrows, loosened, and leveled the soil to facilitate optimal seed-to-soil contact. The seeds used came from certified seed lots (C1 biological category), with a physical purity of 99.80% for Biharia and Katarina and 98.70% for Apache, and a germination capacity of 97%. The thousand kernel weight (TKW) was 42 g for Biharia and Katarina, and 40 g for Apache.

Before sowing, the seeds were mandatorily treated with Austral Plus (500 ml/100 kg seed), a broad-spectrum insecto-fungicide. Sowing took place on October 6, 2023, at a row spacing of 12.5 cm and a sowing depth of 4 cm, with a seeding rate of 390 germinable seeds/m², corresponding to approximately 210 kg seed/ha.

Weed control was achieved through integrated preventive methods and chemical treatments. The main weeds identified were *Sinapis arvensis, Cirsium arvense, Centaurea cyanus, Chenopodium album, Galium aparine, Capsella bursa-pastoris* (dicotyledonous species), and *Avena fatua, Poa annua* (monocotyledonous species). Control of monocotyledonous weeds was achieved with Starane Super (0.4 l/ha), applied on April 24 together with insecto-fungicides. Dicotyledonous weeds were controlled with Biathlon 4D (0.07 kg/ha) and adjuvant Dash HC (1 l/ha), applied at the 6–8 leaf stage on May 11.

Plant protection against diseases was carried out in two stages: the first treatment with Evolus (1 l/ha) on April 24, and the second with Falcon Pro (0.9 l/ha) on May 11, providing preventive, curative, and eradicative protection. Pest control was initially ensured by seed treatment, followed by two chemical treatments: Lamdex Extra (0.3 kg/ha, April 24) and Delmetros 100SC (0.15 l/ha, May 11).

Harvesting was performed on June 28, 2024, with a Deutz Fahr C7206 combine equipped with a cereal header. The grain moisture at harvest ranged between 11.5% and 12.6%. After threshing, the straw was baled, and the grain was transported and stored in designated facilities.

The yield results showed a clear influence of the fertilization regime and variety used. The average yields obtained were: for Katarina, 7,711.4 kg/ha (three fertilizations), 6,425.1 kg/ha (two fertilizations), and 5,689.6 kg/ha (control); for Biharia, 6,543.6 kg/ha (three fertilizations), 5,689.6

kg/ha (two fertilizations), and 4,615.4 kg/ha (control); for Apache, 6,108.7 kg/ha (three fertilizations), 4,848.4 kg/ha (two fertilizations), and 3,365.4 kg/ha (control). These results confirm the benefits of split nitrogen application, especially under challenging climatic conditions and significant water deficits.

Plant density at emergence was determined using standardized methods by counting plants per linear meter over 10 rows with three repetitions for each experimental variant at the first true leaf stage. The average values show a positive influence of fertilization on final crop density.

For Katarina, the average plant density for the three-fertilization variant was 308 plants/m² (min. 27, max. 34 plants), for two fertilizations 302 plants/m² (min. 27, max. 33 plants), and for the control 288 plants/m² (min. 26, max. 34 plants). For Biharia, the values were 305 plants/m² (three fertilizations), 300 plants/m² (two fertilizations), and 283 plants/m² (control), with individual ranges between 26 and 34 plants/m². For Apache, densities were 300 plants/m² (three fertilizations), 299 plants/m² (two fertilizations), and 276 plants/m² (control), ranging from 26 to 33 plants/m².

The results show a clear tendency for increased plant density as fertilization intensity increased across all three varieties. This is attributed to improved germination vigor and seedling emergence, resulting from the additional nutrient (mainly nitrogen) supply applied at sowing. The data also indicate good emergence uniformity, with consistent values between repetitions, highlighting seed quality and effective seedbed preparation.

Spike density at harvest was determined by counting spikes per linear meter over 10 rows, with three repetitions for each variety and fertilization variant. The average values showed a clear influence of fertilization on spike density, demonstrating the positive effect of nitrogen supplementation on tillering capacity and plant survival up to harvest.

For Katarina, spike density was 567 spikes/m² (three fertilizations), 531 spikes/m² (two fertilizations), and 508 spikes/m² (control). For Biharia, values were 532 spikes/m² (three fertilizations), 508 spikes/m² (two fertilizations), and 491 spikes/m² (control). For Apache, values were 509 spikes/m² (three fertilizations), 480 spikes/m² (two fertilizations), and 474 spikes/m² (control). The observed minimum–maximum ranges per row were 44–62 spikes for Katarina, 42–61 spikes for Biharia, and 40–62 spikes for Apache, confirming moderate variability but with a clear tendency for increased spike density with increased fertilization.

These results support the hypothesis that split fertilizer application leads to higher final plant densities at harvest, due to reduced plant mortality and stimulated secondary tillering during the growing season.

To validate the significance of the differences between experimental variants for spike density at harvest, raw data (10 repetitions per variant) were subjected to analysis of variance (ANOVA). The ANOVA test showed extremely significant differences between varieties and fertilization variants ($F(2,87) = 12.28$; $p < 0.0001$). These data confirm that the fertilization regime had a strong impact on spike density at harvest, mainly by stimulating secondary tillering and reducing plant mortality under the severe water deficit conditions recorded in the 2023–2024 agricultural year. A directly proportional relationship between increased fertilization intensity and spike density was consistently observed across all three studied varieties.

The results confirm the hypothesis that split nitrogen application, adapted to wheat phenological stages, optimizes crop architecture and contributes to yield stability under challenging climatic conditions.

To provide a detailed representation of experimental data variability, spike density at harvest was also illustrated using a boxplot diagram (Figure 3). This enabled visualization of the full distribution of the 10 repetitions for each variety and fertilization variant. The boxplot highlights medians, interquartile ranges, and potential outliers, providing a comprehensive picture of result

consistency. A clear trend of higher average density for Katarina, followed by Biharia and Apache, was observed, in line with the previously calculated averages. Slightly higher variability for Biharia was noted, possibly due to greater sensitivity to water stress conditions.

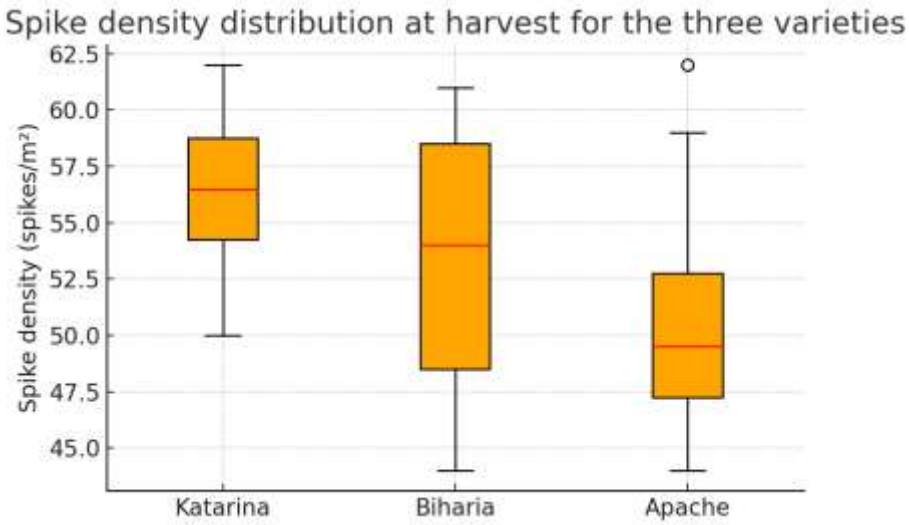

Figure 3 – Boxplot of spike density distribution at harvest for the three wheat varieties

To better understand the relationship between fertilization level and final spike density at harvest, the average data obtained for the three varieties were subjected to linear regression analysis. Fertilization was coded ordinally (0 = control, 1 = two fertilizations, 2 = three fertilizations). The regression model revealed a positive relationship between increasing fertilization intensity and final spike density. The resulting regression line indicated a clear trend of increased crop density with additional fertilizer application. The coefficient of determination ($R^2 = 0.45$) suggests that approximately 45% of the variation in spike density at harvest can be attributed to differences in the applied fertilization regimes.

This result supports the conclusions of the ANOVA test and confirms the hypothesis that phased fertilization, adapted to the phenological requirements of the crop, significantly contributes to reducing plant mortality and stimulating secondary tillering, thereby optimizing crop architecture under water stress conditions.

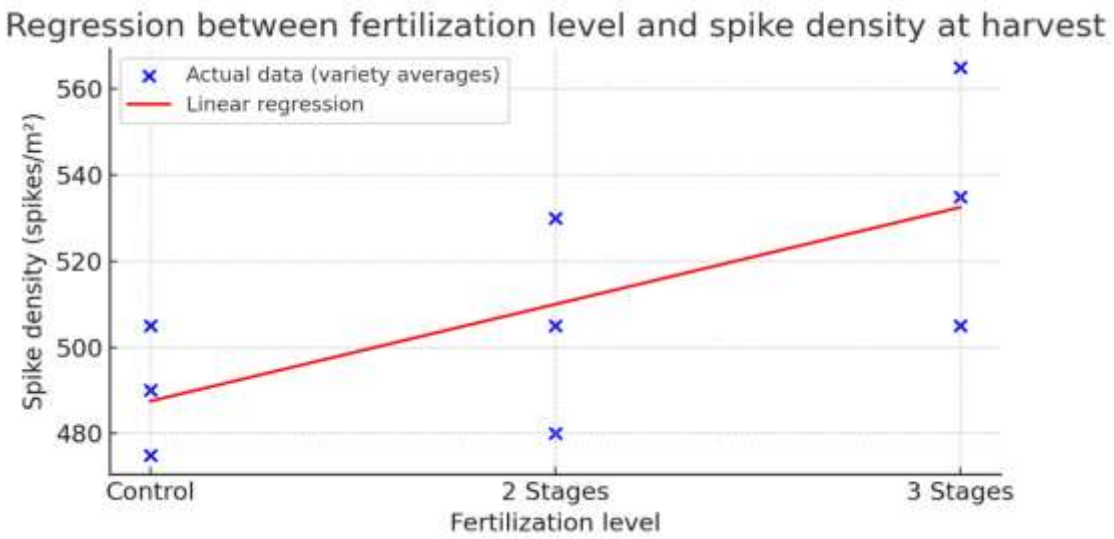

Figure 4 – Regression between fertilization level and spike density at harvest

The determination of the thousand kernel weight (TKW) was carried out by repeated weighing of samples taken from all experimental variants, in order to identify the influence of the fertilization regime on this essential quality indicator. The results showed a clear tendency for TKW to increase with the intensification of fertilization for all three varieties studied. For Katarina, the average values were 44 g (three fertilizations), 42 g (two fertilizations), and 40 g (control). For Biharia, values were 44 g (three fertilizations), 40 g (two fertilizations), and 35 g (control). For Apache, the values were 43 g, 39 g, and 31 g, respectively.

To validate the statistical significance of the differences, raw data were subjected to ANOVA testing. The analysis revealed significant differences between varieties and variants ($F(2,60) = 7.35$; $p = 0.0014$). These results demonstrate the strong influence of split fertilizer application on thousand kernel weight, particularly under the difficult pedoclimatic conditions of the 2023–2024 agricultural year, characterized by precipitation deficit and water stress. It can be concluded that judicious application of nitrogen fertilization, aligned with crop phenological stages, optimizes the grain filling process and contributes to achieving superior grain quality.

To provide a detailed characterization of data variability from the determination of thousand kernel weight (TKW), a visual analysis was performed using a boxplot (Figure 5). This representation highlighted the distribution of values obtained for the three studied varieties: Katarina, Biharia, and Apache. The boxplot reveals clear differences between the three genotypes, with Katarina showing the lowest internal variability, which may indicate superior stability of this trait under unfavorable pedoclimatic conditions. In contrast, Biharia and Apache showed greater dispersion of values, suggesting a stronger reaction to abiotic stress or interaction with the applied fertilization technology.

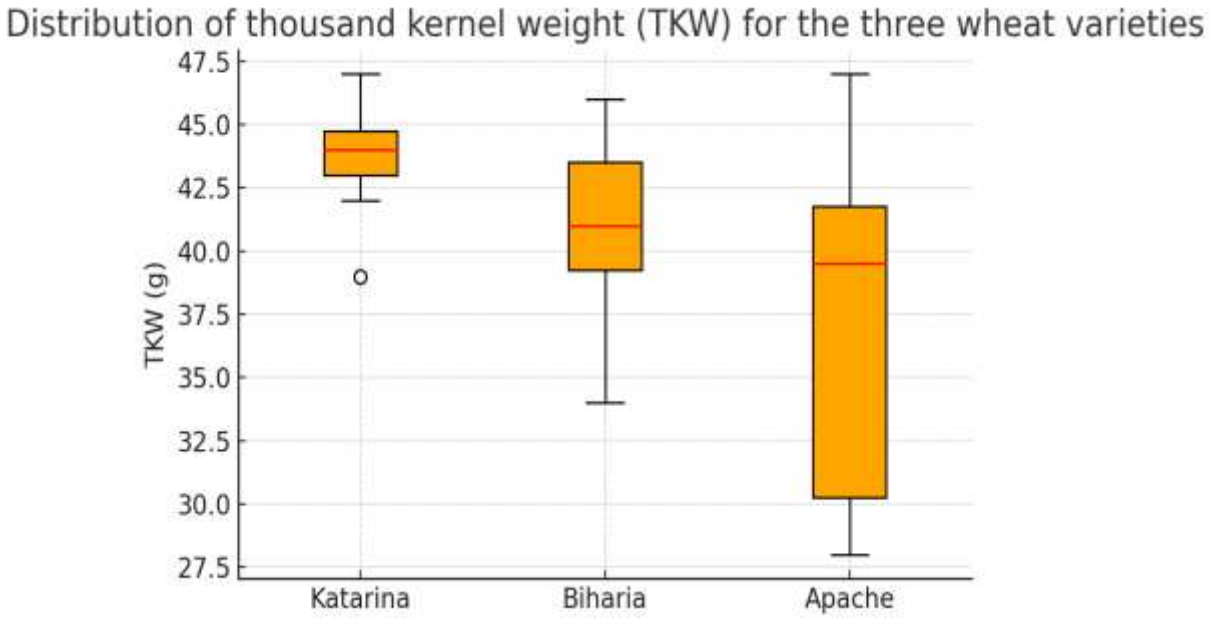

Figure 5 – Boxplot of thousand kernel weight (TKW) distribution for the three wheat varieties

To quantify the relationship between fertilization level and thousand kernel weight (TKW), a linear regression model was applied to the average values obtained for the three varieties and the three fertilization regimes. The results indicated a positive relationship between increased fertilization intensity and higher TKW values. The regression analysis produced a coefficient of determination (R^2) of 0.69, meaning that approximately 69% of the variation in thousand kernel weight can be explained by the applied fertilization level. This demonstrates a strong and predictable influence of the nutritional regime on grain quality. The results are fully consistent with those highlighted by the ANOVA test and the boxplot analysis, further supporting the hypothesis that phased fertilization, adapted to the specific requirements of the crop, plays a decisive role in improving the quality parameters of the wheat harvest.

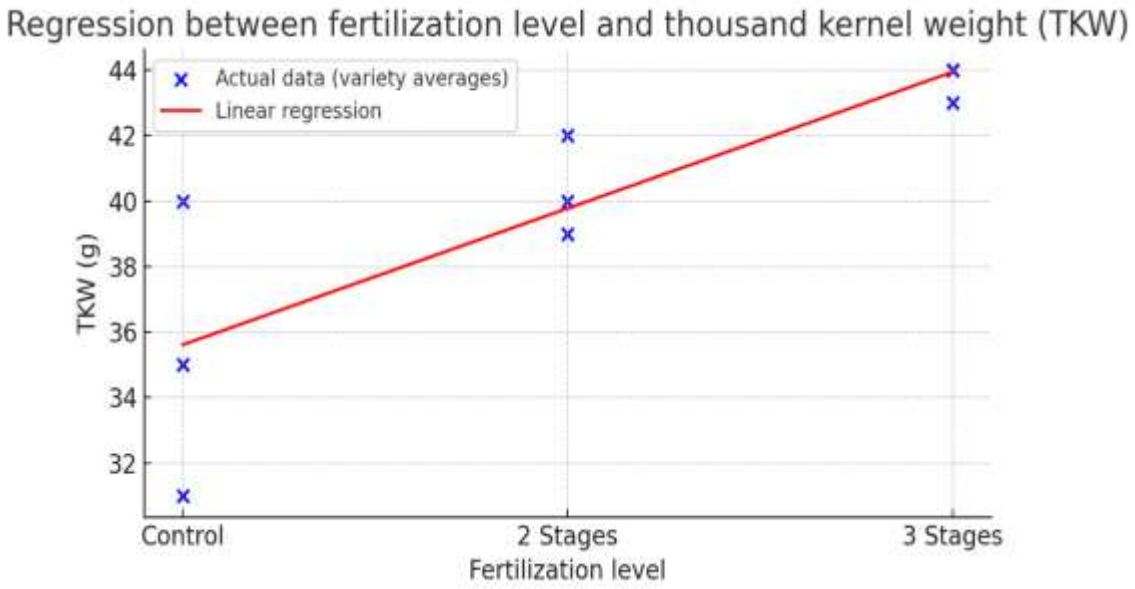

Figure 6 – Regression graph between fertilization level and thousand kernel weight (TKW)

The yield per hectare was determined based on the harvest obtained for each variety and experimental variant. The results showed a clear trend of yield increase with the intensification of the fertilization regime.

For the Katarina variety, yields of 7,711.4 kg/ha (three fertilizations), 6,425.1 kg/ha (two fertilizations), and 5,689.6 kg/ha (control) were recorded. For Biharia, yields were 6,543.6 kg/ha (three fertilizations), 5,689.6 kg/ha (two fertilizations), and 4,615.4 kg/ha (control). For Apache, values of 6,108.7 kg/ha, 4,848.4 kg/ha, and 3,365.4 kg/ha were recorded for the same variants.

To test the significance of the differences between varieties and fertilization variants, an analysis of variance (ANOVA) was applied. Although notable value differences were observed, the statistical test indicated a lack of statistical significance ($F(2,6) = 1.96$; $p = 0.22$). This is explained by the absence of multiple biological replicates for each variant, as the data were obtained from a single experimental plot per treatment. Therefore, these results must be interpreted primarily descriptively, without rigorous statistical validation. Nevertheless, the clear trend of increased yield with increasing fertilization level is evident and correlates with the other agro-productive data previously analyzed (spike density at harvest, number of grains per spike, thousand kernel weight), confirming the effectiveness of phased fertilization even under the difficult climatic conditions of the 2023–2024 agricultural year.

To highlight the trend of yield increase with fertilization level, the average data obtained for the three varieties were analyzed using a linear regression model. The results indicated a positive relationship between the application of phased fertilization and yield values per hectare. The coefficient of determination ($R^2 = 0.58$) showed that approximately 58% of the yield variation can be explained by the administered fertilization level. This suggests a significant influence of the fertilization regime on crop performance, even under the challenging climatic conditions of the 2023–2024 agricultural year.

It is important to note that this analysis is indicative, as it was based on data obtained from a single experimental plot for each treatment. The absence of multiple biological replicates limits the full statistical validity of the model. Nevertheless, the trend illustrated supports the descriptive

observations and is consistent with the results of the other agro-productive analyses conducted in this study.

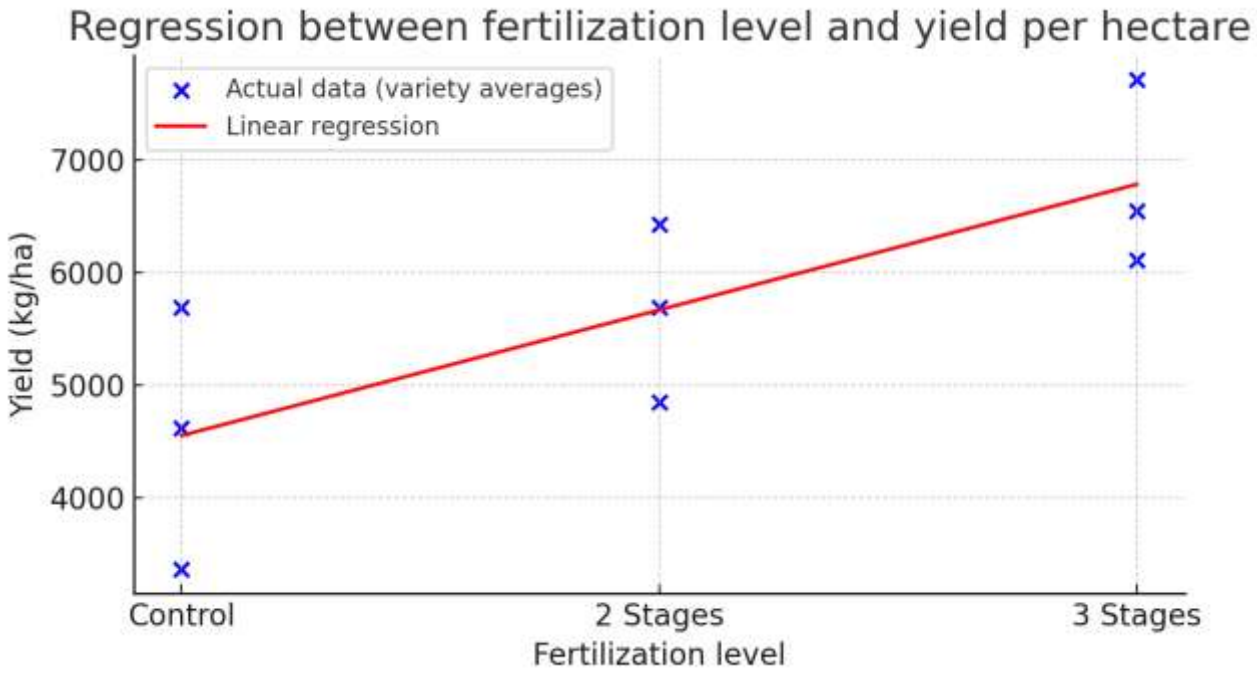

Figure 7 – Regression graph between fertilization level and yield per hectare

The grain quality of wheat was evaluated based on three essential parameters: protein content, wet gluten content, and hectoliter weight (HW). These indicators directly reflect the influence of the applied technological regime and climatic conditions on the baking characteristics of the harvest.

Protein content varied both between varieties and between fertilization variants. Katarina showed the most stable and highest values, ranging between 12.5% and 13.8%, demonstrating superior protein accumulation even under moderate drought conditions. Apache showed a slight decrease in protein content in the control variant (below 12%), suggesting greater sensitivity to the lack of nutritional input. Biharia presented greater variability, with values ranging from 12.1% to 14.3%, indicating better fertilizer use efficiency but also a stronger reaction to fluctuations in moisture and temperature during grain filling.

Wet gluten content, an essential indicator of baking quality, followed a similar pattern to protein content. Katarina was in the superior category, with values between 27.5% and 28.8%, showing stable gluten quality under water stress. Biharia had high values, between 28.6% and 32.3%, but with greater variation between variants, suggesting a complex interaction between nitrogen supply and climatic conditions. In contrast, Apache showed a reduction in gluten content, especially in the control variant (23.7%–24.5%), confirming the negative impact of nutrient deficiency on gluten protein quality.

Hectoliter weight (HW), an indicator of grain filling and commercial quality, recorded values above the minimum threshold (76 kg/hl) for all varieties, with the best results for Katarina (79–81 kg/hl). For Biharia, values ranged between 76–79 kg/hl, while for Apache, the control variant slightly dropped below 76 kg/hl, suggesting incomplete grain filling and greater sensitivity to nutrient and moisture deficiencies.

To provide a synthetic and comparative overview of the performance of the three varieties regarding grain quality, the maximum values obtained for protein content, wet gluten content, and hectoliter weight (HW) were graphically represented (Figure 8).

The graph highlights the superiority of Katarina in terms of stability and high values of hectoliter weight (81 kg/hl) and protein content (13.8%), demonstrating excellent adaptability to moderate drought conditions and the applied fertilization technology. Biharia recorded the highest values for wet gluten content (32.3%), indicating excellent baking quality but showed greater variability in the other parameters. Apache had the lowest maximum values, especially for hectoliter weight (76 kg/hl) and gluten (24.5%), which can be explained by its higher sensitivity to the lack of additional fertilization.

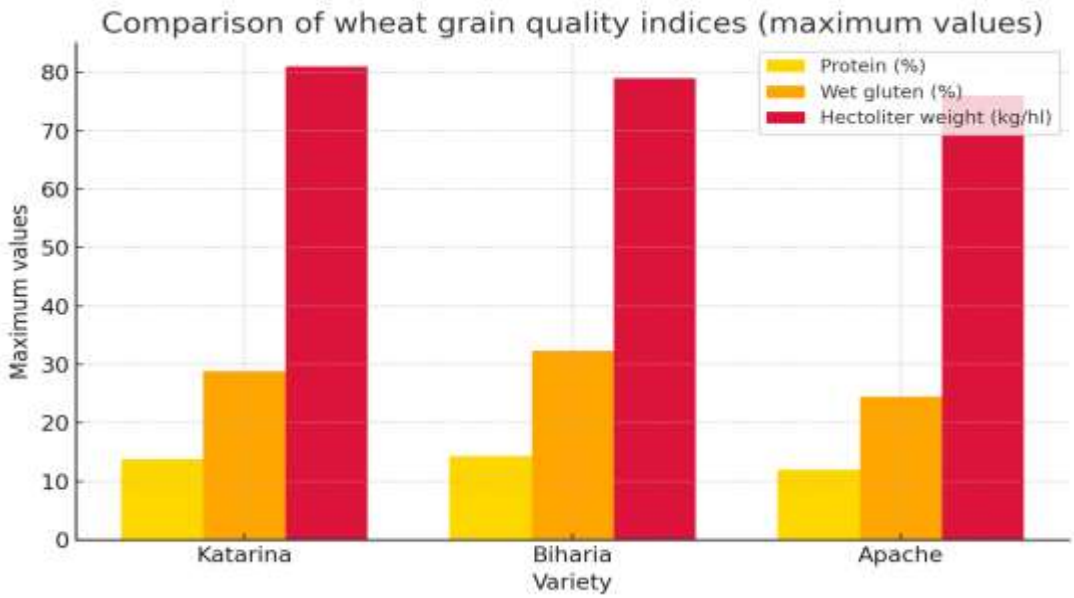

Figure 8 – Graph of the main grain quality indices: protein, wet gluten, and hectoliter weight

CONCLUSIONS

The research conducted at Denagro Martrans, in Gălbinași, Buzău County, during the 2023–2024 agricultural year, aimed to evaluate the agronomic and quality performance of three winter wheat varieties (Katarina, Biharia, Apache) grown under rainfed conditions, in restrictive climatic conditions characterized by severe precipitation deficits and elevated average temperatures.

The results demonstrated a significant influence of the fertilization regime on most of the analyzed parameters. Regarding spike density at harvest, phased fertilization applied in two and three stages led to a significant increase of this indicator, statistically confirmed by ANOVA analysis ($p < 0.0001$). Additional nitrogen application promoted both secondary tillering and reduced plant mortality during the vegetation cycle, resulting in a more uniform and efficient crop architecture.

The thousand kernel weight (TKW), an essential indicator for grain quality, was strongly influenced by fertilization, as shown by the statistical analysis ($p = 0.0014$) and the strong correlation obtained by regression analysis ($R^2 = 0.69$). Split fertilization contributed to improved grain filling and the achievement of higher quality yields, despite unfavorable pedoclimatic conditions.

Although the statistical analysis of yield per hectare did not show significant differences ($p = 0.22$), descriptive data indicated a clear trend of yield increase with increasing fertilization levels. Linear regression showed a positive relationship between fertilization level and yield ($R^2 = 0.58$). Katarina recorded the highest yields, reaching 7,711.4 kg/ha with three fertilizations, followed by Biharia and Apache, highlighting the importance of judicious fertilizer application in maximizing genetic potential.

Grain quality analysis revealed specific differences between varieties. Katarina stood out for its superior stability in protein content (12.5–13.8%) and high hectoliter weight (79–81 kg/hl). Biharia had a high wet gluten content (28.6–32.3%), indicating superior baking quality, but with slightly higher internal variability. Apache, although characterized by good drought tolerance, recorded the lowest values for all quality parameters in the control variant, confirming its higher sensitivity to the absence of additional fertilization.

In conclusion, the study confirmed that adaptation and intensification of phased fertilization can partially compensate for the negative effects of water stress on winter wheat crops, contributing both to yield improvement and grain quality enhancement. These results provide a solid basis for technological recommendations for farmers practicing agriculture under pedoclimatic conditions similar to those of the Gălbinași area and the southeastern regions of Romania.

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BIOCHEMICAL AND AROMATIC CHANGES IN PERSIMMON (DIOSPYROS KAKI L.) FRUITS HARVESTED IN DIFFERENT PERIODS

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ABSTRACT

Persimmon (*Diospyros kaki* L.), originally from China and widely cultivated throughout East Asia, has recently gained popularity in several Mediterranean countries, including Türkiye. This study was conducted to determine the optimum harvest time by evaluating the biochemical and aromatic changes in persimmons at different maturity stages. The research was carried out in a commercial orchard in the Dareski hamlet of the Toroslar district, Mersin Province, focusing on the commonly grown 'Hachia' cultivar. Fruits were harvested at seven-day intervals beginning in the last week of October and analyzed at two distinct maturity stages: unripe and fully ripe. Sugar analysis revealed that glucose and fructose were the predominant sugars in both stages, with significantly higher levels in ripe fruits (8930 and 9166 mg/100g, respectively) compared to unripe ones (6561 and 7213 mg/100g). Total sugar content increased from 13,867 to 18,248 mg/100g, along with an increase in total soluble solid content (TSS) from 14.45% to 19.4%. In terms of organic acids, malic acid was dominant in unripe fruits (22.43 mg/100g), whereas succinic acid significantly increased in ripe fruits (38.27 mg/100g). Citric and ascorbic acid contents also varied notably between the two stages, and titratable acidity rose from 0.86% to 1.14% during ripening. Volatile compound profiling revealed clear maturity-dependent differences. Aldehyde compounds were more abundant in ripe fruits (37.76%), contributing to a richer and more intense aroma, while alcohols and terpenes predominated in unripe fruits (17.44% and 44.98%, respectively), with linalool, nerol, and limonene as major contributors. These findings confirm that the biochemical and aromatic profiles of persimmons are strongly influenced by the maturity stage, which has important implications for flavor development, postharvest quality, and market value. Despite increasing interest among consumers and producers, persimmon production in Türkiye is still limited due to challenges in storage and marketing. The study suggests that harvesting after the second ripening stage may improve product quality and commercial potential under the Mediterranean climate of Mersin.

Keywords: Persimmon, maturity stage, sugars, organic acids, volatile compounds.

INTRODUCTION

Fruit cultivation worldwide is generally concentrated on a limited number of species; however, persimmon (*Diospyros kaki* L.), belonging to the Ebenaceae family, has gradually expanded to many regions of the world and has gained increasing popularity in recent decades (Öz and Özelkök, 2003). Native to China, persimmon was first extensively cultivated in Japan and later established in modern orchards in countries such as the United States (California), Italy, Brazil, New Zealand, Australia, and Israel (Kaynaş & Kuzucu, 2004). The fruit, known for its attractive appearance and pleasant taste, has been given names such as “Japanese persimmon,” “Mediterranean persimmon,” and “cennet hurması” in Turkey (Yıldız Akbulut, 2021). Although the genus *Diospyros* contains numerous species, only four

(*D. kaki Thunb*., *D. lotus* L., *D. virginiana* L., and *D. oleifera* Cheng) are of commercial importance, with *D. kaki* being the most widely cultivated for fresh consumption and processing (Kitagawa & Glucina, 1984; Yıldız et al., 2012). In Turkey, persimmon production has expanded considerably in the last 20–25 years. While production levels were between 15,000–20,000 tons in the early 2000s, it increased to 127,314 tons in 2023 and reached 142,584 tons in 2024, showing a 12% growth within a year (Anonymous, 2025a). This rise reflects both consumer demand and the fruit's adaptation to different climatic regions of Türkiye, including the Mediterranean, Aegean, Marmara, and Black Sea. Persimmons are harvested between September and December, displaying wide variation in fruit shape, size, and skin color. Both astringent and non-astringent cultivars are cultivated; while astringent fruits are unpleasantly bitter when unripe due to soluble tannins, they become sweet and palatable after softening or through specific postharvest treatments (Onur, 1990).

The ripening of persimmon fruit is a complex process involving physiological, biochemical, and structural changes. These changes affect sensory attributes such as color, taste, and texture, determining overall fruit quality (Chitarra et al., 2005). During maturation, chlorophyll degradation and carotenoid accumulation cause color changes from green to yellow, orange, or reddish hues (Kluge et al., 2018; Zhao et al., 2011). Studies have shown that carotenoid content, including lycopene, increases markedly during the mid to late stages of ripening, contributing to the reddish coloration of certain cultivars (Qi et al., 2019). Texture softening is mainly attributed to pectin degradation, cell wall disintegration, and membrane breakdown, while increased intercellular spaces and water loss lead to fruit weight reduction (Salvador et al., 2007; Tessmer et al., 2016; Sapper et al., 2019). Carbohydrate metabolism plays a key role in fruit ripening and sweetness perception. Changes in sugar composition are largely influenced by cultivar-specific enzymatic activity. In general, sucrose content decreases while glucose and fructose increase during ripening due to invertase activity (Bubba et al., 2009; Giordani et al., 2011; Santos et al., 2019). Different developmental patterns have been reported, such as sucrose reduction accompanied by increases in glucose and fructose or stability in certain sugars depending on the cultivar (Santos et al., 2018). Alongside carbohydrates, organic acids, amino acids, phenolics, and choline also undergo significant changes during maturation, especially in the late stages of development (Santos et al., 2018). Nutritionally, persimmon is an important source of ascorbic acid, carotenoids, phenolic compounds, and dietary fiber, contributing to high antioxidant activity and health-promoting effects (Parseker Yönel et al., 2008). Vitamins A and E, tannins, and minerals also enhance its nutritional and functional food value. These properties have contributed to growing consumer demand worldwide. However, challenges such as cultivar identification, determination of optimum harvest time, and effective postharvest handling still cause yield and quality losses (Onur, 1990; Tuzcu & Yıldırım, 2000). A major quality determinant is astringency, resulting from soluble tannins, which decrease during ripening due to polymerization or can be artificially reduced through treatments such as ethylene exposure, CO_2 application, or storage in alkaline solutions (Itoo, 1971; Itoo, 1980; Taira et al., 1997). Considering its nutritional richness, consumer preference, and economic potential, persimmon has become an increasingly valuable fruit crop in Turkey. Nevertheless, studies on its phytochemical and volatile composition under local ecological conditions are still limited. Therefore, this study focuses on the characterization of phytochemical compounds and volatile profiles of the 'Hachiya' persimmon cultivar grown under the ecological conditions of Mersin (Toroslar, Türkiye). The results are expected to provide new insights into ripening behavior, postharvest quality, and flavor development, ultimately contributing to reducing postharvest losses and increasing consumer acceptance.

MATERIALS AND METHODS

Plant Material

Persimmon fruit samples were collected from 20-year-old Hachiya trees planted in 5x5 spacings at various stages of ripening in a producer garden in Darseki village, Toroslar district, Mersin province. The samples were collected twice in 2023, once in October (unripe period) and once in November (ripe period). Fruit samples from three distinct trees and five fruit samples from different sides of the tree (North, South, West, and East) were taken in three repetitions during each harvest period and brought to the Horticulture laboratory of Çukurova University Faculty of Agriculture in an ice box cold chain. After washing the samples in tap water, 1 gram of hand squeezed fresh fruit juice was utilized for each bichemical assay.

Method

Total Soluble Solids (TSS): The SSC of representative fruit samples was measured as °Brix using a digital refractometer (Atago, Japan). A drop of fruit juice was placed on the screen, and the displayed value was recorded as % SSC.

Titratable acidity (TA): A 1 ml sample of fruit juice was titrated with 0.1 N NaOH solution until the pH reached 8.1. The acidity was expressed as a percentage of malic acid (Cemeroğlu 2007).

Individual Sugar and Organic Acid Extraction and Determination

Sugars and organic acids were extracted individually following the procedure described by Mikulic-Petkovsek et al. (2016). Homogenized, unfrozen fruit samples were placed in a test tube containing 4 mL of bi-distilled water, at 1 g. The mixture was continuously mixed on a vortex mixer at room temperature for thirty minutes. After extraction, the samples were centrifuged at 9000 x g for ten minutes at 4°C to obtain the supernatant and then filtered into vials using cellulose membranes. The extracts were stored at -20°C for further investigation. The high-performance liquid chromatography (HPLC) system was equipped with a refractive index detector (Shimadzu RID 20A VP, Kyoto, Japan), an in-line degasser, a pump, a controller, and a 20 µL injection volume automatic injector. Chromatographic separation was performed using a reverse-phase Ultrasphere Coregel-87 C analytical column (300 mm × 7.8 mm i.d., 5 µm, Transgenomic) at a flow rate of 0.6 mL/min, heated to 70 °C, and with the mobile phase being ultrapure water. Data acquisition and processing were performed with Shimadzu Class VP chromatography management software (Kyoto, Japan). Concentrations of individual sugars are reported as percentages of fresh weight (FW) determined using original standards.

Volatile compounds were extracted by solid-phase microextraction (SPME)

Volatile compounds obtained from permisson fruit were studied using three randomly selected fruits at each stage, both unripe and commercially ripe. For each case, 1 gram of homogenized seed tissue was placed in a headspace vial, and then 1 mL of $CaCl_2$ was added. Following a 30-minute 40 °C treatment, the volatile components were extracted using a solid-phase microextraction (SPME) fiber coated with CAR/PDMS (blue fiber). Using the method detailed by Polat and colleagues (2022), the obtained aroma compounds were then analyzed and quantified using a Shimadzu GC-2010 Plus gas chromatography-mass spectrometry (GC/MS) system.).

Statistical Analyses

The experiment was performed with a completely randomised experimental block design in three replications two stages (unripe, ripe) in each replicate. Data were processed with the SPSS package

program version 23.0 (SPSS Inc., Chicago, IL, USA). All data were presented as the mean ± standard error (SE) and analyzed by one-way analysis of variance (ANOVA). (Steel, et al 1997).

RESULT AND DISCUSSION

Sugars are also related to the total soluble solids (TSS) content. In persimmon, this characteristic ranges from 12 to 22 °Brix, due to the great differences between cultivars, maturation, and postharvest treatments, which influence both the content and the profile of these compounds (Kluge et al., 2018). It is important to highlight that the presence of soluble tannins also influence the TSS content. Kim and Ko (1997) observed that astringent fruits with higher levels of soluble tannins presented more TSS than non-astringent cultivars did. When these soluble tannins are no longer present (after a precipitation treatment), the TSS content is affected and there is no clear difference between astringent and non-astringent cultivar types. Therefore, Tessmer et al (2016) suggest that TSS measurement should be used to identify the sugars level only in non-astringent cultivars.

At the ripe stage, the TSS (Total Soluble Solids) value was 19.4% and the titratable acidity was 1.14%, whereas at the unripe stage, the TSS value was 14.45% and the titratable acidity was 0.86% (Table 1; Figure 1). This characteristic positively reflects on the storage period by prolonging the fruit's shelf life. Accordingly, for fruits intended to be stored for longer durations, early harvests, especially those performed in November, are expected to provide significant advantages in terms of storage longevity. When previous studies are examined, it is evident that total soluble solids content (TSS) and titratable acidity (TA) values vary significantly depending on harvest time, cultivar, and ecological conditions. Özkan and Can (2013), in their study on the 'Hachiya' cultivar in Alaşehir (Manisa), reported TSS values of 14.47%, 18.06%, and 20.80% at the first, fourth, and sixth harvests, respectively. In the same study, TA values were determined as 0.19%, 0.16%, and 0.10% at the corresponding harvest times. Similarly, Kaynaş and Kuzucu (2004) reported SSC values of 23.84%, 23.65%, 22.65%, 22.75%, and 22.46% in persimmons harvested at five different times in the Çanakkale region. Yılmaz and Şeker (2024) found that SSC values ranged between 13.62% and 17.67%, being directly associated with harvest time. They noted that cultivars with astringent flesh generally had higher SSC values, such as 17.67% in 'Hachiya' and 15.72% in 'Rojo Brillante'. According to Mowat and Chee (2011), persimmons intended for export in New Zealand must meet industry class standards of an average SSC of 14% at harvest, with a minimum of 12%. In another study, Kılıç et al., (2022) reported SSC values between 18.05% and 20.63% in 'Hachiya', 'Rojo Brillante', 'Hana Fuyu', and 'Fuyu' cultivars grown in Denizli ecological conditions. TA values in the same study were determined to range between 0.14% and 0.28%. Üstün et al., (2017) also investigated different persimmon types and reported SSC values of 14.0–18.9%, total sugars of 12.3–17.1 mg/100 g, reducing sugars of 10.3–16.5 mg/100 g, sucrose of 0.38–1.90 mg/100 g, TA of 0.06–0.14%, and L-ascorbic acid content of 6.8–19.65 mg/100 g. In the study, the SSC was determined as 19.4% and the TA as 1.14% at the ripe stage, whereas at the unripe stage, the SSC was 14.45% and the TA was 0.86%. These findings are consistent with the results reported by Özkan and Can (2013), Yılmaz and Şeker (2013), Kaynaş and Kuzucu (2004), and Kılıç et al., (2022).

Table 1. Contents total soluble solids (TSS) and titratable acidity (TA) of persimmon fruit

	ripe persimmon	**unripe persimmon**
Total soluble solids	19.4±0.10	14.45 ±0.05
Titratable acidity	1.14±0.04	0.86±0.01

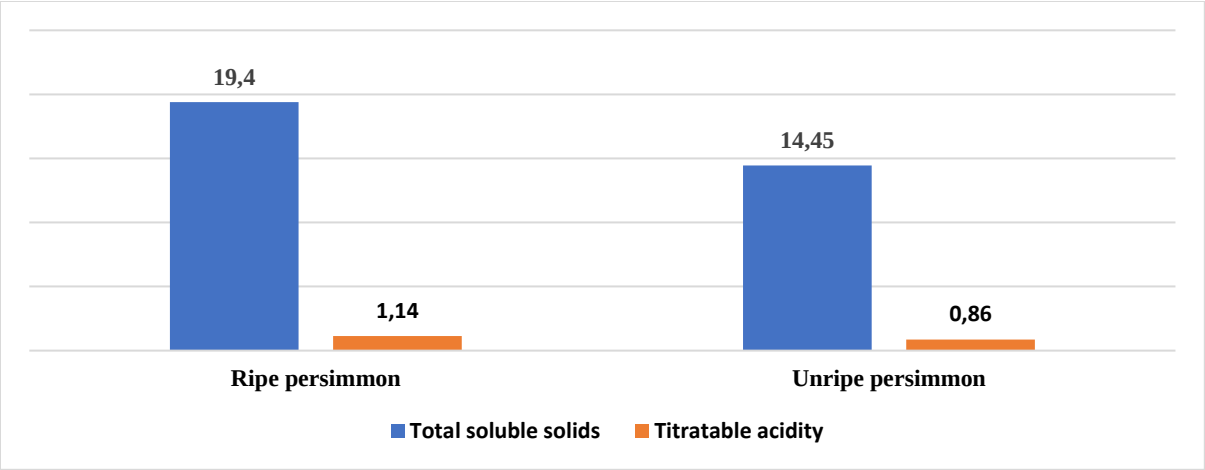

Figure 1. Contents total soluble solids (TSS) and titratable acidity (TA) of persimmon fruit

Sugars

In study, four sugars glucose, fructose, sucrose, and xylose were analyzed and measured for each cultivar (Table 2). Glucose was identified as the predominant sugar, followed by fructose, sucrose, and xylose. As the fruit ripened, starch was enzymatically hydrolyzed into simple sugars such as glucose and fructose, leading to a noticeable increase in sugar content (Yılmaz, and Bostan 2024).

At the ripe stage, the highest level was observed in glucose (95.67 ± 0.15 mg/100 g), followed by fructose (91.66 ± 3.33 mg/100 g), sucrose (89.30 ± 2.30 mg/100 g), and xylose (55.40 ± 8.35 mg/100 g). The total sugar content at this stage was determined as 182.48 ± 7.17 mg/100 g.

At the unripe stage, glucose again showed the highest concentration (72.13 ± 0.08 mg/100 g), followed by fructose (53.42 ± 18.69 mg/100 g), sucrose (65.61 ± 0.76 mg/100 g), and xylose (38.96 ± 7.61 mg/100 g). The total sugar content in the unripe stage was found to be 138.67 ± 27.13 mg/100 g (Table 2;Figure 2). The results of the study suggest that when determining the harvest date of persimmon, it is important to consider when the fruit will be marketed after harvest. If the harvested fruits are intended for storage, the harvest should be carried out during the early stages of fruit development, which corresponds to the second week of November for the Toros region. If the fruits are to be marketed without storage, the harvest should be delayed to later stages; however, in this case, as the fruit approaches the end of the ripening process on the tree, it becomes more attractive in terms of color and taste, but also more susceptible to environmental factors, particularly bird damage. On the other hand, early autumn frosts that may occur in the region during this period can cause chilling injury in the fruits. Fruits harvested in the early stages of development (the first week of November) were found not to reach high-quality edible ripeness, and the astringency caused by tannins was not eliminated in fruits at the edible stage. Robert et al. (2010) reported glucose contents in persimmon ranging from 4.78 to 8.79 mg/100g, fructose from 3.80 to 7.78 mg/100g, and sucrose from 0.91 to 1.22 mg/100g. Similarly, Candir et al., (2009) found that, considering both individual and total sugar concentrations, the cultivar 'Tone Wase' had the highest glucose (8.79 mg/100g), fructose (7.78 mg/100g), and total sugar (17.8 mg/100g) contents. In a study on harvest maturity of persimmon, Gasanov et al., (1973) reported that sugar accumulation during ripening was higher in Khiakume and Tamopan cultivars compared to Tsuronoko and Zendzhimaru. They further noted that the total sugar content varied among cultivars depending on harvest stage, with the highest level found in Emon and the lowest in Tamopan. In the same study, tannin content was determined as 0.164 mg/100g in Khiakume and Zendzhimaru, and 0.083 mg/100g in Tsuronoko, Emon, and Tamopan cultivars. Glew

et al., (2005) demonstrated that the major sugars accumulated in persimmon juice were fructose and glucose, which increased up to 43.55 mg/100g FW and 35.45 mg/100g FW, respectively, during ripening. Sucrose remained relatively low and declined throughout ripening. These values were slightly lower than those reported by Daood et al. (1992) for an unidentified *D. kaki* L. cultivar, which contained higher glucose (11.0 mg/100g) and fructose (10.1 mg/100g) levels. The lowest total sugar content was measured in fully ripe 'O'Gosho', 'Cal Fuyu', and 'Hana Fuyu' cultivars, averaging 10.7 mg/100g. Sweetness is clearly one of the most important internal quality attributes and a key breeding objective in persimmon cultivation. Therefore, promoting cultivars with higher sugar contents is strongly emphasized. The total sugar content in persimmon is comparable to that in apples, which range between 11.5 and 18.3 mg/100g (Hofer et al., 2005). However, in apples, the predominant sugar is fructose, followed by glucose and sucrose (Veberic, Zadravec and Stampar, 2007). In persimmon cultivars with the highest total sugar concentrations, levels can reach 15–23 mg/100g, with glucose being the dominant sugar, followed by fructose and sucrose (Usenik, Fabcic and Stampar, 2008).

Table 2. Sugar content in the persimmon fruit (mg/100 g)

Sugars	Ripe persimmon	Unripe persimmon
Xylose	55.40±8.35	38.96±7.61
Sucrose	89.30±2.30	65.61±0.76
Glucose	95.67±0.15	72.13±0.08
Fruktose	91.66±3.33	53.42±18.69
Total Sugar	182.48±7.17	138.67±27.13

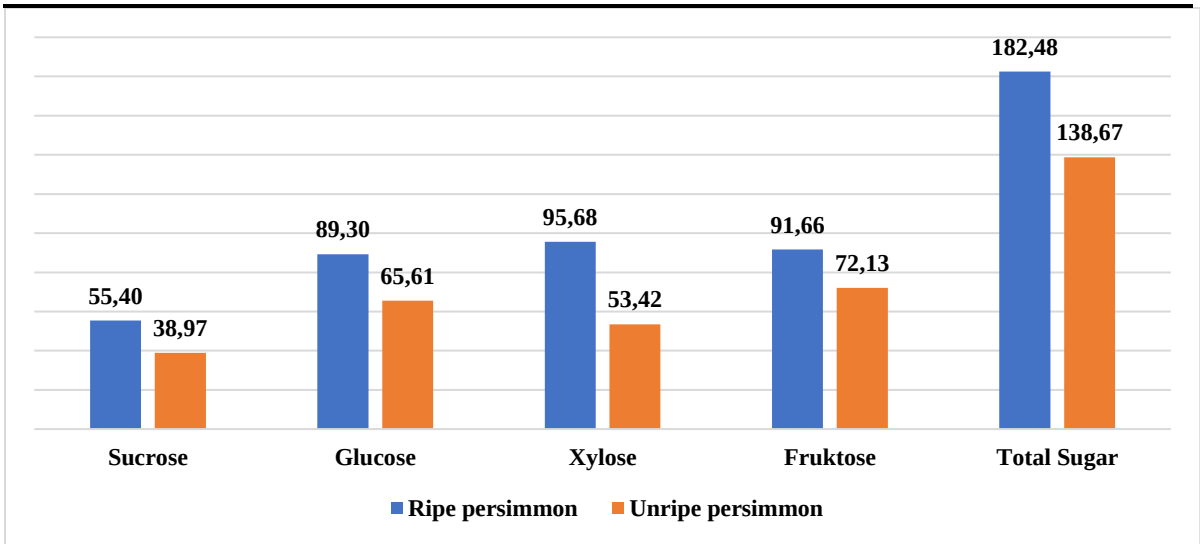

Figure 2. Sugar content in the persimmon fruit

The glucose and fructose levels determined in our study, when compared with the ranges reported by Robert et al., (2010) and Candır et al., (2009) (glucose: 4.78–8.79 mg/100g; fructose: 3.80–7.78 mg/100g), were found. This indicates that varietal/genotypic differences and ecological conditions play a decisive role in sugar accumulation. Our findings, compared with the 15–23 mg/100g levels reported by Gasanov et al., (1973) and Usenik et al., (2008), were at high levels. This result demonstrates that fruit ripening stage and growing conditions have a strong effect on total sugar content The sucrose levels obtained in our study were consistent with / deviated from the findings of Glew et al., (2005), who reported that sucrose remains relatively low and decreases during ripening.

Our findings show that, in terms of sweetness and internal quality characteristics, persimmon exhibits trends similar to those reported in the literature, although differences arise depending on cultivar, growing conditions, and ripening stage. It can be concluded that genotypes with higher sugar accumulation should be preferred in cultivation.

Organic acids

Organic acids play a critical role in defining fruit taste and regulating various physiological processes, with their composition largely dependent on the cultivar. The sugar–acid balance is one of the main determinants of flavor in fruits. In addition, organic acids contribute to nutrient digestion, stimulate blood circulation, and serve as important quality indicators. Their type and concentration vary among fruit species and generally decrease as ripening progresses (Kıralan and Gündoğdu, 2021).

In study, a natural decrease in organic acid content was observed in persimmon cultivars as ripening advanced. The contents of L-ascorbic acid, citric acid, and malic acid were determined in the cultivars, and the results are summarized in Table 5. Among these, succinic acid and malic acid were identified as the predominant organic acids**.**

The sweet taste of a fruit is important for its quality and sensorial acceptance, and both primary metabolites, sugars and organic acids, play an important role. It is estimated that a higher proportion between sugars and organic acids leads to a better perception of sweet taste. (Veberic et al., 2009; Santos et al., 2017). Malic acid exerts an important influence on the sensory characteristics of persimmon (Kluge,et al 2018) and, as identified by some authors, it is one of the main organic acids in this fruit, followed by citric acid and fumaric acids (Veberic et al., 2009; Santos et al., 2017; Ryu et al., 2016).

In ripe fruits, malic acid 18.23 ± 0.50 mg/100 g FW, citric acid 11.29 ± 0.77 mg/100 g FW, and L-ascorbic acid 8.09 ± 0.45 mg/100 g FW. The total organic acid content was determined as 37.62±1.42 mg/100 g (Table 3;Figure 3). In unripe fruits, higher values were recorded: citric acid 10.02 ± 0.16 mg/100 g FW, malic acid 22.43 ± 0.79 mg/100 g FW, and L-ascorbic acid 6.94 ± 0.67 mg/100 g FW (Table 3;Figure 3). The total organic acid content of unripe fruits was calculated as 39.40±0.54 mg/100 g. The observed reduction in total acidity during fruit ripening reflects the typical metabolic changes associated with maturation (Patel et al., 2019).

Robert et al., (2010) reported that the total organic acid content was 68.1 mg/100g in the cultivar 'Jiro', while in the cultivars 'Triumph' and 'Tipo' it was more than twice as high, averaging 14.39 mg/100 g of organic acids. Among the organic acids analyzed, the highest malic acid content was measured in the cultivar 'Triumph' (10.44 mg/100 g FW), whereas the lowest value was found in 'Jiro' (4.01 mg/100 g FW). The malic acid levels in all analyzed cultivars were considerably lower than those reported by Daood et al., (1992), who measured 35.45 mg/100 g FW in ripe fruit of an unidentified persimmon cultivar. Compared to sweet cherry, which contains 35.30–81.20 mg/100 g FW of malic acid (Usenik et al., 2008), the fully ripe fruits of the analyzed persimmon cultivars had much lower malic acid levels. The cultivars contained between 1.96 and 7.01 mg/100 g FW of citric acid, values comparable to those obtained by Daood et al., (1992), who reported 6.57 mg/100 g citric acid in ripe persimmon fruit. In comparison with apple, which contains between 5.20 and 70 mg/100 g FW of citric acid, persimmon is lower in citric acid content. Glew et al., (2005) demonstrated that the major organic acids in date plum fruit were citric and malic acids, which increased during the immature and mid-ripe stages and then decreased in overripe fruit. According to Usenik et al., (2008), ripe persimmon fruit is also rich in these organic acids. However, compared with elderberry, which contains on average 30.80–48.10 mg/100 g of citric acid (Veberic, Jakopic, Stampar and Schmitzer, 2008), persimmon has a significantly lower citric acid content. In other tropical fruits, such as papaya and pineapple, the concentration of citric acid in ripe fruit is much higher (Hernández, Lobo, &

González, 2008). This result is consistent with the low organic acid contents in persimmon reported by Robert et al., (2010) and Daood et al., (1992).

Table 3. Organic acids content in the persimmon fruit (mg/100 g)

	Ripe persimmon	**Unripe persimmon**
Citric Acid	11.29±0.77	10.02±0.16
Malic Acid	18.23±0.50	22.43±0.79
Ascorbic Acid	8.09±0.45	6.94±0.67
Total acid	37.62±1.42	39.40±0.54

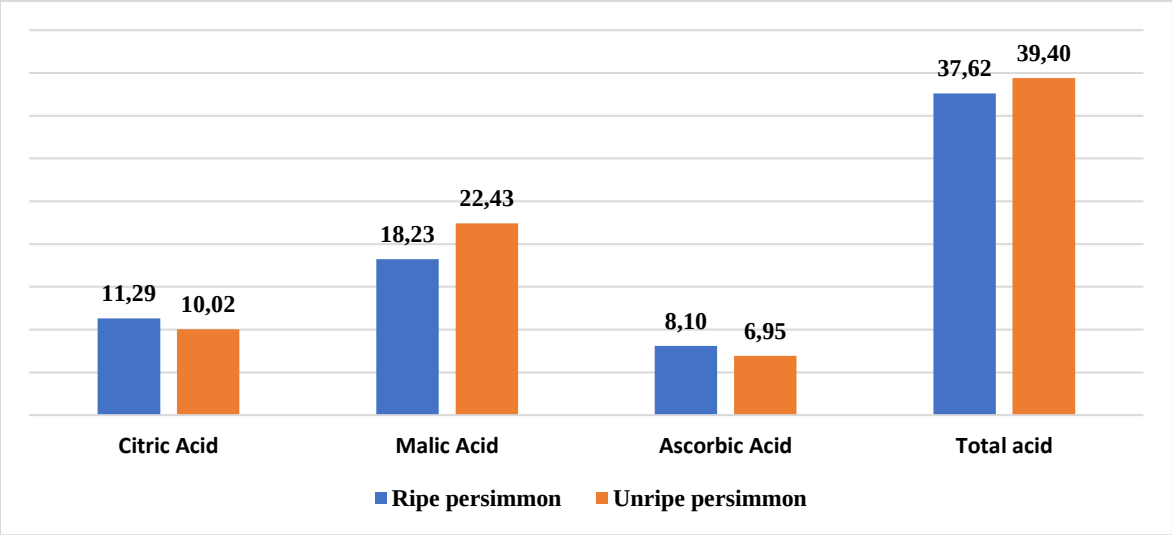

Figure 3. Organic acids content in the persimmon fruit

Volatile Compounds

The volatile compounds identified in persimmon fruits are summarized in Figure 1. Using blue SPME, at the ripe stage the main volatile groups detected were total terpenes (10.39%), total esters (9.37%), total aldehydes (37.76%), total acids (37.08%), total ketones (2.32%), and total alcohols (3.38%)(Figure 4). A total of 31 individual components were identified in the juice, comprising four alcohols, nine aldehydes, one ester, nine acids, three ketones, and five terpenes. Among the extraction methods tested, blue SPME proved to be the most suitable for isolating volatile compounds from persimmon juice.

Aldehydes and acids were found to be the predominant volatile groups in persimmon fruits (Table 4). Key aldehydes detected included hexanal (25.16%), (E)-2-hexenal (2.69%), and trans-2,4-dodecadienal (2.63%), with notably higher levels in ripe fruits. Among acids, acetic acid (22.82%), nonanoic acid (2.62%), and hexanoic acid (2.07%) were the most abundant, also with higher levels in ripe fruits. Within the alcohol group, menthol (1.27%) and 1-octanol (0.82%) were prominent, particularly in ripe fruits. Esters such as 1,2-benzenedicarboxylic acid, diethyl ester (7.26%) were identified as important contributors to aroma and were highly abundant in ripe fruits. Esters are known to impart fruity and floral notes that play a critical role in cultivar differentiation. Regarding terpenes, benzene (isothiocyanatomethyl), benzene, 1-methyl-2-(1-methylethyl)-, and naphthalene, 1,6-

dimethyl-4-(1-methylethyl)- were the major compounds identified. Among them, benzene (isothiocyanatomethyl) was particularly abundant (6.52%) in ripe fruits (Table 4).

At the unripe stage, the main volatile groups detected were total terpenes (44.98%), total esters (1.39%), total aldehydes (10.23%), total acids (17.56%), total ketones (8.40%), and total alcohols (17.44%). A total of 27 individual compounds were identified in the juice, consisting of five alcohols, seven aldehydes, two esters, eight acids, three ketones, and four terpenes. Aldehydes and acids were again found to be the predominant volatile groups in persimmon fruits (Table 3). The key aldehydes detected included hydroxymethyl furfural (6.10%) and 2-furancarboxaldehyde (1.84%), both showing notably higher levels in unripe fruits. Among acids, acetic acid (5.29%), (E)-9-octadecenoic acid (5.10%), and pentadecanoic acid (4.81%) were prominent, also with higher abundance at the unripe stage. Within the alcohol group, linalool (7.10%), geraniol (3.76%), and nerol (3.48%) were prominent, particularly in ripe fruits. The major ketone identified was 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (8.40%). Regarding terpenes, (+)-limonene (42.93%) was the dominant compound identified in unripe persimmon fruits. In the study, it was determined that with increasing ripeness, the amounts of aldehydes, esters, and acids increased, while the amounts of alcohols, ketones, and terpenes decreased. The results of the study revealed that the main factor responsible for changes in the aroma compounds of persimmon fruit was the progression of ripening over time. As the maturity index increased, the composition of aroma compounds also changed. Terpenes were found to increase up to a certain stage of ripening and then decrease in the later stages, with a higher number of terpene compounds identified in the unripe stage. Limonene was identified as the common terpene component in both cultivars.

In persimmon, the total alcohol content within the volatile compound profile decreased during ripening, whereas the proportion of ester compounds increased. In contrast, ketone levels showed a decreasing trend as ripening progressed. As fruits ripen, the quantity and quality of volatile compounds responsible for aroma increase, reaching their maximum at full maturity. The synthesis of aroma compounds is influenced not only by the genetic makeup but also by ecological factors, rootstock, cultivar, altitude, cumulative temperature during the growing season, ripening stage, and storage conditions. The source of aroma compounds largely comes from sugars, proteins, and fats metabolized during ripening. Therefore, aroma formation during ripening occurs not only through enzymatic synthesis but also through the breakdown and transformation of these metabolites. In other words, as the fruit ripens, volatile aroma compounds are primarily produced via metabolic degradation processes and enzyme activity (Perez et al., 1996; Kaynaş et al., 2022).

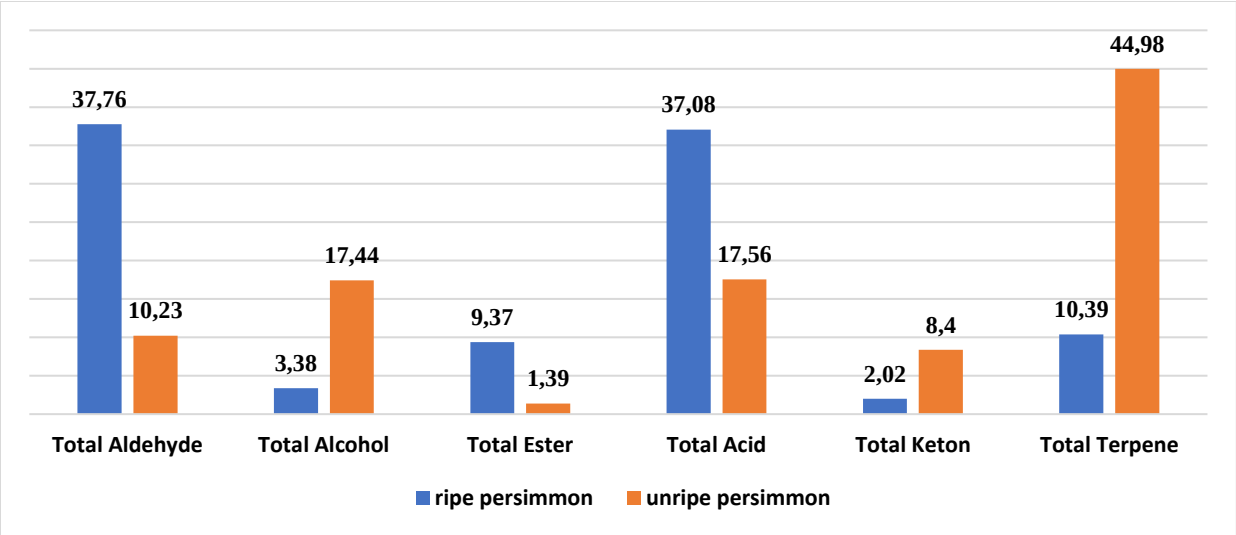

Figure 4. Volatile composition in the persimmon fruit (%)

Table 4. Volatile composition in the persimmon fruit (%)

R.time	Compounds	ripe persimmon	unripe persimmon
	Aldehyde(-al)		
3.061	Hexanal (CAS)	25.16	
5.015	Heptanal (CAS)	0.49	
5.704	2-Hexenal, (E)- (CAS)	2.69	
7.435	Octanal (CAS)	1.19	
10.057	Nonanal (CAS)	1.59	0.5
13.2	Benzaldehyde (CAS)	1.6	
16.091	2-Decenal, (E)- (CAS)	1.39	
18.586	Trans-2-dodecenal	1.02	
26.187	Trans, trans-2,4-dodecadıenal	2.63	
10.735	2-Furancarboxaldehyde (CAS)		1.84
19.286	Hydroxy methyl furfural		6.1
19.649	Neral		0.32
19.974	Dec-2(E)-enal		0.43
20.161	Citral		0.53
21.784	Undec-8-enal <cis->		0.51
	Total	**37.76**	**10.23**
	Alcohol(-ol)		
10.429	2H-Pyranmethanol, tetrahydro-2,5-dimethyl- (CAS)	0.54	
14.342	1-Octanol (CAS)	0.82	0.97
16.145	Menthol	1.27	
30.271	Phenol, 2,4-bıs(1,1-dımethylethyl)-	0.75	
16.8	Linalool		7.14
19.368	Nerol (CAS)		3.48
19.802	Geraniol		3.79
20.478	Phenol, 5-methyl-2-(1-methylethyl)- (CAS)		2.06
	Total Alcohol	**3.38**	**17.44**

	Ester(-ate)		
1.275	Acetic acid, ethyl ester (CAS)	2.11	
30.912	1,2-Benzenedicarboxylic acid, diethyl ester (CAS)	7.26	

Table 4. Volatile composition in the persimmon fruit (%)

R.time	Compounds	ripe persimmon	unripe persimmon
	Ester(-ate)		
16.568	2-Furancarboxylic acid, methyl ester (CAS)		0.87
27.531	Tricosyl heptafluorobutyrate		0.52
	Total Ester	**9.37**	**1.39**
	Acid		
11.971	Acetic acid (CAS)	28.82	5.29
16.26	Butanoic acid (CAS)	0.49	
21.116	Hexanoic acid (CAS)	2.07	0.37
25.494	Octanoic acid (CAS)	1.12	0.57
27.535	Nonanoic acid (CAS)	2.62	
32.158	Benzoic acid (CAS)	0.4	
5.181	Formic acid	1.14	
21.554	Decanoic acid (CAS)	0.42	0.42
24.765	Dodecanoic acid (CAS)		0.38
30.113	(E)-9-Octadecenoic acid,		5.1
30.333	Pentadecanoic acid (CAS)		4.81
33.413	Octadecanoıc acıd		0.62
	Total Acid	**37.08**	**17.56**
	Keton(-one)		
3.902	2-Propanone, 1-methoxy	0.46	
7.834	4-Butoxy-2-butanone	1.56	
7.078	2-Propanone, 1-hydroxy- (CAS)	2.48	
17.822	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one		8.4
	Total Keton	**2.02**	**8.4**
	Terpene (-ene)		
5.135	(+)-LIMONEN	0.45	42.93
6.859	Benzene, 1-methyl-2-(1-methylethyl)- (CAS)	1.34	
25.849	Benzene, (isothiocyanatomethyl)-	6.52	0.55
27.991	Naphthalene, 1,6-dimethyl-4-(1-methylethyl)- (CAS)	1.29	
14.455	beta.-Myrcene	0.79	0.78
16.038	gamma.-Terpinene		0.72
	Total Terpene	**10.39**	**44.98**

Besada et al., (2013) reported a high-resolution volatile profiling of fruits from 12 persimmon cultivars, identifying 67 compounds. This profiling was used to characterize the different astringency types of persimmon fruit before and after deastringency treatment. Fruits that do not naturally accumulate astringent compounds (PCNA type) exhibited high levels of 3(2H)-benzofuranone, whereas this compound was not detected in any astringent-type fruits (non-PCNA). In addition, at harvest, PCNA cultivars showed higher accumulation of benzeneacetaldehyde and lipid-derived aldehydes (hexanal, heptanal, octanal, and decanal) compared to non-PCNA fruits. Wang et al., (2012)

reported that gas chromatography–mass spectrometry (GC-MS) analysis of the extract led to the positive identification of 50 compounds, among which aldehydes emerged as the most important class of volatile compounds. Gas chromatography–olfactometry (GC-O) analysis of the aroma extract revealed that 32 compounds had an aroma impact. The six most intense aroma-impact compounds were methional, (E)-2-Hexenal, Phenylacetaldehyde, (E,Z)-2,6-Nonadienal, Hexanal, and Furaneol. The aroma qualities of these compounds largely explain the aroma profile of persimmon fruit, which was described as fresh peach, a touch of orange, sweet, woody, floral, green, and potato-like. The unambiguous identification of trace aroma-impact compounds – (E,E,Z)-2,4,6-Nonatrienal and (E,Z,Z)-2,4,7-Decatrienal – was achieved by applying a solid-phase extraction procedure for the selective isolation of carbonyl compounds using GC-MS.

CONCLUSION

The study observed a significant increase in TSS values and a decrease in titratable acidity (TEA) throughout the ripening process. Harvesting: For optimum quality, high TSS (18–20%) and low TEA (0.1–0.2%) values should be targeted at harvest. Therefore, producers are advised to pay attention not only to rind color but also to the sugar/acid balance. In terms of sugar composition, glucose and fructose levels increase with ripening, while sucrose levels gradually decrease. This can be explained by the high activity of the invertase enzyme (Bubba et al., 2009; Santos et al., 2019). Sugar-acid balance: Sweetness (high glucose + fructose) and low acidity (reduction of malic and citric acid) play important roles in consumer taste. Therefore, post-harvest ripening methods (CO_2, ethylene, limewater) should be used to improve flavor balance. In terms of organic acids, it has been observed that malic and citric acid levels gradually decrease during the ripening process, resulting in a decrease in acidity and an increase in sweetness perception. In terms of volatile compounds, aroma compounds, particularly aldehydes, esters, and furaneol, have been found to vary with ripening and contribute to the fruit's characteristic aroma (Wang et al., 2012; Besada et al., 2013). Storage conditions: Because persimmon is climacteric, low-temperature and controlled-atmosphere storage conditions are important for maintaining the sugar-acid balance in the fruit and preventing aroma loss. The Hachiya variety, with its high sugar and aroma profile, can be utilized in drying, juice, and functional food products to create value-added products. The TSS, TA individual sugars, organic acids, and aroma values obtained from the analyses were found to be at sufficient levels in the final harvest period in terms of fruit edibility. Detailed examination of sugar and organic acid metabolism in different genotypes, correlation with consumer taste, and monitoring of changes during storage are recommended.

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PRESENCE OF NOSEMOSIS IN HONEY BEES IN ISPARTA PROVINCE, TÜRKİYE

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ABSTRACT

Isparta province is a province where both continental and Mediterranean climates are experienced together, as it is located in the continental interior of the Mediterranean Region. So, winters are cold and summers are hot and dry. Geographically located in the lake region, this province has both mountains and flat areas. It is known that geographical conditions affect the spread of bee diseases and increase the incidence of bee losses. For this reason, researching beekeeping and bee diseases in Isparta province is very important. In this study, the first findings on the presence of Nosemosis in bee colonies in Isparta province are presented. Nosemosis was detected in two of the examined beehives. Five of the 194 live bee samples and four of the 180 dead bee samples were infected with nosema agents. The infection rate varies between 11.4% and 3.8%. The infection average is 2.4%. However, the number of sampled beehives needs to be increased to represent the entire Isparta region, other pathogens and parasites need to be investigated and each needs to be identified at the species level.

Keywords: Honey bee, Disease, Nosemosis, Isparta, Türkiye

INTRODUCTION

Beekeeping is rapidly increasing worldwide due to the diversity of bee product production. Türkiye has an important potential in beekeeping in terms of its rich plant diversity and suitable ecology. However, this wealth of natural resources is not being utilized sufficiently in Türkiye. The Aegean, Black Sea and Mediterranean regions of Türkiye are the most important regions for beekeeping in terms of both the number of hives and the share of production (Yıldız and Fakir 2019). Isparta province is located in the continental interior of the Mediterranean Region and experiences both continental and Mediterranean climates. So, winters are cold and summers are hot and dry. Geographically located in the lake region, this province has both mountains and flat areas. In addition, most of the beekeepers in Isparta province practice migratory beekeeping. In this case, it further increases the spread of bee diseases, causes stress in bees and increases the incidence of bee losses (Ratia, 2012). Additionally, Isparta has 36,924 colonies, 256 tons of honey production, and 6.92 kg/colony honey yield, ranking 60th in Türkiye's honey production (TÜİK, 2025). For this reason, researching beekeeping and bee diseases in Isparta province is very important. In this study, it was aimed to present the first findings to determine the current status of Nosemosis presence in bee colonies in Isparta province.

MATERIAL AND METHOD

Bee samples were collected from 5 apiaries in Isparta region. Total 374 adult bee samples, 180 dead and 194 living bees were provided in April and May 2025. After macroscopic examination, dead and living adult bees were dissected in Ringer's solution and wet smears were prepared. Host fat body, malpighian tubules, gut epithelium, and hemolymph were examined for the presence of pathogens under a light microscope at × 400–1000 magnification (Yaman et al. 2019). When an infection with the pathogen was observed, a part of the material was used for the preparation of Giemsa-stained smears. For this, the slides were air-dried and fixed with methanol, then stained with freshly prepared 5% solution of Giemsa stain and reexamined under the microscope by using the oil immersion lens. The spores detected by the light microscopy were measured andphotographed using a microscope with a digital camera and Soft Imaging System (Yaman and Sağlam 2023; Yaman et al. 2023).

RESULTS AND DISCUSSION

In this study, the first findings were presented to determine the presence of Nosemosis, the most important harmful disease of honey bees, in bee colonies in Isparta province.

Spore of the pathogen is the most characteristic stage in the examined bees. Light microscopic examinations of the living and dead bees revealed the presence of the pathogen spores in the intestine and the body cavity. Signs of infection spores were confirmed using the Giemsa staining technique (Figure 1). Mature fresh and Giemsa stained spores were measured. Mature fresh spores measured 4.46 ± 0.22 x 2.53 ± 0.21 (n=25) and Giemsa stained spores 4.12 ± 0.49 x 2.33 ± 0.21 µm (n=25).

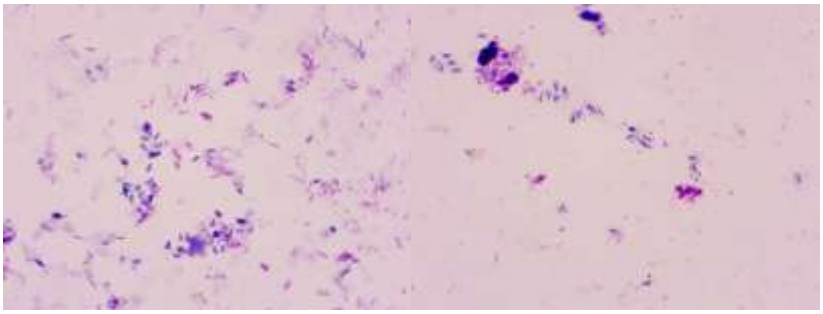

Figure 1. *Nosema* spp. Giemsa stained spores (1000X).

Nosemosis was observed in 9 of 374 adult worker bees examined in Isparta province, and the average infection rate was determined as 2.4% (Table 1). During the study, the disease was observed in two (3.15%) of the examined five localities in Isparta province. The highest nosemosis was determined in Central 5.6% rates respectively. Five of the 194 live bee samples examined and four of the 180 dead bee samples examined were infected with nosema agents. The infection rate ranged from 11.4% to 3.8%. Nosemosis detected in the city center was detected in a bee sample taken from a migratory beekeeper, while Nosemosis detected in the Gelendost district was detected in a bee sample taken from a stationary beekeeper.

Table 1. Table 1. Occurrence of Nosemosis in Isparta province, Türkiye.

Location	Living bee samples	Nosema	%	Dead bee samples	Nosema	%	Total
Centre	35	4	11,4	107	4	3,8	5.6
Isparta/ISUBÜ West Campus-1	33	-	0	8	-	0	-
Isparta/ISUBÜ West Campus-2	44	-	0	27	-	0	-
Aşağıgökdere	58	-	0	18	-	0	-
Gelendost	24	1	4,2	20	-	0	2.3
Total	194	5	2,6	180	4	2,2	2.4

In Isparta, where migratory beekeeping is practiced by many beekeepers, the number of permanent beekeepers is quite low. Many migratory beekeepers relocate their colonies more than twice a year. However, it is also a known fact that relocating colonies multiple times can cause stress in honeybees (UNEP, 2010). Moving colonies between locations with different climates and geographic structures does not directly cause bee mortality. However, by creating stress in bees, it triggers other factors that cause bee mortality. According to Ratia (2012), many factors influence bee losses, and stress related to migratory beekeeping is considered one of these factors (Özgür, 2012). In addition, the increase in Nosema disease in the country and around the world should be considered as infected beekeeping materials (Klee et al., 2007), the inclusion of carrier colonies and queen bees, and migratory beekeeping activities (Giersch et al., 2009; Whitaker et al., 2010; Büyük et al., 2014)

Nosemosis, one of the most common diseases seen in honeybees in Turkey and the world, is a fatal disease that causes serious decreases in honey yield, damages the country's economy, and causes colony losses (Yaman et al., 2015; Sinpoo et al., 2018; Tel et al. 2021).

Numerous studies have been conducted to identify nosemosis in Turkey, and its presence has been detected in various provinces (Kutlu, 1988; Aydın, 2006; Ütük et al. 2010; Yaman et al. 2015; Ütük et al. 2016; Yaman and Sağlam 2023; Yaman et al. 2023). To determine the prevalence of nosemosis among honeybees in the Kars region, 343 honeybees were examined, and 54 were determined to be infected with nosemosis (Topçu and Özkan Arslan 2004). In a study examining the presence, distribution, and pathogens of nosemosis in the Ordu region, the disease was detected in all 10 localities sampled. The infection rate was determined to range from 25 to 85% (Yaman et al. 2015). Zerek et al. (2020) examined adult bee samples from 450 hives in Hatay province for nosemosis. They

reported that 20% of the hives were infected with Nosema. Yaman and Sağlam (2023) examined the presence of nosemosis in bee colonies in Bolu province. The study found that 190 of the 3030 bee samples examined were infected with Nosema spp., and the average nosemosis rate was 6.3%.

According to the literature review, there is no other study in Isparta province, except for the master's thesis by Özgür (2012). This preliminary study, conducted by us based on current information, is of great importance for the current status of beekeeping in Isparta province. The current status of nosemosis, which has caused colony losses worldwide in recent years and has been detected in Isparta province, will be determined by molecular and microscopic identification and by investigating its distribution and density in Isparta, to determine whether it is caused by *Nosema apis* or *N. ceranae*.

CONCLUSIONS

The present study determined that bee colonies in Isparta were infected with *Nosema* spp. Also demonstrates that beekeepers in the Isparta region should be comprehensively informed about the necessary measures for monitoring and control of nosemosis.

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EFFECTS OF CHITOSAN COATED IRON OXIDE AND SILVER NANOPARTICLES ON GROWTH AND BIOCHEMICAL CHARACTERISTICS OF SNAPDRAGON (ANTIRRHINUM MAJUS)

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ABSTRACT

Snapdragon (*Antirrhinum majus*) with a colorful flowers and aesthetic appeal, is a perennial plant belonging to the *Scrophulariaceae* family. Factorial arrangement based on a completely randomized design with four replications were conducted to investigate the effects of chitosan coated iron oxide nanoparticles (chitosan coated IONPs) (0, 50, 100, and 150 µM) and silver nanoparticles (AgNPs) (0, 50, 100, and 150 mg/L-1) on morphological and biochemical traits of the ornamental Snapdragon plant under greenhouse conditions. Measured indices included plant height, Branches Number, flower number and diameter, stomatal conductivity, total phenolic content, and chlorophyll fluorescence indices ($Fv/F_o$and Fm). The results indicated that the interaction effects of chitosan coated IONPs and AgNPs, especially at the concentrations of (100–150 µM) and (100–150 mg/L-1) mg/L respectively, significantly improved vegetative growth, flower production, and physiological indices. Increases in $Fv/F_o$and Fm enhanced photosynthetic efficiency and overall plant growth. Additionally, phenolic content was increased under AgNP treatments by activating the antioxidant defense system. These findings revealed that individual and combined use of nanoparticles can enhance Snapdragon performance, with potential applications in sustainable and high-quality ornamental plant production. Standardized testing protocols and regulatory oversight further complicates the evaluation of risks versus benefits.

Key words: Chlorophyll fluorescence, Nanoparticles, Snapdragon, Stomatal conductivity

INTRODUCTION

Snapdragon (*Antirrhinum majus*) with a colorful flowers and aesthetic appeal, is an important ornamental perennial plant belonging to the *Scrophulariaceae* family and native to the Mediterranean region. This plant cultivated as an annual in cold climates and as a biennial in temperate areas. In recent decades, nanotechnology has emerged as a revolutionary scientific advancement, opening new avenues for agriculture and sustainable resource management. Nanoparticles, due to their high surface area and unique physicochemical properties, have become promising tools for improving plant growth, pest control, and abiotic stress resistance. Among these nanoparticles, iron oxide (IONPs) and silver nanoparticles (AgNPs) have attracted attention due to their nutrient delivery capabilities and strong antimicrobial properties, respectively. Their application as bio-pesticides, protective agents, antimicrobials, and nutritional supplements has revolutionized agricultural practices. Studies showed that AgNPs can enhance plant growth parameters. In *Melissa officinalis*, significant increases in root and shoot biomass and length were observed under AgNP treatment, with maximum anthocyanin content at 60 ppm, attributed to time-dependent activation of antioxidant enzymes (Shahvali Bar &

Esmaeilzadeh Bahabadi, 2019). Other studies have shown that low concentrations of AgNPs can strengthen secondary metabolite production, such as flavonoids, thereby improving antioxidant capacity in plants (Jadczak et al., 2020; Liu et al., 2020). Chitosan, a natural polymer derived from chitin, not only stabilizes nanoparticles but also reduces their toxicity, enabling safer use in biological systems. On the other hand, chitosan-coated iron oxide nanoparticles is a biocompatible and provide stability against aggregation, making them suitable for managing environmental stress. In *Mentha piperita* under drought stress, chitosan coated IONPsNPs improved chlorophyll fluorescence and photosynthetic indices (Torabi Giglou et al., 2022). This study investigates the synthesis, action mechanisms, and growth-promoting potential of IONPs and AgNPs nanoparticles in Snapdragon. The findings suggest that combining nanoparticles with chitosan coating may offer a novel strategy to enhance Snapdragon growth, stress tolerance, and ornamental or medicinal value

MATERIAL AND METHOD

Snapdragon seeds were prepared and cultivated under greenhouse conditions at Mohaghegh Ardabili University. Treatments included two nanoparticle types: AgNPs and chitosan coated IONPs, each at four concentrations: 0 (control), 50, 100, and 150 (mg/L-1 for AgNPs and µM for chitosan coated IONPs). Treatments were applied via foliar spraying at three growth stages. Seeds were first sown in trays and then transplanted to pots containing a 1:1:1 mixture of organic matter, sand, and garden soil. The experiment carried out in a completely randomized factorial design with four replications. The measured morphological traits were plant height (±1 mm), branch and flower number, and flower diameter (±0.1 mm). For biochemical traits, total phenol, Chlorophyll fluorescence (Fv/F0 and Fm), and Stomatal conductivity were measured. Data were analyzed using SAS 9.1 software. Means were compared using Duncan's multiple range test, and graphs were prepared using Excel.

RESULTS AND DISCUSSION

Analysis of variance showed that both individual and interactive effects of chitosan coated IONPs and AgNPs significantly ($p < 0.01$) affected the plant height, branch and flower number, and flower diameter (Table 1). Biochemical indices including Fv/F, Fm, stomatal conductivity, and total phenol content also significantly affected by chitosan coated IONPs and AgNPs (Table 2).

Table 1. Variance Analysis of the Effects of chitosan Coated IONPs and AgNPs on Morphological Traits of Snapdragon
*, ** and significant at the %5 and %1 and not significant probability levels, respectively.

Sources of Variation	DF	Plant Height	Branches Number	Flowers Number	Flower diameter
Chitosan coated IONPs	3	45.92**	24.87**	518.5**	59.16 **
AgNPs	3	11.49*	56.70**	108.62 **	48.41 **
Chitosan coated IONPs × AgNPs	9	26.88**	31.02**	**91.23	107.62**
Error	48	3.83	2.43	7.80	4.09
Coefficient of variation	_	6/6	10.86	13.38	6.99

Table 2. Variance Analysis of the Effects of chitosan Coated IONPs and AgNPs on Biochemical Traits of Snapdragon

Sources of Variation	DF	Phenol	stomatal conductivity	Fm	Fv/F0
Chitosan coated IONP	3	470.04**	2085.5**	6396.7**	0.814**
AgNPs	3	87.95**	406.7*	37819.9*	5.46**
Chitosan coated IONP × AgNPs	9	23.55**	888.2**	44867.2*	1.39**
Error	48	1.26	239.7	14128.3	0.141
Coefficient of variation	_	10.17	11.002	6.20	8.99

*, ** and significant at the %5 and %1 and not significant probability levels, respectively.

PLANT HEIGHT

The results indicated that a significant increase in plant height was observed in plants treated with chitosan coated iron oxide nanoparticles at 50 µM and silver nanoparticles at 50 mg/L, However increasing the concentrations of silver nanoparticles (AgNPs) and chitosan coated iron oxide nanoparticles (chitosan coated IONPs) to 100 and 150 mg/L and µM, respectively, hadn't a positive effect on the final plant height. Increasing in the concentrations of chitosan coated iron oxide nanoparticles had the positive effect on final plant height, particularly at 100 µM, in combination with silver nanoparticles at 50 mg/L, which appeared to promote flower height (measured as the total plant height up to the last petal) (Figure 1A). The results showed that the lower concentrations of chitosan coated IONPs and AgNPs (0 mg/L and µM) had the lowest effects on plant height.

BRANCHES NUMBER

In the evaluation of the effects of chitosan coated iron oxide nanoparticles and silver nanoparticles on the branch numbers, the results were consistent with those observed for final plant height. The highest branches number was achieved in the treatment combining chitosan coated iron oxide nanoparticles at a concentration of 100 µM with silver nanoparticles at 50 mg/L (Figure 1B).

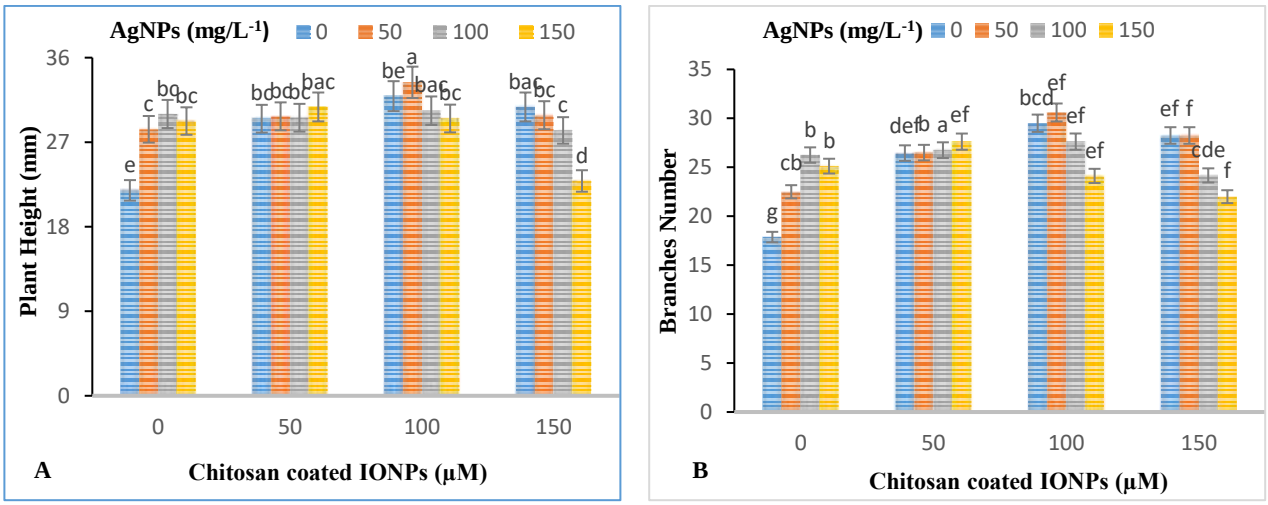

Figure 1. Interaction effects of chitosan Coated IONPs and AgNPs on Plant Height (A) and Branches Number (B) of Snapdragon

NUMBER OF FLOWERS

According to Figure 2A, by increasing the concentration of chitosan Coated IONPs and AgNPs the flower number was increased and the highest number of flowers was observed in chitosan-coated iron oxide nanoparticles at the concentration of 100 µM combined with 150 mg/L silver nanoparticles followed by the chitosan Coated IONPs at 150 µM of and 50 mg/L of AgNPs. The lowest flower number was in control (0 concentration of both chitosan coated IONPs and AgNPs).

Fv/F0

The results of experiment demonstrated that increasing the concentration of silver nanoparticles (AgNPs) across all levels led to an enhancement in the Fv/Fm ratio. The highest Fv/Fm value was observed at 150 mg/L of silver nanoparticles combined with 150 µM of chitosan coated IONPs. Conversely, a high concentration of iron oxide nanoparticles (150 µM) in the absence of silver nanoparticles resulted in a decline in this parameter followed by control (Figure 2B).

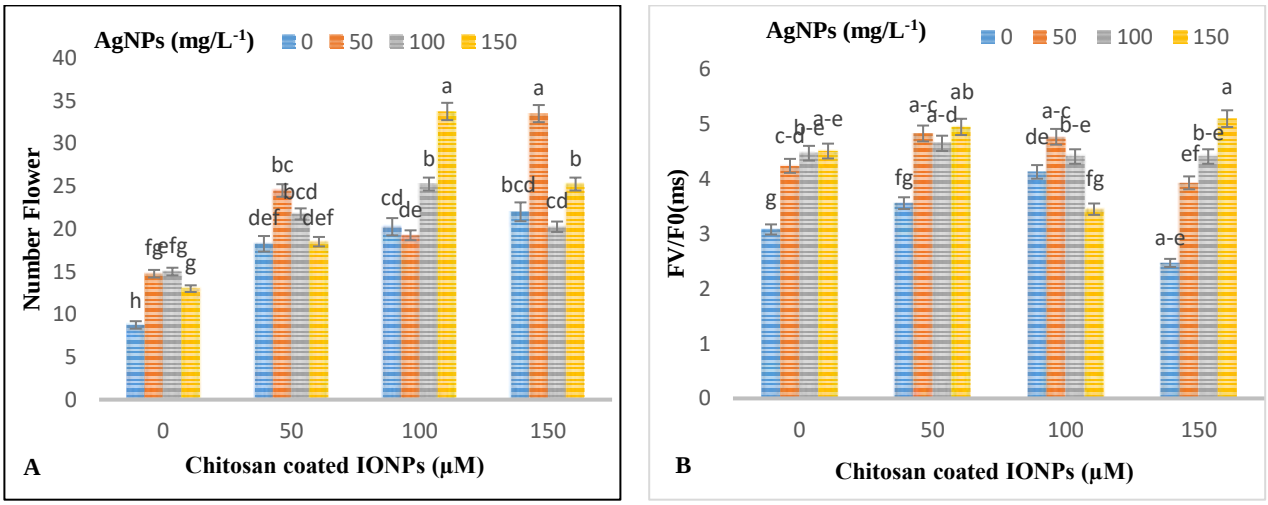

Figure 2. Interaction effects of chitosan Coated IONPs and AgNPs on Flower number (A) and Fv/F0 Ratio (B) in Snapdragon

Fm

The comparison of mean values across traits revealed that a moderate concentration of iron (50 µM) played a significant role in enhancing the Fm (maximum fluorescence). However, the highest Fm value was achieved from 150 mg/L of silver nanoparticles (AgNPs). Increasing the concentrations of chitosan coated iron oxide nanoparticles combined with various concentrations of silver nanoparticles, resulted in a reduction of Fm (Figure 3A).

STOMATAL CONDUCTIVITY

The mean comparison of data showed that the use of chitosan coated iron nanoparticles at moderate concentrations (50 and 100 µM) combined with various concentrations of silver nanoparticles had a positive effect on stomatal conductivity in the leaves of snapdragon. However, this value was

relatively lower compared to control plants, with the highest stomatal conductivity observed in the control group (Figure 3B).

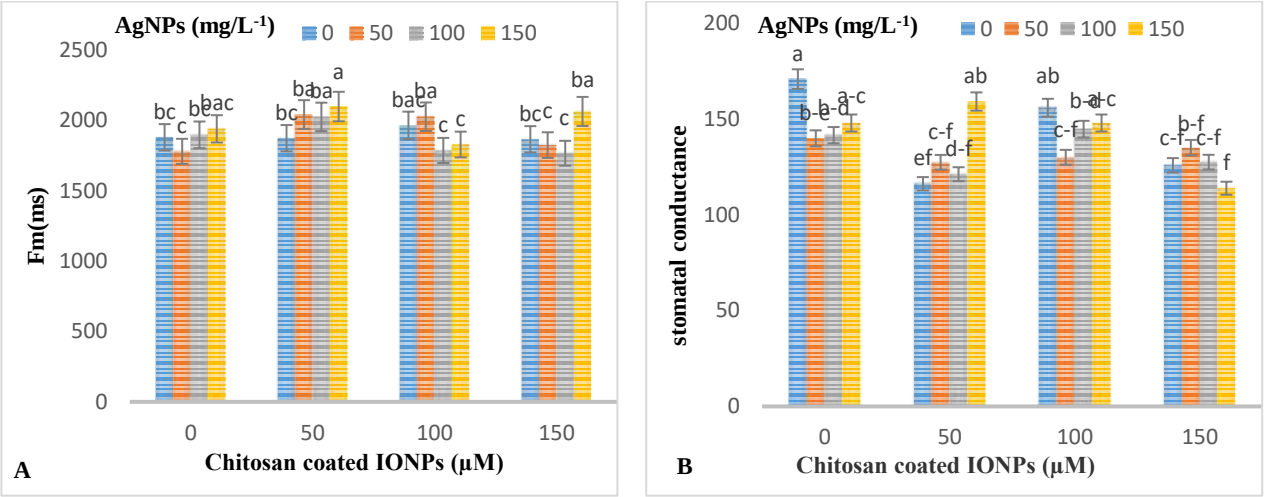

Figure 3. Interaction effects of chitosan Coated IONPs and AgNPs on the Fm (Maximum Fluorescence) (A) and Stomatal conductivity (B) in Snapdragon

TOTAL PHENOL

The results of experiment revealed that by increasing in the concentration of chitosan coated iron oxide nanoparticles (IONPs) from 0 to 150 mg/L, the phenol content increased significantly ($p < 0.01$) across all treatments. The mean comparison showed that the highest total phenol levels were observed in chitosan coated IONPs at the rate of 100 and 150 mg/L combined with higher concentrations of silver nanoparticles (AgNPs). In contrast, the control treatment without IONPs and AgNPs showed the lowest phenol content (Figure 4A).

FLOWER DIAMETER

According to the results obtained from the effects of different concentrations of chitosan-coated iron oxide nanoparticles and silver nanoparticles, as presented in Figure 4B, the lowest number of flowers was recorded in the control treatment (0 mg/L silver nanoparticles and 0 µM chitosan coated iron oxide nanoparticles), while the highest number was observed in the treatment with 150 mg/L silver nanoparticles combined with 0 µM chitosan-coated iron oxide nanoparticles. Moreover, the findings indicated a direct correlation between the increase in flower number and the elevated concentrations of both types of nanoparticles.

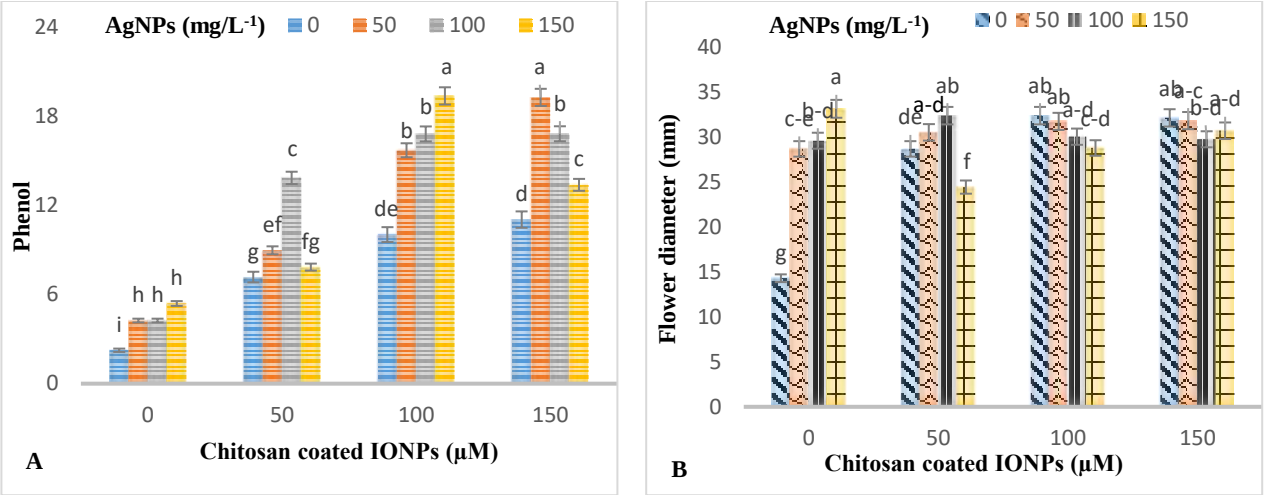

Figure 4. Interaction effects of chitosan Coated IONPs and AgNPs on total phenol content (A) and flower diameter (B) in snapdragon

DISCUSSION

According to the results of this experiment, chitosan coated IONPs at the rate of 100–150 µM significantly enhanced vegetative growth and flower production in Snapdragon which or consistent with the results of Rahmatizadeh et al. (2019) research, they results revealed that iron nanoparticles could increase the plant height, biomass, and Fe content in tomato plants. Torabi Giglou et al. (2023) conducted a study to investigate the effects of chitosan coated IONPs on peppermint and they found that this treatment could improve the photosynthetic capacity. In this study the similar results was obtained and nanoparticles enhanced chlorophyll fluorescence and stomatal conductivity of snapdragon. AgNPs played a synergistic role by increasing total phenolic and activating antioxidant systems, consistent with Ribeiro et al. (2023), who observed ROS-mediated defense activation via Ag/Fe nanoparticle combinations. Chitosan, as a biopolymer coating, not only stabilized nanoparticle delivery but also contributed to plant health. Hence, the combined use of chitosan coated IONPs and AgNPs created a synergistic effect, significantly improving morphological traits, photosynthetic performance, and biochemical defenses in Snapdragon, highlighting their potential in sustainable ornamental horticulture.

CONCLUSIONS

This study demonstrated that the combined application of chitosan-coated iron oxide and silver nanoparticles, particularly at moderate to high concentrations, can effectively improve growth and physiological traits in Snapdragon. These nanoparticles enhanced plant height, branching, and flowering while also increasing chlorophyll fluorescence and phenolic compounds.

These findings support the strategic use of biocompatible nanoparticles in ornamental plant management as a promising approach for boosting performance and resilience, particularly within sustainable and eco-friendly agricultural systems.

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PRODUCTION AND MODES OF ACTION OF BACTERIOCINS PRODUCED BY ENTEROCOCCAL STRAINS

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ABSTRACT

Bacteriocins are ribosomally synthesized peptides that typically exhibit antimicrobial activity against closely related bacterial species. Their production is considered a significant biochemical feature, particularly due to their role in biopreservation applications, such as the prevention of food spoilage and contamination. Various bacterial species, including enterococci, are capable of producing bacteriocins. In recent years, increasing attention has been directed toward the identification of novel bacteriocin-producing strains, their characterization, and the exploration of the potential applications of these peptides.

In this study, the bacteriocin production capacities of *Enterococcus faecium* DP8.3, DP9.3, and *E. mundtii* DP35.1 strains, previously isolated from traditional Turkish cheeses, as well as the modes of action of the bacteriocins produced by these strains, were evaluated. The analyses revealed that all strains began producing bacteriocins at the 5th hour of incubation at 37°C, reaching maximum production levels by the 8th hour. Bacteriocin production exhibited a positive correlation with cell density. The modes of action of the bacteriocins were tested against vancomycin-resistant *E. faecium* ATCC 51559. The bacteriocins produced by *E. faecium* DP8.3 and DP9.3 exhibited bactericidal effects, whereas the bacteriocin produced by *E. mundtii* DP35.1 demonstrated a bacteriostatic effect. The results demonstrate the potential application of *E. faecium* DP8.3 and DP9.3 strains in food systems due to their inhibitory effects. Additionally, the bacteriocin produced by *E. mundtii* DP35.1 appears to be a promising candidate for further investigation, particularly regarding its potential synergistic effects when combined with various antibiotics or other antimicrobial bacterial strains. Moreover, further studies are recommended to evaluate the applications of the bacteriocins produced by *E. faecium* DP8.3, DP9.3, and *E. mundtii* DP35.1 in the food industry.

Keywords: *Enterococcus*, Bacteriocin production, Mode of action

INTRODUCTION

Preservation methods are employed in the food industry to extend the shelf life of products by preventing spoilage and reducing the occurrence of food-related health risks (Singh, 2018). In recent years, growing consumer awareness and the increasing demand for healthy eating have led to heightened interest in natural preservatives as alternatives to chemical additives. In parallel with these developments, research and development efforts focused in biopreservation techniques have accelerated considerably. Although various techniques are employed for biopreservation, the use of beneficial bacteria to maintain and enhance product quality is among the prominent approaches. In this context, the shelf life of products can be extended through the use of various microorganisms or antimicrobial compounds derived from lactic acid bacteria (LAB). In addition to their role in the

development of characteristic taste, aroma, and textural properties of food products, LAB also produce compounds such as organic acids, hydrogen peroxide, ethanol, and bacteriocins, which contribute to their preservative effects (O'Connor et al., 2020; Kasimin et al., 2022).

Bacteriocins are ribosomally synthesized antimicrobial peptides. Although they are typically effective against closely related microorganisms, certain broad-spectrum bacteriocins are capable of inhibiting a wide range of microbial species (O'Connor et al., 2020). Bacteriocins synthesized by enterococci are known as enterocins. These compounds, composed of low molecular weight peptides, are classified into four distinct classes (Franz, et al., 2007). Enterocins exhibit specific antimicrobial activity, effectively inhibiting the growth of Gram-positive bacteria and spoilage microorganisms (Moreno et al., 2006).

Bacteriocins exhibiting optimal biopreservative potential are characterized by their safety for human consumption, minimal effect on the human microbiota, effectiveness against foodborne pathogens and spoilage microorganisms, and stability within the food matrix which they are applied (O'Connor et al., 2020). Moreover, bacteriocin-producing LAB are regarded as promising alternatives to antibiotics, owing to their capacity to survive in the gastrointestinal environment, suppress pathogenic and competing microbes, modulate host immune response, and mitigate both inflammation and oxidative stress, thereby contributing to improved gut health (Dobson et al., 2012; Dahiya et al., 2017; Westfall et al., 2017; Bäuerl et al., 2017). The growing interest in bacteriocins is further supported by several factors: the increasing consumer demand for minimally processed foods free from chemical additives (Silva et al., 2018); their capacity to modulate the human microbiota (Garcia-Gutierrez et al., 2019); their potential to serve as natural alternatives to antibiotics in response to rising antimicrobial resistance (Cotter et al., 2013); and their application as bacteriocin-producing probiotic cultures aimed at improving animal health and reducing infections (Vieco-Saiz et al., 2019).

The investigation of the production and mode of action of bacteriocins is of great importance due to their potential contributions to enhancing food safety, controlling antibiotic-resistant pathogens, and developing natural alternatives to synthetic additives. In this context, the present study examined the production and mode of action of bacteriocins produced by *E. faecium* DP8.3 and DP9.3, and *E. mundtii* 35.1, which were previously isolated from traditional Turkish cheeses.

MATERIAL AND METHOD

Bacterial strains and growth conditions

The bacteriocin-producing strains *E. faecium* DP8.3, DP9.3, and *E. mundtii* DP35.1 used in this study were previously isolated from traditional Turkish cheeses obtained from the Isparta province of Türkiye (Akpınar Kankaya, 2024). In the experiments, the strains were cultivated in de Man Rogosa and Sharpe (MRS) broth (Biokar, France), at 37°C for 24 h prior to use. All strains were stored at -32°C with 20% (v/v) sterile glycerol.

Bacteriocin production

For bacteriocin production, active cultures were inoculated at 1% (v/v) into 100 mL of MRS broth and incubated at 37°C for 12 h. During the incubation period, the optical density (OD) at 600 nm was measured at 1-h intervals, and antimicrobial activity was assessed concurrently. Bacteriocin activity was quantified as arbitrary units per milliliter (AU/mL), using vancomycin-resistant *E. faecium* ATCC 51559 as the indicator strain (Gök Charyyev et al., 2019).

Mode of action of bacteriocin

The mode of action of bacteriocins produced by *E. faecium* DP8.3, DP9.3, and *E. mundtii* DP35.1 was determined by adding 20 mL of pH-adjusted (pH 6.0) cell-free supernatant (CFS) to 100 mL cultures of vancomycin-resistant *E. faecium* ATCC 51559 at the early exponential growth phase. Cultures were incubated at 37°C for 12 h, with optical density (OD) at 600 nm measured at hourly intervals. A culture of *E. faecium* ATCC 51559 without bacteriocin addition served as the control (Todorov and Dicks, 2005).

RESULTS AND DISCUSSION

Within the scope of this study, bacteriocin production by *E. faecium* and *E. mundtii* strains, previously isolated from traditional Turkish cheeses, was investigated. It was observed that all isolates initiated bacteriocin production at the 5th h of incubation in MRS broth at 37°C, reaching maximum production at the 8th h.

In analyses determining the bacterial growth of *E. faecium* DP8.3 via OD measurements at 600 nm, the initial OD was recorded as 0.035 and increased to a maximum of 1.701 by the 11th h of incubation. Bacteriocin activity produced by *E. faecium* DP8.3 reached its peak at 400 AU/mL against *E. faecium* ATCC 51559 at the 8th h, when the OD was 1.274 (Figure 1). Although OD continued to increase up to the 12th h, bacteriocin production remained stable at 400 AU/mL during this period.

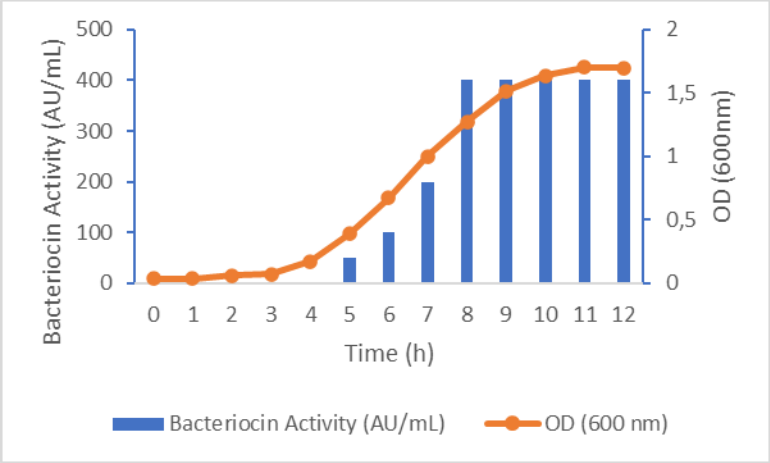

Figure 1. Growth curve of *E. faecium* DP8.3 at 37°C under uncontrolled pH conditions (●) and bacteriocin production expressed as AU/mL (■)

Similarly, bacteriocin production by *E. faecium* DP9.3 commenced at the 5th h and reached a maximum activity of 200 AU/mL at the 8th h (Figure 2). The initial OD of *E. faecium* DP9.3 was 0.042, increasing to 1.612 by the 12th h of incubation. At the 8th h, when the maximum bacteriocin activity against *E. faecium* ATCC 51559 was determined as 200 AU/mL, the OD was 1.110. From that point onward, bacteriocin activity remained constant at 200 AU/mL.

For *E. mundtii* DP35.1, bacteriocin production also began at the 5th h of incubation. The initial OD was recorded as 0.066 and increased to 1.736 by the 12th h (Figure 3). Bacteriocin production, against *E. faecium* ATCC 51559 reached a maximum level of 400 AU/mL at the 8th h of incubation. After this point, bacteriocin activity remained stable until the 12th h of incubation.

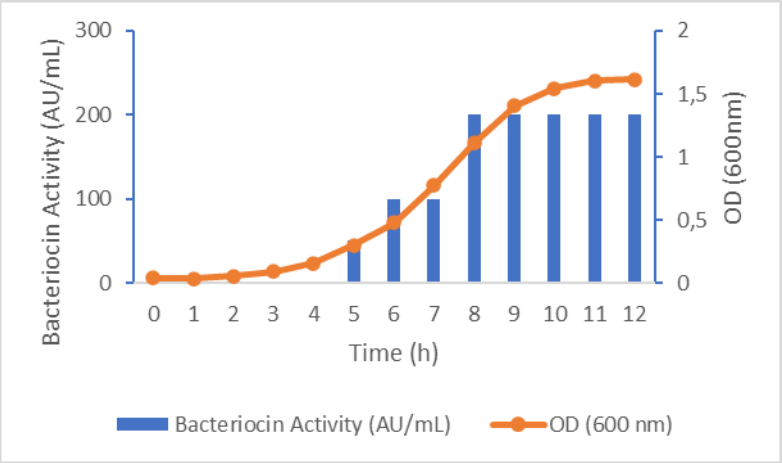

Figure 2. Growth curve of *E. faecium* DP9.3 at 37°C under uncontrolled pH conditions (●) and bacteriocin production expressed as AU/mL (■)

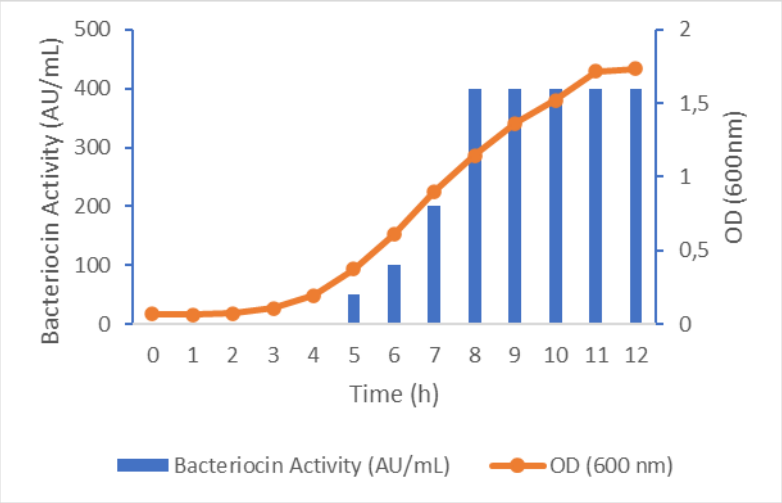

Figure 3. Growth curve of *E. mundtii* DP35.1 at 37°C under uncontrolled pH conditions (●) and bacteriocin production expressed as AU/mL (■)

The initiation of bacteriocin production during the growth phase and its reaching a maximum level at the end of this phase in all strains indicate that the bacteriocins produced follow primary metabolite kinetics. This observation consistent with previous studies reporting primary metabolite kinetics for bacteriocins produced by *Enterococcus* species (Gök Charyyev et al., 2019; Altınkaynak and Tuncer, 2020; Mancini et al., 2025). Similarly, Gök Charyyev et al. (2019) reported that *E. faecium* YT52, isolated from boza, began bacteriocin production at the 2nd h of incubation, with maximum production reaching 6400 AU/mL at the end of the logarithmic growth phase (7th h). Likewise, *E. mundtii* YB6.30, isolated from sucuk, was reported to initiate bacteriocin production at the 4th h of incubation, with activity increasing in parallel with cell density and peaking at the 9th h (Altınkaynak and Tuncer, 2020). Valledor et al. (2022) found that bacteriocin production by *E. faecium* ST20Kc and ST41Kc, isolated from kimchi, started during the early logarithmic growth phase (3 h after inoculation), and that the highest level of bacteriocin production was detected at the 6th h of incubation, with an activity of 12800 AU/mL against *L. monocytogenes* ATCC 15313. Mancini et al. (2025) documented that bacteriocin activity of *E. faecium* RM12, a raw milk isolate, was first detected at the

3rd h of incubation against *L. monocytogenes* ATCC 19115, and reached a maximum level of 16000 AU/mL at the 9th h.

In studies investigating the modes of actions of bacteriocins produced by *E. faecium* DP8.3, DP9.3, and *E. mundtii* DP35.1 against *E. faecium* ATCC 51559, CFS containing bacteriocins were individually added to early exponential phase cultures of *E. faecium* ATCC 51559. The OD of the cultures was measured hourly over a 12-h period. Following the addition of CFS from *E. faecium* DP8.3 at the 3rd h of incubation, no further increase in OD was observed in the treated culture, whereas the control culture showed a continuous increase in OD (Figure 4).

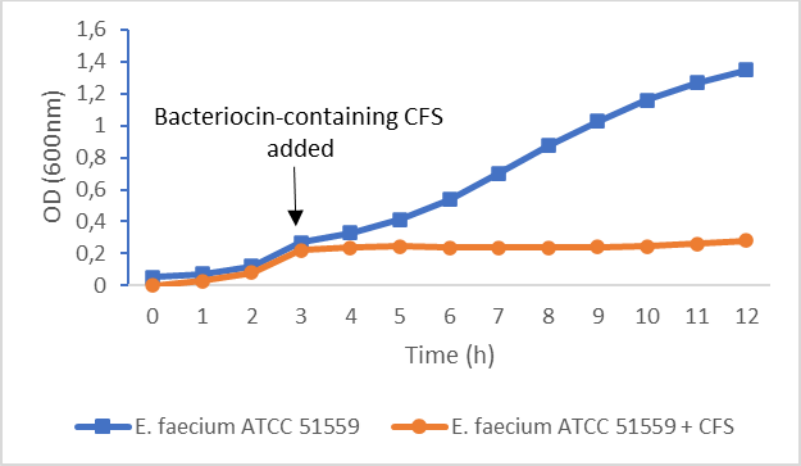

Figure 4. Mode of action of CFS from *E. faecium* DP8.3 on the growth of *E. faecium* ATCC 51559 in the absence (■) and presence (●) of CFS

In the investigation of the bacteriocin produced by *E. faecium* DP9.3, CFS containing the bacteriocin was added to the *E. faecium* ATCC 51559 culture at the 3rd h of incubation. In the group treated with bacteriocin-containing CFS, a decrease in OD was observed until the 8th h, after which it remained constant (Figure 5). In contrast, the control group exhibited a continuous increase in optical density throughout the incubation period.

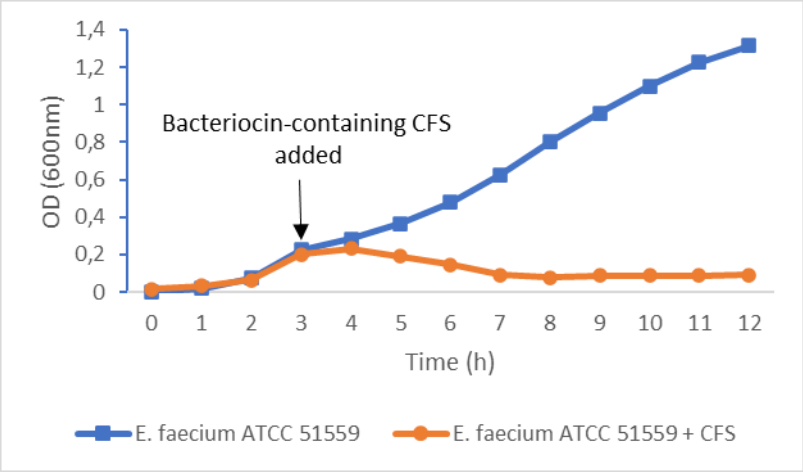

Figure 5. Mode of action of CFS from *E. faecium* DP9.3 on the growth of *E. faecium* ATCC 51559 in the absence (■) and presence (●) of CFS

CFS containing the bacteriocin produced by *E. mundtii* DP35.1 was added to the *E. faecium* ATCC 51559 culture at the 3rd h of incubation. In the treated group, cell density declined until the 9th h, followed by a renewed increase beginning at the 10th h (Figure 6). In contrast, the OD of the untreated *E. faecium* ATCC 51559 control culture exhibited a continuous increase throughout the incubation period.

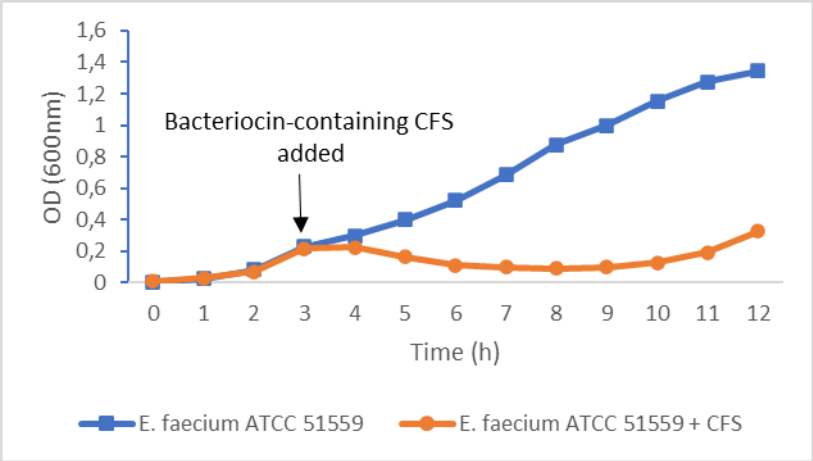

Figure 6. Mode of action of CFS from *E. mundtii* DP35.1 on the growth of *E. faecium* ATCC 51559 absence (■) and presence (●) of CFS

Upon examining the modes of action of the bacteriocins produced by all strains, it was determined that those produced by *E. faecium* DP8.3 and DP9.3 exhibited bactericidal activity. The bactericidal activity of these bacteriocins suggest their potential application as biopreservatives in the food industry. Similarly, studies conducted by various researchers have reported that bacteriocins produced by different enterococcal strains exhibit bactericidal effects against sensitive indicator bacteria, consistent with the data obtained in this study (Gök Charyyev et al. 2019; Altınkaynak and Tuncer 2020; Valledor et al. 2022; Meral Aktaş, 2024; Mancini et al., 2025). For example, Gök Charyyev et al. (2019) reported that the addition of CFS derived from *E. faecium* YT52, isolated from boza, to an early exponential phase culture of *L. monocytogenes* ATCC 15813 resulted in growth inhibition. Altınkaynak and Tuncer (2020) observed a decrease in the OD of *E. faecium* ATCC 51559 upon the addition of bacteriocin-containing CFS from *E. mundtii* YB6.30, isolated from sucuk, indicating a bactericidal effect. Valledor et al. (2022) reported that the OD of *L. monocytogenes* ATCC 15313 and *E. faecalis* 200A remained unchanged following treatment with bacteriocin-containing CFSs from *E. faecium* ST20Kc and ST41Kc, isolated from kimchi, suggesting bactericidal activity. In another study, the addition of bacteriocins produced by *E. faecium* H108 and H206, isolated from raw bovine milk, were found to immediately inhibit the growth of *L. monocytogenes* ATCC 7644 (Meral Aktaş, 2024). Mancini et al. (2025) also reported that no significant increase in OD was observed in *L. monocytogenes* ATCC 19115 cultures treated with the CFS of *E. faecium* RM12, a raw milk isolate, in comparison to untreated controls.

On the other hand, the bacteriocin produced by *E. mundtii* DP35.1 demonstrated a bacteriostatic effect against *E. faecium* ATCC 51559, which may be attributed to an insufficient bacteriocin concentration. Similarly, Qiao et al. (2020) reported that low concentrations of bacteriocin produced by *E. faecium* TJUQ1 were unable to completely eliminate *L. monocytogenes* CMCC 1595. In a different study, Pingitore et al. (2012) reported that the bacteriocins CRL35 and ST88Ch, produced by *E. mundtii* CRL35 and *E. faecium* ST88Ch, caused an extended lag phase when added to 3-hour-old cultures of *E. faecalis* ATCC 19443 and *Lb. sakei* ATCC 15521. Due to its bacteriostatic effect,

further investigation is warranted into the combined use of this bacteriocin produced by *E. mundtii* DP35.1 with antibiotics or other inhibitory strains, as well as its potential applications in the food industry.

CONCLUSIONS

In this study, the production and modes of action of bacteriocins produced by *E. faecium* DP8.3, DP9.3, and *E. mundtii* DP35.1 strains were investigated. All strains initiated bacteriocin production at the 5th h of incubation, reaching maximum levels by the 8th h. To evaluate their modes of action against vancomycin-resistant *E. faecium* ATCC 51559, CFS containing bacteriocins were added to *E. faecium* ATCC 51559 cultures at the 3rd h of incubation. The results demonstrated that *E. faecium* DP8.3 and DP9.3 exhibited bactericidal effects, whereas *E. mundtii* DP35.1 showed a bacteriostatic effect. The bactericidal activity observed in *E. faecium* DP8.3 and DP9.3 against the vancomycin-resistant strain is particularly significant, given the increasing global concern over antibiotic-resistant pathogens and their impact on food safety and public health. These findings highlight the promising potential of these strains as natural biopreservative agents. In contrast, the bacteriostatic effect of *E. mundtii* DP35.1 suggests that its efficacy may be enhanced through synergistic application with other antimicrobial agents or hurdles, warranting further investigation.

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IN VITRO ANTIOXIDANT AND ANTIDIABETIC OF MAESA KAMERUNENSIS MEZ. PLANT EXTRACT

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ABSTRACT

The genus *Maesa* Forssk., belong to the Myrsinaceae family and comprising some 150 species distributed throughout the Old World. In this study, the antioxidant, and antidiabetics properties of (hexane, ethyl acetate, methanol, n-butanol and water) extracts of *Maesa kamerunensis* that grows naturally in the Cameroon were investigated. $ABTS^+$ scavenging, *β*-carotene-linoleic acid, Cupric-reducing antioxidant capacity (CUPRAC), DPPH·scavenging, and metal chelating assays were performed to evaluate antioxidant activities of the extracts. The enzyme inhibitory potential of extracts was investigated against key enzymes involved in antidiabetics (α-amylase and α-glucosidase). The spectrophotometric methods were used to determine the total phenolic, flavonoid, ascorbic acid, *β*-carotene, and lycopene contents. Among the extracts tested, the crude extract of stems showed the highest antioxidant activity in CUPRAC ($A_{0.50}$: 9.34±0.71 µg/mL), ABTS·+ (IC_{50}: 3.27±0.25 µg/mL), DPPH (4.09±0.45 µg/mL), *β*-carotene linoleic acid (IC_{50}: 6.18±0.25µg/mL) assays. At 200 µg/mL, the ethyl acetate extract of *M. kamerunensis* was more active against *α*-glucosidase and *α*-amylase enzymes with the IC_{50} at 10.61±0.31 and 12.07±0.37 respectively, follow by the methanol extract of fruits with IC_{50} at 28.65±0.44 and 22.20±0.13 respectivly. This study contributes to the traditional therapeutic uses of *M. kamerunensis* and emphasizes its value in the development of new therapeutic agents exhibiting antioxidant and anti-diabetic activity.

Keywords: *Maesa kamerunensis*, antidiabetic potential, antioxidant proprieties,

INTRODUCTION

Since ancient times, food and natural products have been used in different parts of the world as traditional medicine, depending on their high therapeutic efficacy in treating various diseases. To date, using natural products offers high biocompatibility with marginal side effects. Thus, they have contributed to the development of up to 50% of the approved drugs in the market (Kamarudin et al. 2017). Traditional herbal medicines have played a fundamental role in preventing and treating various ailments since ancient times, which are rich sources for new drug discovery (Shendge and Belemkar 2018). The prescribed drugs derived from higher plants have increased by 25% in the last few decades (Khaliq 2017). The WHO has declared that 11% of the 252 drugs are from plant sources, while various synthetic drugs have natural precursors (Saleem, et al. 2019). Especially with the worldwide spread of diseases such as diabetes mellitus (DM), cardio-metabolic, cancer, and Alzheimer's diseases (AD) are

being focused on safe and consistent natural drugs over synthetic ones and side effects (Mocan et al. 2016).

Diabetes is a multifactorial metabolic disorder characterized by aberrant carbohydrate metabolism and high glucose levels. Alarmingly, the WHO has predicted diabetes to be the 7th leading cause of death by 2030, with the expected rise in the diabetic population to 418 million in 2025 and 552 million by 2030 (Federation 2019). Diabetes mellitus is an important metabolic problem characterized by insulin deficiency or no response to insulin in cells. Diabetes mellitus is categorized as a complex metabolic disease associated with irregular insulin production, developing insulin resistance, and β-cell malfunction. The intestinal absorption and breakdown of carbohydrates are majorly regulated by the enzymes α-amylase and α-glucosidase. A major intention is to discover natural α-amylase and α-glucosidase enzyme inhibitors. Inhibitors of α-amylase and α-glucosidase are becoming the target enzymes for possible drug development. These enzymes play a key role in hydrolyzing long-chain carbohydrates and disaccharides into monosaccharides, respectively (Jhong et al. 2015). The literature has a number of documentation that identify plant extracts as a viable source to relieve diabetes and through inhibitory actions against amylase and glucosidase. Despite these findings, there is still an ongoing need to study novel plant sources as antidiabetic medications. The aim of this study was to investigate the antioxidant and antidiabetics activities of various extracts obtained from fruits and the stems of *M. kamerunensis.*

MATERIAL AND METHOD

The fruits, leaves and the stems bark of *Maesa kamerunensis* was collected in Bangang, West Region of Cameroon, in July 2020. Authentication was performed in the Cameroon National Herbarium in Yaounde, where our sample was deposited under the voucher number 56111/HNC.

Extraction

The materials were dried in the shade at room temperature, then crushed. The powder resulting (3.1 Kg; 1.7 kg and 2.2 kg respectively of fruits, leaves and stems bark respectively) was extracted by maceration, three times, with MeOH. After filtration, the filtrate was evaporated dry under reduced pressure to give a crude extract of 115.1 g (fruits), 85 g of leaves and 33.5 g stems bark extract. The crude extract of the fruits and the stems bark of *M. kamerunensis* has been partitioned with hexane, ethyl acetate and n-butanol using liquid-liquid extraction. We obtained hexane; ethyl acetate, n-butanol and aqueous exttracts.

Antidiabetic activity

α-Amylase and α-glucosidase inhibition assay

The α-amylase inhibitory activity was measured using by starchiodine method with slight modifications (Küçükaydın et al., 2021). α-amylase (from porcine pancreas) solution (0.1 units/mL) was prepared with phosphate buffer (20 mM pH = 6.9 phosphate buffer prepared with 6 mM NaCl). Then, 50 mL of α-amylase solution and 25 mL of sample solution were mixed in a 96-well microplate. The mixture was pre-incubated for 10 min. at 37 °C. Following pre-incubation, 50 mL of starch solution (0.05 %) was added and incubated for 10 min. at 37 °C. HCl (0.1 M, 25 mL) and Lugol (100 mL) solutions were added to complete the reaction and absorbance was measured at 565 nm in 96-well microplate. Acarbose was used as standard.

The *α*-glucosidase inhibitory activity was determined according to Kim et al. (2010) with modifications (Küçükaydın et al., 2021). 50 mL of *α*-glucosidase (from *Saccharomyces cerevisiae*) (0.1 units/mL) in phosphate buffer (0.01 M pH = 6.0), 25 mL of PNPG (4-N-nitrophenyl-a-Dglucopyranoside) in phosphate buffer (0.01 M pH = 6.9), 50 mL of phosphate buffer (0.01 M pH = 6.9) and 10 mL of sample solution were mixed in a 96-well microplate. The solution was incubated for 20 min. at 37 °C.

Antioxidant assay

Samples were investigated for antioxidant activities with regards to lipid peroxidation inhibition, CUPRAC, DPPH* and $ABTS^{*+}$ scavenging and ferrous ion chelating assays as described by Çayan *et al.*, 2019. α-Tocopherol, BHA and EDTA were used as reference compounds. The results of antioxidant activity are stated as IC_{50} and $A_{0.50}$ (effective concentration providing 0.50 absorbance for CUPRAC assay) values.

RESULT AND DISCUSSION

Antioxidants activities

The crude extracts of fruits (MKFM) and the stems bark (MKSM), the ethyl acetate fractions (MKFE; MKSE), the aqueous fractions (MKSW; MKFW) and the hexane fractions (MKFH) of *M. kamerunensis* were evaluated for their antioxidant activities. The results are grouped in the **Table 1** below.

Table 1: Antioxidant activity of the extracts by β-Carotene-linoleic acid, $DPPH^{\bullet}$, $ABTS^{\bullet+}$, CUPRAC and metal chelating assays[a]

Antioxidant Activity						
		β-Carotene-linoleic acid assay	**$DPPH^{\bullet}$ assay**	**$ABTS^{\bullet+}$ assay**	**CUPRAC assay**	**Metal chelating assay**
		IC_{50} (µg/mL)	**IC_{50} (µg/mL)**	**IC_{50} (µg/mL)**	**$A_{0.50}$ (µg/mL)**	**IC_{50} (µg/mL)**
	MKFH	50.67±0.84	45.17±0.21	29.95±0.25	53.85±0.86	80.19±0.39
	MKFB	44.47±0.61	26.37±0.51	17.33±0.60	35.83±0.49	105.2±0.91
	MKSB	38.44±0.76	25.36±0.62	20.23±0.36	31.79±0.81	81.78±0.80
	MKFE	26.73±0.59	13.05±0.83	8.05±0.53	18.79±0.90	57.90±0.25
	MKSM	6.18±0.25	4.09±0.45	3.27±0.25	9.34±0.71	16.72±0.17
	MKFM	50.21±0.29	25.67±0.29	6.40±0.12	36.71±0.57	54.35±0.73
	MKSW	20.09±0.18	22.50±0.88	17.63±0.21	23.61±0.40	35.27±0.70
	MKFW	>200	>200	>200	>200	>200
Std	**α-Tocopherol**	2.25±0.07	38.15±0.45	35.50±0.56	61.40±0.75	NT[b]
Std	**BHA**	1.40±0.03	19.82±0.33	12.80±0.08	25.50±0.43	NT[b]
Std	**EDTA**	NT[b]	NT[b]	NT[b]	NT[b]	5.60±0.45

[a] Values represent the means ± SEM of three parallel sample measurements ($p < 0.05$). [c] NT: not tested.

From this table, the crude extract of stem's bark (MKSM) presented the lowest value of IC_{50} using β-Carotene-linoleic acid assay with IC_{50} at 6.18±0.25µg/mL compraring to the value of references, IC_{50}=2.25±0.07 µg/mL for α-Tocopherol and IC_{50}=1.40±0.03 µg/mL for BHA. With $DPPH^{\bullet}$ assay, it

showed the value IC_{50} at 4.09±0.45 µg/mL comparing to the standart at IC_{50}= 38.15±0.45 µg/mL for α-Tocopherol and IC_{50}=19.82±0.33 µg/mL for BHA. This extract exhibited the lowest value of IC_{50} at 3.27±0.25 µg/mL when tested to $ABTS^{\bullet+}$ assay and was more active than the value of standard. When used CUPRAC assay, it showed the IC_{50} at (9.34±0.71 µg/mL) comparing to the value of standard at IC_{50}=61.40±0.75 µg/mL and IC_{50}=25.50±0.43 µg/mL for the α-Tocopherol and BHA respectively. This extract has also been evaluated using Metal chelating assay and it showed the value of IC_{50} at 16.72±0.17 µg/mL) comparing to the refrence's (EDTA) value at IC_{50}=5.60±0.45 µg/mL.
The ethyl acetate fraction (MKFE) of *M. kamerunensis* exhibited the moderated activities. It presented the value of IC_{50} at 13.05±0.83 µg/mL for the $DPPH^{\bullet}$ assay and 8.05±0.53 µg/mL for the $ABTS^{\bullet+}$ assay. It was more active than the crude extract. This can be explain by the manifestation of antagonistic effet.
The crude extract of fruit (MKFM) showed the lowest value of IC_{50} at 6.40±0.12 using the $ABTS^{\bullet+}$ assay comparing to the one of references at 35.50±0.56 and 12.80±0.08 for α-Tocopherol and BHA respectively. It were more active than the reference. With DDPH and CUPRAC assays, it exhibited moderate activities with the low value of IC_{50} in relation to standard's one. The hexane fraction (MKFH) showed generally the higher value of IC_{50} comparing to the value of standard in the all assay done. The aqueous fraction of stems bark (MKSW) presented the moderate potent antioxidant activities with the IC_{50} at 23.61±0.40 using CUPRAC assay and the aqueous fraction of fruits (MKFW) showed weak activities for all assays.

Antidiabetic activity

Antidiabetic activities of the extracts were performed using ***α*** -amylase and ***α*** -glucosidase enzymes, which are key enzymes involved in the hydrolysis of carbohydrates and intestinal absorption respectively. ***α*** -Amylase and ***α***-glucosidase inhibitory activities of extracts and fractions are given in Table 2.

***α*-amylase inhibitory activity**

The best *α*-amylase inhibitory activity was observed in the ethylacetate fraction with IC_{50} at 12.07±0.37 µg/mL followed by the methanol extract of the fruit of *M. Kamerunensis* with IC_{50} at 22.20±0.13 µg/mL. The n-butanol fraction showed an IC_{50} at 66.91±0.85 µg/mL and its crude extract at 70.11±0.90 µg/mL Table 2.

***α*-Glucosidase inhibitory activity**

The *a*-glucosidase inhibitory activity results of the crude methanol extracts, and fractions from *M. kamerunensis* are given in Table 2. It was seen that all obtained extracts exhibited *α*-glucosidase inhibitory activity in a dose-dependent manner. The water fraction of stems demonstrated the highest inhibitory effect on *α*-glucosidase with the IC_{50} at 22.34±0.51 µg/mL, follow by crude extract and n-butanol fractions with IC_{50} at 22.71±0.69 and 22.80±0.35 µg/mL compared to acarbose (22.34±0.51 µg/mL).

Table 2: Antidiabetic inhibitory activities of the samples[a]

Samples/ Standards	Anti-diabetic activity			
	***α*-glucosidase**		***α*-amylase**	
	IC_{50} (µg/mL)	Inhibition (%) (at 200 µg/mL)	IC_{50} (µg/mL)	Inhibition (%) (at 200 µg/mL)
MKFH	26.45±0.38	82.88±0.80	21.08±0.35	85.31±0.86
MKFB	27.09±0.37	82.36±0.93	30.87±0.46	80.58±0.75
MKSM	22.71±0.69	85.33±0.43	109.4±0.63	61.33±0.56

MKFM	>200	28.65±0.44	>200	22.20±0.13
MKFE	>200	10.61±0.31	>200	12.07±0.37
MKFW	23.61±0.78	84.23±0.58	91.74±0.77	67.86±0.55
MKSW	22.34±0.51	86.28±0.75	75.22±0.45	70.11±0.90
MKSB	22.80±0.35	85.53±1.12	94.90±0.86	66.91±0.85
Acarbose	22.34±0.51	57.70±0.75	32.50±0.45	83.10±0.27

From these results, the stems extract of *M. kamerunensis* could have the relation between it antioxidant and antidiabetics activities.

CONCLUSIONS

In this study, the antioxidant and antidiabetics inhibitory properties of various extracts of the fruits and stems of *M kamerunensis*, which is naturally distributed in West region of Cameroon, were investigated. According to *β*-carotene-linoleic acid, ABTS+, CUPRAC DPPH free radical scavenging and iron chelating methods, the methanol extract of stems exhibited higher antioxidant activity than all other extracts. The water extracts of stems showed significant *α*–glucosidase inhibition activity. The ethyl acetate extract from the fruits has higher α-amylase inhibitory activity among all extract, even than acarbose. This study represents the first report on the detailed analysis of various parts of *M. kamerunensis* for their antioxidant and in vitro antidiabetic enzyme inhibitory activities. Our results showed that this species has promising bioactivities. They can be used as a natural source of antioxidant, antidiabetic agent in food, cosmetic and pharmaceutical industries. Therefore, further studies are needed to isolate and identify antioxidant and antidiabetic components from these species.

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ANTIDIABETIC AND ANTIOXIDANT ACTIVITY OF TWO SAPONINS ISOLATED FROM M. KAMERUNENSIS MEZ.

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ABSTRACT

Diabetes mellitus (DM) represents a global health problem. It is the most common of the endocrine disorders and is characterized by chronic hyperglycemia due to relative or absolute lack of insulin secretion or insulin actions. According to the World Health Organization projections, the diabetes population is likely to increase to 300 million or more by the year 2025. Complementary and alternative approaches to diabetes management such as isolation of phytochemicals with anti-hyperglycemic activities from medicinal plants is therefore imperative. The current study aim to identify new compounds by a systematic study of the anti-oxidant and anti-diabetic activity of stems of *M. kamerunensis*. Phytochemicals were isolated from the methanolic extract of stem parts of the plant by column chromatography and identified by nuclear magnetic resonance spectroscopy and mass spectroscopy. A new triterpenoid saponin glycoside was isolated. The isolated phytochemicals were identified as 3*β*-O-{([*α*-L-rhamnopyranosyl-(1→2)-*β*-D-galactopyranosyl-(1 → 3)]-[*β*-D-galactopyranosyl-(1 → 2)]-*β*-D-glucopyranosyl)}-22*α*-(2-methylbutanoyl)-13*β*,28-oxido-olean-16*α*,28*α*-diol (**1**) and 3*β*-O-{([α-L-rhamnopyranosyl-(1→2)-*β*-D-galactopyranosyl-(1→3)]-[β-D-galactopyranosyl-(1→2)]-β-D-glucuronopyranosyl)}-21*α*-angeloyloxy,22-*(E)-cinnamoyl*)-13*α*,28-oxido-olean-16*β*,28*β*-diol (**2**). For the antioxidant activities, compound **1** showed the higher value of IC_{50} at 124.8±1.15 µg/ml for *β*-Carotene-linoleic acid assay and 185.3±1.10 for $ABTS^{\bullet+}$ assay. For antidiabetic potential activities, compound **1** elicited stronger *α*-glucosidase (IC_{50} = 36.80±0.73) and moderate *α*-amylase (IC_{50} = 28.05±0.54 g/ml) inhibitory activities as compared to acarbose. Overall, the data suggested that the saponin isolated from *M. kamerunensis* has potentials as natural antioxidants and antidiabetics.

Keywords: *M. kamerunensis*; saponin; antioxidant activity; antidiabetic activity; *α*-Glucosidase; *α*-amylase.

INTRODUCTION

Medicinal plants and their derived components have provided potent drugs for modern medicine including vincristine, quinine, morphine, and digoxin to fight against life-threatening diseases such as diabetes, cancer, coronary heart diseases, HIV/Aids, and neurological conditions [Adhami et al., 2018]. Plant-derived molecules are also significant as pharmacological probes, drug prototypes and drug precursors that lead to the discovery of bioactive and innovative compounds or drugs [Parveen et al., 2018]. Therefore scientists are actively seeking new compounds from natural and traditional resources. *Maesa kamerunensis* belong to the *Maesa* genus and it is an important member of the Myrsinaceae familly. Several species of the genus *Maesa* have many uses in traditional medicine and presented diverses biological activities among antidiabetic, antitumor, antibiotic, piscicidal, antifungal, antimutagenic and antifungal (Foubert et al., 2016). It has also been reported that *Maesa* species contain flavonoid, triterpenoid and saponines as majors secondary metabolites compounds (Foulbert et al., 2016; Simbawee et al., 1999). Saponins, the natural glycosides of steroid or triterpene are pharmaceutical and nutraceutical agents mostly present in plant leaves and roots. These possess hypocholesterolemic, antidiabetic, anticoagulant, hepatoprotective, anticarcinogenic, hypoglycemic, neuroprotective, immunomodulatory, anti-inflammatory, antimicrobial and antioxidant activity, leading to significant concentration on their benefit to be used as vaccine adjuvants (Koneri *et al*., 2014; Thao *et al*., 2014; Astuti *et al*., 2011). Estimation of total saponins in plants is a routine practice that needs to be more elaborated in terms of antioxidant and antidiabetic efficacy of isolated saponins. Phytochemicals are isolated by extraction with numerous polarity based solvents and these constituents exhibit variation in their chemistry and ultimately solubility in specific solvent (Sultana *et al*., 2009). Despite the natural abundance and widespread use, data on biological activities of saponins in different solvents and the isolated saponins is limited.

Researchers are working hard to explore not only the causative factors of diabetes but also to find new molecules to target those factors in order to develop novel therapies. Among the common causative factors of diabetes several, such as auto-oxidation of glucose that results from the free radical formation, receptor tyrosine kinases, including insulin receptor and epidermal growth factor receptor, mediate protein tyrosine phosphatase (PTPs), which are involved in the down-regulation of various cellular signaling transduction mechanisms, and the enzyme glucosidase may be considered as an initial target to screen out potential antioxidant and antidiabetic molecules in order to find innovative molecules via various ways [Parthiban et al., 1995; Abdelsalam et al., 2019]. The purpose of the present study was to investigate the phytochemical composition of n-butanol fraction of stems of *M. kamerunensis* and the evaluation of their antioxidant and antidiabetic activities.

MATERIALS AND METHODS

Plant materials

The stems bark of *Maesa kamerunensis* was collected in Bangang, West Region of Cameroon, in July 2020. A botanist of the Cameroon National Herbarium in Yaoundé performed authentication, where our sample was deposited under the voucher number 56111/HNC.

Extraction

The materials were dried in the shade at room temperature, then crushed. The powder resulting (2.2 kg) of stems bark was extracted by maceration, three times, with MeOH. After filtration, the filtrate was evaporated dry under reduced pressure to give a crude extract of 33.5 g stems bark extract. For antioxidants, antidiabetics, we used methods previously described by Küçükaydın et al., 2021.

Isolation

The most active extract, the methanol extract of the stems (33.0 g) of *M. kamerunensis* was suspended in 300 mL of water and then partitioned with ethyl acetate and n-butanol (BuOH). The resulting n-BuOH fraction were concentrated to dryness to give crude extract. It was subjected to a silica gel column with gradient elution of $CHCl_3$/MeOH/H_2O.The fractions have been collected and combined under the basis of TLC yielding six fractions FA-FF. Fraction C (5 g) was subjected to sephadex LH20 and eluted with MeOH. Five subfractions was grouped under the basis of TLC indexed FC1-FC5. Fraction FC2 (1.1 g) was further subjected to a revered phase silica gel column with isocratic elution of H_2O/MeOH, yielding cinq fractions FC2A-FC2E. Subfraction FC2B was purified using sephadex LH20 and eluted with MeOH isocratically to give compound **1** (15 mg). Fraction E was chromatographed to C-18 reversed phase silica gel column eluting with a stepwise gradient mixture of H_2O/MeOH to obtain three fractions FE1-FE3. The repeated column chromatography column using C-18 reversed phase silica gel of fraction FE2 eluted with isocratic H_2O/MeOH, followed by Sephadex LH-20 allowed us to obtain compound **2** (12.2 mg).

Biological activities

Antidiabetic activity

α-Amylase and α-glucosidase inhibition assay

The *α*-amylase inhibitory activity was measured using by starchiodine method with slight modifications (Küçükaydın et al., 2021). The α-glucosidase inhibitory activity was determined according to Kim et al. (2010) with slight modifications (Küçükaydın et al., 2021).

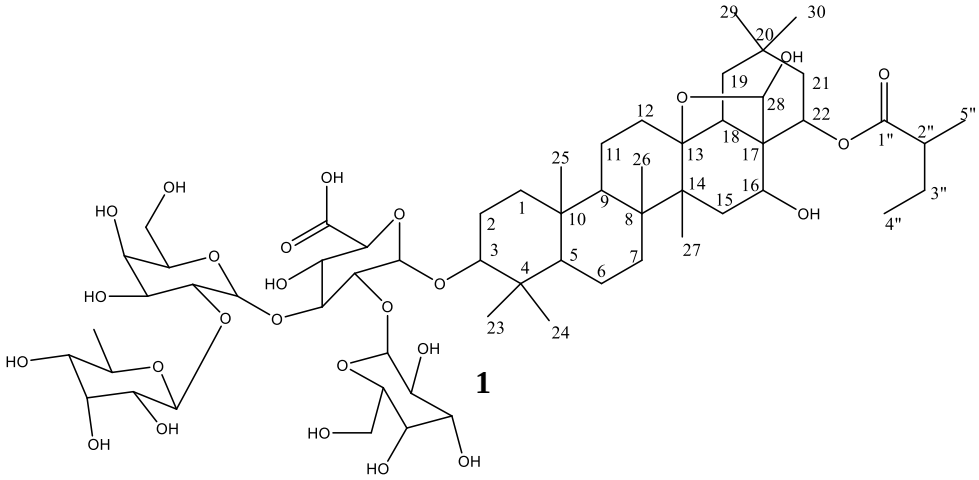
RESULTS AND DISCUSSION

In the search for new antioxidant and anticholinesterase compounds from plant materials, we discovered that methanol of the stems *M. kamerunensis* presented, higher activities that the methanol fruits extract suggesting that the extract contains antioxidant and anticholinesterase compounds. When that MeOH extracts were partitioned by ethyl acetate and n-BuOH, the resulting n-BuOH layer showed a strong activity comparing to the ethyl acetate, water and even standard tested.

Structural Elucidation of the Active Compounds Isolated from *M. kamerunensis*

The HRESIMS spectrum of compound **1** showed a molecular ion at m/z 1220.6190 $[M]^-$, 1219.6151 $[M - H]^-$, which is consistent with the molecular formula $C_{53}H_{96}O_{26}$ (calculated m/z 1220.6190) and $C_{53}H_{95}O_{26}$ (calculated m/z 1219.6112) respectively. The Fragment peaks appeared at *m/z* 1073 $[M – 147]^-$ and 911 $[M - 147 - 162]^-$ due to the losses of a rhamnose terminal unit, and two hexose units respectively. The ^1H and ^{13}C NMR spectra showed that compound **1** is a triterpenoid glycoside with four sugar moieties. The interpretation of its spectroscopic and spectrometric data allowed us to assign it structure **1** and was unambiguously characterized as 3*β*-O-{([*α*-L-rhamnopyranosyl-(1→2)-*β*-D-galactopyranosyl-(1 → 3)]-[*β*-D-galactopyranosyl-(1 → 2)]-*β*-D-glucopyranosyl)}-22*α*-(2-methylbutanoyl)-13*β*,28-oxido-olean-16*α*,28*α*-diol.

Compound (**2**) was obtained as white powder, had a molecular formula of $C_{68}H_{100}O_{28}$, and determined from its negative ESIMS and ^{13}C DEPT NMR data. ^{1}H and ^{13}C NMR spectra indicated that compound **2** had the same sugar arrangement as that of **1.** The aglycon of both compounds **2** was identical to that of maesargentoside IV [Foubert et al., 2016]. 1D and 2D NMR spectra of compounds **5** revealed the same position (C-21) of esterification with angelic acid, and showed the presence of a cinnamoyl group at position 22 of the aglycon. Based on the coupling constant (16.0 Hz) between the olefinic protons H-2 and H-3, the (*E*)-configuration of the cinnamoyl group was established. Compound **2** could be identified as 3*β*-O-{([α-L-rhamnopyranosyl-(1 → 2)-*β*-D-galactopyranosyl-(1 → 3)]-[*β*-D-galactopyranosyl-(1 → 2)]-*β*-D-glucunopyranosyl)}-21*β*-angeloyloxy-22*α*-(*E*)-cinnamoyloxy-13*β*,28-oxidoolean-16*α*,28*α*-diol.



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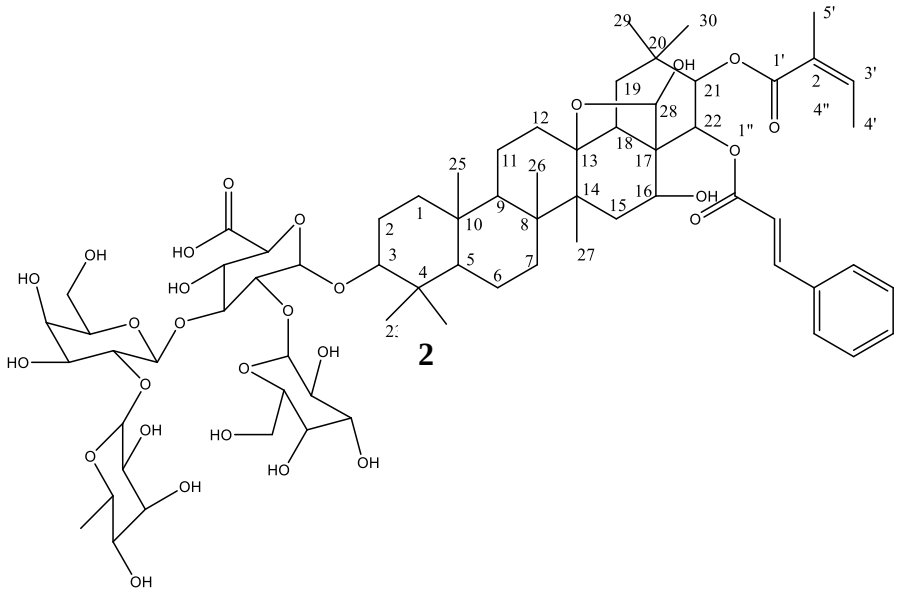

2

Scheme: structures of saponins isolated from *M. Kamerunensis*

Biological activities

In vitro antioxidant activity

As seen in Table 1, saponins as well as the standard antioxidant BHT scavenged the ABTS radical in a concentration dependent-manner.

The **compound** (**1**) showed the value of IC_{50} at 166.1±0.93 mL using *β*-Carotene-linoleic acid and the 185.3±1.10 mL using $ABTS^{\bullet+}$ assay and compound **2** presented the IC_{50} >200 mL using all methods. This could be due to the fixation of 2-methybutanoyl group at the position C-21 of compound 1.

Table.. Antioxidant activity of the isolated compounds by *β*-Carotene-linoleic acid, $DPPH^{\bullet}$, $ABTS^{\bullet+}$, CUPRAC and metal chelating assays[a]

		Antioxidant Activity				
		β-Carotene-linoleic acid assay	**$DPPH^{\bullet}$ assay**	**$ABTS^{\bullet+}$ assay**	**CUPRAC assay**	**Metal chelating assay**
		IC_{50} (µg/mL)	**IC_{50} (µg/mL)**	**IC_{50} (µg/mL)**	**$A_{0.50}$ (µg/mL)**	**IC_{50} (µg/mL)**
	MKSD	166.1±0.93	>200	185.3±1.10	>200	>200
	MKSK	>200	>200	>200	>200	>200
Std	**α-Tocopherol**	2.25±0.07	38.15±0.45	35.50±0.56	61.40±0.75	NT[b]
Std	**BHA**	1.40±0.03	19.82±0.33	12.80±0.08	25.50±0.43	NT[b]
Std	**EDTA**	NT[b]	NT[b]	NT[b]	NT[b]	5.60±0.45

[a] Values represent the means ± SEM of three parallel sample measurements ($p < 0.05$).
[c] NT: not tested.

Antidiabetics activities

Our results displayed that saponins extract forms *M. kamerunensis* having inhibitory potentials enzymes. *M. kamerunensis* saponin strongly inhibited *α*-glucosidase activity and inhibition of *α*-amylase activity (Table 2). The inhibitory activity elicited by compound **1**, IC_{50} 36.80±0.73 µg/ml on α-glucosidase was stronger than that of the standard, acarbose (57.70±0.75 mg/ml). While in the case of α-amylase, it displayed a better inhibition with IC_{50} at 28.05±0.54 µg/ml compared to the value of reference (acarbose, 83.10±0.27). The compound **2** inhibited *α*-glucosidase with the value of IC_{50} at 48.70±0.85 µg/ml and 33.74±0.77 for the α-amylase activity.

Table 2: Antidiabetic inhibitory activities of the samples[a]

	Anti-diabetic activity			
	***α*-glucosidase**		***α*-amylase**	
Samples/ Standards	**IC_{50} (µg/mL)**	**Inhibition (%) (at 200 µg/mL)**	**IC_{50} (µg/mL)**	**Inhibition (%) (at 200 µg/mL)**
MKSD	>200	36.80±0.73	>200	28.05±0.54
MKSK	>200	48.70±0.85	>200	33.74±0.77
Acarbose	128.5±0.62	57.70±0.75	32.50±0.45	83.10±0.27

[a] Values represent the means ± SEM of three parallel sample measurements ($p < 0.05$).
NT: not tested.

The reduction of activities could be due to the fixation of 2-methylbuatnoyl group at position C-21.

CONCLUSION

The aim of this work was to isolate and purify secondary metabolites from one medicinal plant in Cameroon: *M. kamerunensis* and evaluate their antioxidant and antidiabetics activities. During the phytochemical investigations, two saponines were isolated and characterized using chromatographic and spectroscopic techniques, particularly 1D and 2D NMR and mass spectrometry. Compound **1** showed the highest antioxidant and antidiabetics activity. The results of this study clearly demonstrated that saponins from *Maesa kamerunensis* stems exhibit free radical scavenging activities and possess reducing power making it an excellent candidate in the treatment of diseases in which ROS has been implicated. It can be uses to prevent diabetic complications. Hence, the above findings have given scientific evidence to saponins, a class of phytochemical from *M. kamerunensis* stems as potent antioxidant that can be employed in the management of diabetes.

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CONFLICT OF INTEREST
There are no conflicts of interest.

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CORIANDRUM SATIVUM L.: A REVIEW ON BOTANICAL CHARACTERISTICS AND AGRICULTURAL SIGNIFICANCE

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ABSTRACT

Coriander (*Coriandrum sativum* L.) is an annual, medicinal, and aromatic plant species belonging to the Apiaceae family. Widely used in traditional medicine and the food industry as a spice and flavoring agent, coriander is noted for its anti-inflammatory, antimicrobial, and digestive-regulating properties. Morphologically, coriander grows to a height of 30–60 cm, with a cylindrical and usually branched stem. The lower leaves are petiolate, broad, and lobed; the middle leaves are sessile; and the upper leaves are narrower, thread-like, and deeply lobed. The flowers are pink or white, and the fruits are spherical, 3–5 mm in diameter, and rich in essential oils. In Turkey, coriander is primarily cultivated in the Central Anatolia and Mediterranean regions, while globally it is widely grown in countries such as India, Iran, and Mexico.
This review aims to examine the medicinal and aromatic uses, botanical characteristics, and current cultivation status of coriander in Turkey and worldwide, based on the recent literature.

Keywords: Coriandrum sativum, Medicinal Plant, Review

INTRODUCTION

Medicinal and aromatic plants have played a significant role in human life throughout history due to their use for both nutritional and therapeutic purposes. Today, these plants are used across a range of fields, from the food industry to pharmaceutical products. Coriander (*Coriandrum sativum* L.), one of medicinal and aromatic plants, is an annual medicinal and aromatic species belonging to the Apiaceae family. It is widely used in traditional medicine and the food industry, and its fruits and leaves contain nutritionally and pharmacologically valuable components (Gil et al., 2002; Sahib et al., 2013). Evidence of the medicinal and aromatic properties of coriander, also known as "kişniş", "kuzbere" and "aşotu" in Turkey, dates back to 1500 BC. First used by the Egyptians, extracts and essential oils derived from the seeds and other parts of this plant are reported to have various pharmacological effects. Among these effects, sedative, muscle-relaxant, antimicrobial, antioxidant, anti-inflammatory, analgesic, and antispasmodic properties stand out. Because of these qualities, coriander has been considered an important medicinal and aromatic plant, valued for centuries in both

folk medicine and the pharmaceutical industry (Özel et al., 2010). Although the natural distribution area of coriander is Southern Europe and the Mediterranean Basin, it is now grown in many regions of the world thanks to its ability to adapt to different climatic conditions (Hazeri et al., 2021). On a global scale, Russia, various Asian countries, and Morocco stand out in coriander production; in particular, South Asian countries hold a leading position in terms of production volume (Ulutaş Deniz et al., 2018). In Turkey, the plant naturally occurs in the Central, Eastern, and Southeastern Anatolia regions, while its cultivated production is concentrated in specific areas, primarily the provinces of Mardin, Gaziantep, Burdur, Erzurum, Denizli, and Isparta (Kan, 2007). Statistics on coriander cultivation area and production amount in our country have been recorded since 2012. While 1 ton of production was achieved on only 11 decares of cultivation area in 2012, by 2016 the cultivation area had expanded to 503 decares and production had increased to 42 tons. Certain fluctuations were observed in the following years, and especially in 2019, the cultivation area decreased to 155 decares, and the production amount decreased to 12 tons (TUIK, 2019). However, from 2020 onwards, production began to show an upward trend again, with the cultivation area increasing to 2,455 decares and production reaching 188 tons that year. This data demonstrates that the production potential and market value of coriander in Turkey have been steadily increasing in recent years. However, it is noteworthy that production decreased to 101 tons and the cultivation area decreased to 1841 decares in 2024. This situation reveals that fluctuations in production continue and that sustainability in coriander cultivation may vary depending on various environmental, economic, or agricultural factors (TUIK, 2024).

Table 1. Coriander plant cultivation areas and production quantities in Turkey.

Production year	Area (Decare)	Production (Ton)
2015	150	11
2016	503	42
2017	410	29
2018	405	29
2019	155	12
2020	2 455	188
2021	2 612	253
2022	1 571	204
2023	1 932	222
2024	1 841	101

(TUIK, 2024)

Coriander is an annual herbaceous plant that can grow to a height of 30–60 cm, with thin, spindle-shaped roots, an upright stem, and alternate leaves. The lower leaves are broadly segmented and petiolate, the middle leaves are segmented and short-stemmed, and the upper leaves are stemless, thin and threadlike, and have deep lobes. The stem is erect, gnarled, and marked with longitudinal striations, branching primarily in its upper portions. The flowers of the plant are white or pinkish and

are arranged in an umbrella shape at the ends of the branches. Each flower also contains five petals, five sepals, five stamens, and a two-part stigma. The plant flowers in June–July and produces round fruits consisting of two pericarps; the fruits are yellowish brown, spherical, and their diameter varies between 3–5 mm (Baytop, 1984; Diederichsen, 1996; Baydar, 2009; Burdock and Carabin, 2009). In Turkey, coriander cultivation is carried out mainly in hot and arid climate regions. The plant exhibits optimal growth in well-drained, calcareous, sandy-loam soils with a light texture and neutral to slightly alkaline pH (Beyzi and Gürbüz, 2010; Verma et al., 2011). Cultivation is generally carried out in October–November. For optimal growth, the plant requires temperatures between 20–26 °C. The frequency and amount of irrigation vary depending on the climate, soil characteristics, and soil moisture. (Singh et al., 2015).

Coriander has two subvarieties: *C. sativum* L. var. *macrocarpum* and *C. sativum* L. var. *microcarpum*. The primary criteria for distinguishing these varieties are thousand-seed weight and fruit diameter. Those with a fruit diameter greater than 3 mm and a thousand-seed weight greater than 10 g are classified as macrocarpum, while those with a fruit diameter of 3 mm or smaller and a thousand-seed weight less than 10 g are classified as microcarpum (Diederichsen, 1996). While the entire coriander plant is suitable for consumption, the most preferred parts from a nutritional perspective are the fruit and leaves. Rich in essential oils (0.03–2.6%), phenolic compounds, and flavonoids, coriander is noted for its antimicrobial, antioxidant, and anti-inflammatory activities (Nadeem et al., 2013; Laribi et al., 2015). The volatile oil content of mature, dried coriander seeds ranges from 0.03% to 2.6%, whereas the fixed oil content varies between 9.9% to 27.7%. The seeds are particularly rich in volatile oils and polyphenols, while the leaves are notably rich in vitamins (Bhat et al., 2014). Approximately 40% of the essential oil is present in the fruit peel, while 60% is located in the seeds (Özel et al., 2009). The stems, leaves, and fruits of the plant contain linalool-rich aromatic compounds, which contribute to its characteristic aroma and flavor, while the leaves and fruits also serve as an important nutritional source, providing protein, vitamins, minerals (Ca, P, Na, Zn), and carbohydrates. Moreover, the fruits contain valuable fatty acids, including petroselinic, linoleic, oleic, palmitic, and stearic acids (Kassahun, 2020). The high content of vitamin C (160 mg/100 g), vitamin A (β-carotene 12 mg/100 g) and vitamin B12 (60 mg/100 g) in the leaves makes coriander more valuable from a nutritional point of view (Girenko, 1982; Prakash, 1990). The main components of coriander essential oil are linalool (67.7%), α-pinene (10.5%), γ-terpinene (9.0%), geranyl acetate (4.0%), camphor (3.0%) and geraniol (1.9%), which are responsible for both the characteristic aroma of the plant and its potential functions in medicinal and therapeutic products. All these characteristics highlight the strategic importance of coriander in terms of both agricultural production and commercial value. However, the essential oil composition of coriander is not constant and varies significantly depending on many factors, such as genotype, climatic and seasonal conditions, geographical characteristics, planting time, cultivation methods, plant developmental stage, fertilizer applications, harvest period, abiotic stress factors, and storage duration and conditions (Arganosa et al., 2008; Özel et al., 2010).

Coriander is an annual medicinal plant widely used throughout the world due to its medicinal properties as well as its nutritional value (Laribi et al., 2015). Similarly, it is used in our country due to its appetite-stimulating, digestive system-regulating, cough-suppressing, and headache-relieving

impacts (Ulutaş Deniz et al., 2018). Essential oils and fatty acids extracted from dried fruits are commercially utilized in the food, beverage, plastics, cosmetics, and detergent industries. In addition, they play a role in storage processes due to their insecticidal properties and heavy metal-binding potential (Kassahun, 2020).

Coriander leaves are known to contain higher levels of bioactive compounds and have strong antioxidant properties (Chithra and Leelamma, 1999; Melo et al., 2002; Wangensteen et al., 2004). Antioxidants are known as molecules that prevent or repair cellular damage caused by oxidative stress in living organisms (Msaada et al., 2017). In biological systems, reactive oxygen species (ROS), such as hydroxyl radicals and superoxide anions, can induce DNA damage and oxidize cellular lipids and proteins (Li et al., 2015; Peng et al., 2014; Xu et al., 2017). Oxidative stress-induced damage can be mitigated through increased intake of exogenous antioxidants, which function as free radical scavengers by inhibiting the initiation or propagation of oxidative chain reactions (Baiano and del Nobile, 2015). In general, a balance is achieved between oxidation and antioxidation by clearing these radicals by the antioxidant system in the body. Natural antioxidants of plant origin are mostly in the form of polyphenols (flavonoids, phenolic acids, anthocyanins, stilbenes and lignans), carotenoids (carotenes, xanthophylls) and vitamins (vitamins E and C) (Manach et al., 2004; Baiano and del Nobile, 2015). The antioxidant capacity of the plant is primarily attributed to its pigments, which are particularly rich in carotenoids. Studies have demonstrated that the carotenoids in coriander extract possess a strong potential to scavenge hydroxyl radicals, thereby protecting cells from oxidative damage (Divya et al., 2012). In addition to conferring nutritional benefits to the plant, these phytochemicals function as antioxidants, contributing to the extension of food product shelf life and offering protection against free radical-induced damage in humans.

CONCLUSIONS

Coriander *(Coriandrum sativum* L.*)* is an important spice as well as a medicinal plant due to its nutritional and aromatic properties. The composition of essential oils in its leaves, stems, flowers, and fruits is influenced by species, cultivation conditions, environmental factors, and post-harvest processing (Mandal and Mandal, 2015). Therefore, a comprehensive scientific evaluation of coriander's essential oils and bioactive compounds will contribute to the more effective, safe, and sustainable use of the plant in food, pharmaceutical, and health applications. Owing to its high antioxidant content, coriander stands out both as a functional food additive and as a natural therapeutic agent, and a more detailed investigation of its biological effects would provide significant contributions to the fields of food science and health.

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MULTI-SITE EVALUATION OF NITROGEN DYNAMICS IN INHIBITOR-ENRICHED UREA APPLICATIONS UNDER FIELD AND CONTROLLED CONDITIONS

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ABSTRACT

In this study, the soil nitrogen transformation dynamics of inhibitor-enriched urea formulations were investigated in both protected tomato cultivation (field trial in Söğüt, Bilecik), unplanted pot trials (controlled conditions in Antalya), and in an open field pepper plantation in Dörtdeğirmen, Gediz. A total of six formulations, including uncoated urea and a Max 46 reference formulation, were evaluated for total nitrogen (N), ammonium (NH_4^+), and nitrate (NO_3^-) contents at different time points. In field trials, soil samples were collected on days 7 and 16 after application. In pot trials, three samplings were carried out over 14 days. The results revealed that Formula 3 maintained higher total nitrogen and ammonium levels, consistently with lower nitrate formation, and demonstrated superior nitrogen use efficiency. Controlled pot trials confirmed the field findings and highlighted the compensatory effects of inhibitors in delaying nitrification. These results highlight the potential of inhibitor formulations to reduce nitrogen loss, extend fertilizer life, and contribute to sustainable agriculture.

Keywords;Urea inhibitor, nitrogen efficiency, ammonium, nitrate, tomato greenhouse, pot trial, NBPT

INTRODUCTION

Agricultural soils contribute significantly to global anthropogenic nitrous oxide (N2O) emissions. Approximately 50–60% of anthropogenic N2O emissions are attributed to direct or indirect emissions from nitrogen (N) fertilization in agricultural systems (Tian et al., 2020; Canadell et al., 2021). These N2O emissions from agricultural soils primarily arise from microbially mediated processes in the soil, such as denitrification, nitrification denitrification, nitrification, and dissimilar nitrate reduction (Braker and Conrad, 2011; Butterbach-Bahl et al., 2013). Nitrogen productivity in agricultural systems is critically affected by the rapid conversion of urea to nitrate, which in turn leads to significant leaching and volatilization. To prevent this, inhibitors such as NBPT (N-(n-butyl)thiophosphoric triamide) are added to urea fertilizers to retard nitrification and preserve nitrogen in the ammonium form. The microbial processes responsible for N2O production depend on soil oxygen (oxic/anoxic) conditions and the availability of N and carbon (C) in the soil (Chen et al., 2014). Since N2O emissions vary with local conditions such as climate, management practices, and soil type (Ruser et al., 2017), site-specific N2O reduction strategies are essential. Enhanced efficiency fertilizers (EEFs) containing inhibitors such as nitrification inhibitor [NI], urease inhibitor [UI] and dual inhibitors [NIUI], controlled-release urea (CRU) and controlled-release bulk blend fertilizer (CRBF) have been proposed as an effective approach to maintain high crop yields and reduce reactive N (Nr) losses (Li et al., 2017; Lan et al., 2022a). This study evaluates the performance of six inhibitor-based urea formulations in three different experimental settings: an open-field protected tomato greenhouse

(with crops) in Söğüt and controlled pot trials (without crop effects) in Antalya. The Triple approach allows comprehensive evaluation of fertilizer behavior in both real-world and isolated environments in an open-field pepper plantation in Gediz Dörtdeğirmen region.

MATERIAL AND METHOD

In the inhibitor synthesis project, field trials are being conducted across different land and climate conditions to monitor developmental processes. Within the scope of the project, field trials were conducted at different locations, tailored to different climates and soil types. Two different plant species, namely tomatoes and peppers, were tested in two open fields. Additionally, empty pot trials were conducted in Antalya. Six urea inhibitor formulations were tested on tomatoes in a closed greenhouse in the village of Çaltı, Söğüt, Bilecik, in open fields tested according to different soil types. Six urea inhibitor formulations were used in open field trials in Dörtdeğirmen village, Gediz district, Kütahya. Pot trials were conducted at the YLDZ Laboratory in Döşemealtı, Antalya, and six urea inhibitor formulations were tested. Care was taken to leave a 20 cm fertilization space between plants in the test areas. The fertilization method used was an equal amount of product, totaling 10 g, on each plant.

a.) Söğüt b.) Gediz c.) Antalya

Figure 1. Application Areas pictures a.)Söğüt b.)Gediz c.)Antalya

Figure 2. Photographs of the areas where Urea Inhibitor fertilizer was applied in Kütahya Gediz Dörtdeğirmen application areas

Figure 3. Photographs of the areas where Urea Inhibitor fertilizer was applied in Bilecik Söğüt Çaltı application areas (3 month time skip)

Figure 4. Photographs of the areas where Urea Inhibitor fertilizer was applied Döşemealtı application areas (1 empty 6 prescriptions 2 repeated trials)

RESULTS AND DISCUSSION

For nitrogen to be in its most permanent (persistent) form in the soil:

In inhibitory urea studies, high ammonium and relatively low nitrate indicate that nitrogen is retained in the soil for longer periods.

• High Total Nitrogen means a generally higher amount of nitrogen in the soil, meaning an abundant nitrogen source.

• High Exchangeable Ammonium because ammonium retains its cation binding sites in the soil for longer periods, making nitrogen more resistant to leaching and loss.

• Low Exchangeable Nitrate because nitrate is a very mobile form, easily leached by water, and not persistent in the soil. Therefore, a low exchangeable nitrate is advantageous for persistence.

• For "persistence," in particular: • High Total Nitrogen • High Exchangeable Ammonium (the permanent nitrogen form) • Low Exchangeable Nitrate

If we examine the formulas we use based on these findings,

Table 1. Bilecik Söğüt Çaltı 1 empty 6 prescription application Sampling results

Bilecik Söğüt Çaltı 1 empty 6 prescription application 1. Sampling results					Bilecik Söğüt Çaltı 1 empty 6 prescription application . Sampling results				
#	SAMPLE	Total Nitrogen (N)	Ammonium nitrate	Exchangeable Nitrate	#	SAMPLE	Total Nitrogen (N)	Ammonium nitrate	Exchangeable Nitrate
1	Ref.	0,44	0,04	0,34	1	Ref.	0,53	0,10	0,25
2	1.Formula	0,53	0,06	0,38	2	1.Formula	0,45	0,09	0,33
3	2.Formula	0,69	0,27	0,33	3	2.Formula	0,45	0,09	0,31
4	3.Formula	1	0,33	0,21	4	3.Formula	0,60	0,11	0,25
5	4.Formula	0,32	0,16	0,67	5	4.Formula	0,48	0,08	0,22
6	5.Formula	0,61	0,13	0,30	6	5.Formula	0,61	0,10	0,31
7	6.Formula	0,52	0,06	0,27	7	6.Formula	0,41	0,06	0,70
8	Max46	0,53	0,06	0,38	8	Max46	0,74	0,08	0,26

Among the first trial results, in Figure 3. area the third formula has the highest total itrogen and ammonium content, making it the most persistent nitrogen in the soil. The second test was conducted with samples taken 16 days later. It appears that NBPT successfully delays nitrate conversion. Nitrate levels are very controlled in the third formula.

Table 2. Kütahya Gediz Dörtdeğirmen 1 empty 6 prescription application Sampling results

#	SAMPLE	Total Nitrogen (N)	Ammonium nitrate	Exchangeable Nitrate
1	Ref.	0,29	0,01	0,28
2	1.Formula	0,17	0,06	0,38
3	2.Formula	0,24	0,27	0,33
4	3.Formula	0,25	0,33	0,21
5	4.Formula	0,16	0,16	0,67
6	5.Formula	0,28	0,13	0,30
7	6.Formula	0,25	0,06	0,27
8	Max46	0,27	0,06	0,38

To interpret the results in the Gediz Dörtdeğirmen region:

The low total nitrogen in Formula 1 indicates nitrogen loss. The slightly high ammonium level suggests that the inhibitory effect may have been partially maintained. However, the low nitrate level indicates that nitrification remains limited, meaning there is some inhibitory effect. Formula 2 yielded results very close to the reference formula. Total nitrogen: Only 0.03% lower – acceptable nitrogen loss. Ammonium level: Same as the reference the inhibitory effect is successful. Nitrate level: Slightly lower this indicates that the conversion of NH_4^+ to nitrate is suppressed, meaning the inhibitory effect

is maintained. Formula 3 generally yields results close to the reference: Total nitrogen: 0.25% Low nitrogen loss, which is positive. Ammonium: 0.01% Lower than the reference This indicates that the inhibitory effect is weakened. Nitrate: 0.24% Almost the same as the reference, which indicates that nitrification is limited. The inhibitory effect is moderate. Formula 4 showed a weak inhibitory effect: Total nitrogen is very low (0.16%) This low level indicates that nitrogen is being lost and is not being retained sufficiently in the system. Ammonium level is low NH_4^+ has been converted, limiting its inhibitory effect. Nitrate is also low Nitrification may not have occurred completely, but this may also be due to low total nitrogen. Formula 5 appears strong in terms of nitrogen preservation, but its inhibitory effect is weak. Total nitrogen (0.28%) is higher than the reference, which is positive (nitrogen is well preserved). Ammonium (0.01%) is low – its inhibitory effect is weak. Nitrate (0.27%) is high – its nitrification rate is higher than the reference. Its inhibitory effect is weak, but it performs best in terms of total nitrogen. Formula 6 shows good nitrogen preservation (0.25%), with only a 0.02% loss. Ammonium is low (0.01%), indicating a slight weakening of the inhibitory effect. Nitrate is very close. (0.24%), indicating that the NH_4^+ , NO_3^- conversion is limited. It has a moderate inhibitory effect. Consequently, it was determined that Formula 3 achieved maximum efficiency.

Table 3. Antalya Application and Sampling Schedule

Antalya Döşemealtı 1 empty 6 prescription application 1. Sampling results					Antalya Döşemealtı 1 empty 6 prescription application . Sampling results				
#	SAMPLE	Total Nitrogen (N)	Ammonium nitrate	Exchangeable Nitrate	#	SAMPLE	Total Nitrogen (N)	Ammonium nitrate	Exchangeable Nitrate
1	Ref.				1	Ref.	0,43	0,25	0,18
2	1.Formula	0,94	0,14	0,21	2	1.Formula	0,46	0,16	0,30
3	2.Formula	1,29	0,19	0,22	3	2.Formula	0,40	0,17	0,23
4	3.Formula	1,15	0,15	0,19	4	3.Formula	0,47	0,18	0,29
5	4.Formula	1,20	0,18	0,19	5	4.Formula	0,40	0,17	0,22
6	5.Formula	1,13	0,18	0,19	6	5.Formula	0,39	0,19	0,20
7	6.Formula	1,01	0,17	0,24	7	6.Formula	0,41	0,21	0,20
8	Max46	1,13	0,14	0,15	8	Max46	0,50	0,22	0,28

Antalya Döşemealtı 1 empty 6 prescription application 1. Sampling results				
#	SAMPLE	Total Nitrogen (N)	Ammonium nitrate	Exchangeable Nitrate
1	Ref.	0,50	0,22	0,28
2	1.Formula	0,56	0,24	0,32
3	2.Formula	0,47	0,23	0,24
4	3.Formula	0,49	0,25	0,25
5	4.Formula	0,48	0,22	0,26
6	5.Formula	0,48	0,22	0,26
7	6.Formula	0,48	0,25	0,23

8	Max46	0,43	0,20	0,23

Date Procedure Type Description / Detail
- Fertilizer application and irrigation 10 g fertilizer + 200 mL water per pot
- 1. Soil sampling ~10 g from each pot was taken for analysis
Irrigation 150 mL water per pot
- Irrigation 200 mL water per pot
- 2. Soil sampling ~10 g from each pot was taken for analysis
Irrigation 150 mL water per pot
- Irrigation 200 mL water per pot
- 3. Soil sampling ~10 g from each pot was taken for analysis
- Irrigation 200 mL water per pot

Total Nitrogen Retention in the Antalya potted plant-free trials:
Formulas generally retain nitrogen at or above the reference level. The lowest nitrogen rate is in the reference (0.43%), while other formulas significantly prevented nitrogen loss.
Inhibitory Effect:
The strongest inhibitory effect is seen in Formula 6 and Formula 3. These formulas exhibit high NH_4^+ values and an NH_4^+/NO_3^- ratio of 1 or above.
Nitrification Level:
Controlled nitrification ensures a slow and balanced release of nitrogen. A slight increase in Formula 1 may indicate rapid release.
Slow Release and Balanced Performance: If the goal is slow release and minimizing nitrogen loss, Formula 3 and Formula 6 stand out. Formula 3 offers a more balanced profile, while Formula 6 provides a slightly stronger inhibitory effect and slows nitrification. Because the Witness Formula has no inhibitory effect, nitrogen undergoes nitrification more rapidly and has a higher release rate. Performance closest to the reference: Formula 5 and Formula 4. Slow release and strong inhibitor: Formula 6 and Formula 3. Best nitrogen retention: Formula 1 (but nitrification is slightly faster).

CONCLUSION

Inhibitor-enriched urea formulations can improve nitrogen retention and balance nitrification in both field and controlled conditions. Formulations 3 and 6 are strong candidates when slower release is desired; Formula 1 is excellent at retaining total N, but rate/timing adjustments may be required to reduce NO_3^- accumulation. Formula 3 yielded the best results against the established commercial product. Further multiseason trials with yield and quality endpoints are recommended to refine usage guidelines based on soil type and climate.

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PROPOLIS AGAINST BOTRYTIS CINEREA: A REVIEW OF STUDIES COMBINING IN VITRO AND IN VIVO TESTS

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ABSTRACT

Botrytis cinerea is a necrotrophic fungal pathogen that is responsible for substantial losses in a wide range of plant species. The detrimental impacts of chemical fungicides on human health and the environment have given increase the interest in alternative control strategies. In this review, the antifungal effects of different propolis extracts on this pathogen were evaluated based on studies that combined *in vitro* and *in vivo* tests. The findings of the *in vitro* experiments demonstrated that propolis extracts have the capacity to inhibit various developmental stages of this pathogenic fungus such as mycelial growth, sporulation, and conidial germination. However, the effectiveness of this inhibition was changed with concentration of the extract and the method of preparation. In this context, it has been demonstrated that extracts prepared based on the use of ethanol at high concentration and sonication method have a stronger antifungal effect. *In vivo* test demonstrated that the treatment of propolis to the fruits of plants including strawberries, grapes, blueberries and tomatoes led to a reduction in both the disease incidence and infection area by the pathogen, although this effect exhibited the variability with plant species, the application method and dosage. Consequently, while propolis reveals considerable antifungal potential against *B. cinerea*, additional researches are required on the standardization of extracts, the optimization of doses, the determination of application timing and *in vivo* tests on different plant species to increase its applicability in agricultural practices.

Keywords: *Botrytis cinerea*, propolis extract, antifungal effect, *in vitro* and *in vivo* tests

INTRODUCTION

Propolis is a complex natural compound that is formed by honeybees (*Apis mellifera*) by combining the natural substances they collect from plant buds, resins and leaf sap with their own enzymatic secretions. The word 'propolis' is derived from the Greek word's 'pro', meaning 'front or entrance', and 'polis', meaning 'city-colony', which indicates that it was used in its hive defense. Propolis is characterised by its flexibility, softness and stickiness at high temperatures, while it becomes hard and brittle when cooled. Due to these properties, it is used by honeybees to close cracks and holes and strengthen the structure of beeswax combs in order to keep the temperature and humidity inside the hive constant throughout the year (Sforcin, 2007).

From antiquity to the present, propolis has been utilized as an antiseptic and disinfectant for oral and skin illnesses in ancient Greece, primarily in traditional medicine, and by the Egyptians during mummification procedures (Havsteen, 1983; Burdock, 1998). This particular bee product, which has a wide range of uses, has been shown to contain over 300 chemical compounds (Huang et al., 2014). The chemical composition of raw propolis typically consists of 50% plant resins, 30% waxes, 10% essential and aromatic oils, 5% pollen, and 5% other organic substances (Bankova, 2005). However, the main chemical groups in propolis resin are phenolic acids and their esters, terpenes, flavonoids,

aromatic aldehydes and alcohols, stilbenes, fatty acids, and β-steroids (Watanabe et al., 2011; Ahangari et al., 2018; Przybylek and Karpinski, 2019). Despite the chemical diversity of propolis, comprehensive research has revealed the existence of at least six main types (Ghallab et al., 2024).

Figure 1. The main types of propolis (Ghallab et al., 2024).

Following the demonstration of the anticancer, antioxidant, anti-inflammatory, antibiotic, antifungal and antihepatotoxic properties of propolis by Ghisalberti (1979), there has been an increase in the number of modern controlled experimental studies on the chemical structure and biological activities of this substance. *Botrytis cinerea*, the causal agent of gray mold disease in several plant species and a major cause of yield losses both in the field and post-harvest (Williamson et al., 2007; Dean et al., 2012), took a place among the fungal pathogens against which propolis was tested. The ability of this fungal pathogen for development of resistance exhibited by this fungal pathogen to fungicides has rendered the use of alternative compounds to control it, such as propolis, increasingly significant.

Some of the studies have been conducted *in vitro* to investigate the impact of propolis on *B. cinerea* (Sapieha-Waszkiewicz et al., 2011; Curifuta et al., 2012; Yang et al., 2016; Ezazi and Davari, 2018; Wuaden et al., 2018; Ouahab et al. 2023; Erdoğan and Güzel, 2025). However, the results of *in vivo* tests involving plant parts are more significant in determining the practical applicability of this compound. In this review, the effect of propolis against this pathogen was discussed in the light of the findings obtained from both *in vitro* and *in vivo* stages, with emphasis placed on the highest effects observed in the studies. Within this context, *in vitro* studies are incorporated into the text according to their chronological sequence, while *in vivo* studies are presented in tabular form. Articles belonging to the research conducted until today were obtained by using many databases in Tekirdağ Namık Kemal University library.

Studies on The Effects Of Different Propolis Extracts and Applications *on Botrytis cinerea*

In the study conducted by Darweesh (2010), samples of propolis collected from Baghdad, Iraq, were prepared using 96% ethyl alcohol and subsequently added to Potato Dextrose Agar (PDA) medium at doses of 1.25, 2.5, and 5 mg/ml. The researcher then has determined the pathogen's colony diameter and mycelial growth rate seven days after placing a mycelial disk of the pathogen in the center of each petri dish. In the study, the same concentrations were also added to Potato Dextrose Broth (PDB) medium and mycelial dry weights were measured after 14 days of incubation at 25°C. *In vitro* tests of the study revealed that a concentration of 5 mg/mL of propolis extract led to a 100%

reduction in both the colony growth and the mycelial dry weight in PDA and PDB media. This dose was applied to eggplant leaves and cucumber fruit, and its efficacy against the pathogen *in vivo* was tested (Table 1). In addition, the chemical composition of propolis was analysed using infrared spectroscopy, and the presence of flavonoids was determined.

In a study conducted by Moura et al. (2016), extracts of two different types of propolis (green and brown) prepared with ethanol were added to PDA medium, which were present in 9 cm Petri dishes at doses of 0.5% and 2.5%, and the mycelial disk of the pathogen was placed on it. Petri dishes were incubated at 25°C and the colony diameter of the pathogen was measured at 48, 72 and 96 hours after inoculation. Following a thorough evaluation of the study's findings, it was determined that both 0.5% and 2.5% doses of green propolis exhibited a higher antifungal effect against the pathogen in comparison to brown propolis. Findings from the study indicated that a 2.5% dose of green propolis led to a 33.67%, 43.90%, and 43.23% inhibition in colony development at 48, 72, and 96 hours after inoculation, respectively; these values were higher in comparison to the inhibition rates of brown propolis at same dose. In the study, both propolis were tested using strawberry fruit *in vivo* at both doses (Table 1).

Table 1. Some studies conducted under *in vivo* conditions against *Botrytis cinerea*

Treatment	**Scope**	**Result / Effectiveness**	**Author (Year)**
Application of propolis ethanol extract (5 mg/ml) to eggplant leaves and cucumber fruits at different times before and after inoculation [dropping of conidia suspension (1×10^6 conidia/mL) into wounds opened on leaves and fruits] of the pathogen	Determination of the effect of application time (both 24, 48 hours before and after inoculation) on the growth of *B. cinerea*	The treatments made 24 and 48 hours before inoculation significantly slowed down disease development.	Darweesh (2010)
Spraying of a conidia suspension of the pathogen at a concentration of 1×10^5 conidia/mL after one minute immersion of strawberry fruits in solutions of green and brown propolis at 0.5% and 2.5% doses	Control of pathogen development in the cultivar (cv) Camarosa with different propolis doses	No difference was observed between the two propolis extracts in terms reducing disease incidence, with an overall inhibition rate of 12.5% being determined. The 0.5% dose of green propolis inhibited the disease severity at the highest rate (23.08%).	Moura et al. (2016)
After covering cherry tomatoes with pullulan film containing propolis ethanol extract (5% and 10%), the inoculation of the pathogen conidia suspension (10µl) to the wound	Determining the protection of the application by measuring the pathogen population on tomatoes	There was no reduction in the population of coated pathogens after 14 or 21 days.	Pobiega et al. (2020)
Inoculation of pathogen (10 µL) into wounds opened	Evaluation of the effect of different propolis	In the study, after 15 days, Portuguese propolis	Diallo (2021)

with a pipette tip after immersing grapes berries in propolis solutions (15 g/L) for 2 minutes (min)	types (Portuguese, Brazilian red) on the decay area caused by *B. cinerea*	(~16.3%) was found to be effective in red grapes and Brazilian red propolis in white grapes (~21.5%) were found to be effective, even if at low rates.	
Treatment	**Scope**	**Result / Effectiveness**	**Author (Year)**
Immersion of strawberry fruits in propolis extract (20-, 40- and 60-mL/L) for 30 da, keeping at 25ºC for 24 hours and inoculation of conidia suspension of the pathogen	Monitoring the rate of disease incidence at different times (24, 48, 72 hours), determining the PAL (phenylalanine ammonia-lyase) enzyme activity and total phenolic substance amount, which play a role in resistance	No significant decrease in disease incidence was observed at any time point at any of the three doses, and an increase was observed at higher doses. Although the total phenol content of the fruit decreased, PAL enzyme activity increased by only 7.61% after 72 hours.	Stefanski et al. (2024)
Inoculation of 5µl of conidia suspension of the pathogen into the fruits using a sterile needle; for volatile compounds, the placement of the inoculated fruits on sterile papers with 100 µl of extract; for non-volatile compounds, treating the extracts (5 µl) to the wounds on the fruit arranged on the grid one hour after inoculation.	Evaluation of the effect of volatile and non-volatile compounds in extracts on disease incidence	PFvg1 extract exhibited strong antifungal activity (~60-80%) for both compounds. PFvg2 followed it.	Sadallah et al. (2025)

Pobiega et al. (2020) determined the minimum inhibitory concentration (MIC) value for *B. cinerea* as 2 mg/mL and the minimum fungicidal concentration (MFC) value as 8 mg/mL as a result of serial dilution tests prepared with propolis ethanol extract (EEP). In the conducted study, additionally, pullulan films containing 5% and 10% EEP were prepared, and 6 mm diameter discs were cut from these films and placed on Sabouraud agar media spread with conidia suspension. Cellulose discs coated with EEP at the same concentration have been used as the control group. The experimental findings revealed that no inhibition zones were observed in any of the treatment groups. The *in vivo* tests of the study were carried out on cherry tomato fruits (Table 1).

In a study performed by Diallo (2021), the antifungal effects of three distinct propolis ethanol extracts (Portuguese propolis, Brazilian green propolis, and Brazilian red propolis) on *B. cinerea* G3 isolate were investigated. For the present study, the extracts were prepared at doses of 0.5 g/L, 1.5 g/L, 5 g/L, and 15 g/L, and were compared with a commercial fungicide and a fungicide containing the active ingredient tebuconazole. Both agar disk diffusion and well diffusion methods were used in the *in vitro* experiments. In the disk diffusion assay, square dishes containing 50 mL of PDA were prepared, and a conidial suspension with a density of 1×10^6 conidia/mL was spread evenly onto the

medium using a sterile swab. Subsequently, a total of nine filter paper disks were placed on the surfaces, and 100 µL of the extract was dropped onto each disk. In the well diffusion method, the same volume (100 µL) of solution was applied to each well (nine wells punched with the back of a sterile Pasteur pipette on the agar) and the experiments were conducted separately for different extracts. The dishes were then subjected to an incubation process at a temperature of 25°C for a period of five days. Following the conduction of the experiments, it was reported that none of the propolis species examined in both methods demonstrated antifungal efficacy at the applied doses. *In vivo* tests were conducted utilising extracts at a concentration of 15 g/L and grape berries. (Tablo 1).

Stefanski et al. (2024) examined the effects of an ethanol extract of brown propolis on mycelial development, sporulation and conidia germination of *B. cinerea* under *in vitro* and in *vivo* conditions. In the *in vitro* experiments, propolis extract was added to PDA medium at doses of 20, 40, and 60 mL/L. Petri dishes with a 5 mm diameter *B. cinerea* mycelium disk placed in the center were then incubated at 25°C for 6 days. Colony growth was measured daily, and conidia counts were performed at the conclusion of the sixth day. In the study, it was indicated that mycelial growth rates were significantly reduced and that sporulation was absent at doses of 40 and 60 mL/L. *In vivo* studies on strawberry fruits demonstrated that the three doses were ineffective in reducing disease incidence (Table 1.).

Sadallah et al. (2025) evaluated the antifungal activity of propolis samples [PFvg1 (Friuli Venezia Giulia), PFvg2 (Friuli Venezia Giulia), PU3 (Umbria), PV4 (Veneto), PT5 (Tuscany)] obtained from different geographical coordinates of various Italian regions against *B. cinerea* isolate obtained from grape berries, both *in vitro* and *in vivo* (Table 1). In the study, samples of propolis were prepared by extraction with a range of solutions of ethanol (30%, 60% and 90% v/v). *In vitro* tests were carried out in which the conidia suspension of the pathogen (1×10^5 conidia/mL) was spread on PDA medium, 80 µL of extract was applied to the wells (5 mm in diameter) on the medium, and the inhibition zones were measured after 72 hours. The findings of this test demonstrated that the widest inhibition zone was observed in the use of PU3 propolis extract, which was prepared with 90% ethanol. The researchers also had ascertained the EC_{50} value for volatile and non-volatile compounds of each extract based on conidia germination using increasing doses (0, 3.2, 6.5, 12.5, 25, 50, 100, 150 and 200 ppm) of propolis extracts obtained by sonication with 90% ethanol for 24 hours. For tests involving volatile compounds, filter papers were placed on conidia suspensions spread on PDA medium, and the relevant dose of propolis extract was applied to each. For non-volatile metabolites, volatile compounds (100 µl) were added one hour after the pathogen spread onto the medium. Petri dishes were subjected to an incubation process at a temperature of 20°C for a duration of 48 hours. The outcomes of these experimental tests had indicated that PFvg2 (EC_{50}: 1.36 mg/ml) was identified as the most effective extract in regard to volatile compounds, while PU3 (EC_{50}: 2.24 mg/ml) was determined to be the most effective extract in relation to non-volatile compounds. In *in vivo* tests, the EC_{50} value of each extract was used as the dose.

CONCLUSION

The *in vivo* studies referenced in this review demonstrate the significant antifungal potential of propolis extracts against *B. cinerea*. However, the extent of this efficacy is dependent on numerous factors, including the chemical composition of the propolis, the extraction method, the type of solvent utilised, the dose, the timing of application and the target plant tissue. The extant literature reveals that research studies have examined the efficacy of propolis extracts on the plants at post-harvest stage, with promising results obtained. The efficacy of propolis under *in vivo* conditions has been proven by significant reductions in disease incidence and lesion development, particularly in high-value crops

with perishable properties, including strawberries, grapes, blueberries, cucumbers and tomatoes. The fact that propolis is not only used in the context of agricultural products but also in human health, with its antimicrobial, antioxidant and anti-inflammatory properties, renders it a versatile and safe product. In this context, the utilisation of propolis, a substance that has been demonstrated to be effective in both human health and in the inhibition of plant pathogens, particularly in the control of fungal diseases in the post-harvest period in fruits, will not only result in a reduction of product loss but also contribute to food safety by eliminating the risk of chemical residues. In conclusion, propolis extracts, with their naturality, environmentally friendly composition, and multiple biological activities, have the potential to become a significant component of sustainable agricultural practices in the future to control postharvest diseases. However, further studies on various fruits and vegetables are needed to integrate this potential into agricultural practice.

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IMPACT OF MASTITIS PATHOGENS ON BLOOD ANTIOXIDANT ENZYMES IN TRANSITION DAIRY COWS

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ABSTRACT

Dairy cows in early postpartum experience significant metabolic stress that increases their susceptibility to mastitis. This study examined the correlation between mastitis pathogens and the activity of superoxide dismutase (SOD) and glutathione peroxidase (GPX) in blood serum. Over a two-year study, udder health and antioxidant enzyme activity were assessed across three physiological periods, from the 21st day before calving up to the 42nd day of lactation. During the trial period, each cow in lactation was observed daily for clinical signs of mastitis. Categorisation of the observed population was done following screening for mastitis using udder clinical examination, the California Mastitis Test, and bacteriological culturing. The measurement of the SOD and GPX activity in blood serum was done with spectrophotometric assays expressed as mU/mg protein. Mastitis pathogens were isolated from 43.83% of udder quarters with milk secretion disorder, and the dominantly isolated microorganisms were grouped as contagious: *Streptococcus agalactiae* (19.14%) and *Staphylococcus aureus* (6.17%) or environmental: *Enterococcus spp.* (8.02%), *Pseudomonas aeruginosa* (7.41%), *Escherichia coli* (1.85%) and *Aspergillus niger* (1.23%). The results indicated that contagious mastitis pathogens had a statistically significant influence on the SOD and GPX activity in the blood serum of transition dairy cows. The relationship between oxidative stress, anti-oxidative enzymes, and mastitis in dairy cows underscores the importance of managing oxidative levels to enhance udder health and prevent mastitis.

Keywords: dairy cows, antioxidative enzymes, mastitis.

INTRODUCTION

The oxide-reduction process in animal organisms is an essential biochemical phenomenon underpinning energy production, detoxification, and signalling. In animal cells, reduction reactions occur predominantly in the mitochondria, where oxygen is sequentially reduced to water through the electron transport chain. At the final step, the enzyme cytochrome c oxidase catalyses the reduction of oxygen, a process that is critical for ATP synthesis and overall cellular energy metabolism (Schieber & Chandel, 2014). Simultaneously, a balance is maintained between oxidation and reduction reactions to prevent the buildup of reactive oxygen species (ROS), thereby protecting cells from oxidative damage (Mladenov et al., 2023). Furthermore, the interplay between reductive and oxidative processes is not only central to metabolic functions but also plays a role in redox signalling. This redox balance is vital, since oxidative stress can lead to cellular dysfunction if not tightly regulated. These

mechanisms are intricately regulated through enzyme-catalysed pathways that maintain redox homeostasis, thereby protecting cells from damage while allowing necessary physiological functions to proceed effectively (Schieber & Chandel, 2014).

Oxidative stress in animal systems is a phenomenon that results from an imbalance between reactive oxygen species (ROS) production and the capacity of antioxidant defences. This imbalance can damage cellular proteins, lipids, and nucleic acids, thereby contributing to various physiological and pathological conditions in animals. When ROS levels exceed the buffering capacity of endogenous antioxidants, oxidative damage occurs, which can disrupt normal cellular function and lead to disease states (Sies, 2015).

Dairy cows, particularly during the peripartum period, exhibit significantly increased oxidative stress levels due to metabolic disturbances, which predispose them to intramammary infections and subsequently mastitis (Turk, 2017).

The most significant connection between oxidative stress and mastitis lies in the inflammatory response. During mastitis, the immune system responds to bacterial invasion by releasing inflammatory mediators and immune cells that produce reactive oxygen species (ROS) as a defence strategy. Although this helps fight infection, an overproduction of ROS can lead to oxidative stress, resulting in tissue damage and amplified inflammation. This harmful cycle can impair immune function, making the cow more vulnerable to further infections. Increases in oxidative products such as malondialdehyde (MDA), an indicator of lipid peroxidation, have been observed in cows with mastitis, reinforcing the notion that these oxidative markers correlate with the severity of the disease (Rizzo et al., 2013; Mavangira et al., 2015). Studies indicate that bacterial endotoxins increase the expression of pro-inflammatory cytokines in mammary tissues, which can elevate oxidative stress levels (Guo et al., 2020). Different mastitis pathogens may elicit varying responses in terms of oxidative stress and antioxidative enzyme activity.

The primary objective of this study was to establish a connection between mastitis pathogens and oxidative stress through the activity of SOD and GPX in blood samples collected from dairy cows during the transition period from late gestation to early lactation.

MATERIAL AND METHOD

In this study, the activity of the antioxidant enzymes SOD (superoxide dismutase) and GPX (glutathione peroxidase) in the blood serum of dairy cows was used as a biomarker of oxidative stress.

During the study, the health status of the mammary gland in early-lactating cows and changes in antioxidant enzyme activity in blood serum were monitored to explore a potential relationship between enzyme activity and udder health.

The blood samples for determination of SOD and GPX activities were collected in three physiological periods (PP): the dry period 21 days before calving (transition period 21 - TP_21), the period from the beginning of lactation until the 21st day in lactation (lactation period 21 - LP_21) and the period from 22nd to 42nd day in lactation (lactation period 42 - LP_42). The methodology was described in detail in the previously published paper by Nakov et al. (2023).

During the trial period, each cow in lactation was observed daily for clinical signs of mastitis. The California Mastitis Test (CMT) was used to detect quarters with abnormal milk secretion. Milk

samples from CMT-positive quarters were aseptically collected for microbiological culturing, following National Mastitis Council guidelines (NMC, 2004). The isolated microorganism species were classified as either contagious or environmental pathogens. Based on clinical examination, CMT results, and bacteriological cultures, cows were categorised into four groups: healthy, those with persistent milk secretion disorder (MSD), and those with intramammary infection (IMI) caused by contagious or environmental pathogens.

The measurement of the SOD and GPX activity in blood serum was done with spectrophotometric assays expressed as mU/mg protein. SOD activity was measured using kinetic analysis according to Gao et al. (1998), while GPX measuring was used method modified according to Chen et al. (2000). The methodology was described in detail in the previously published paper by Nakov et al. (2023).

Multivariate General Linear Model (GLM) was used to determine the influence of mastitis pathogens on changes in SOD and GPX activity in blood serum.

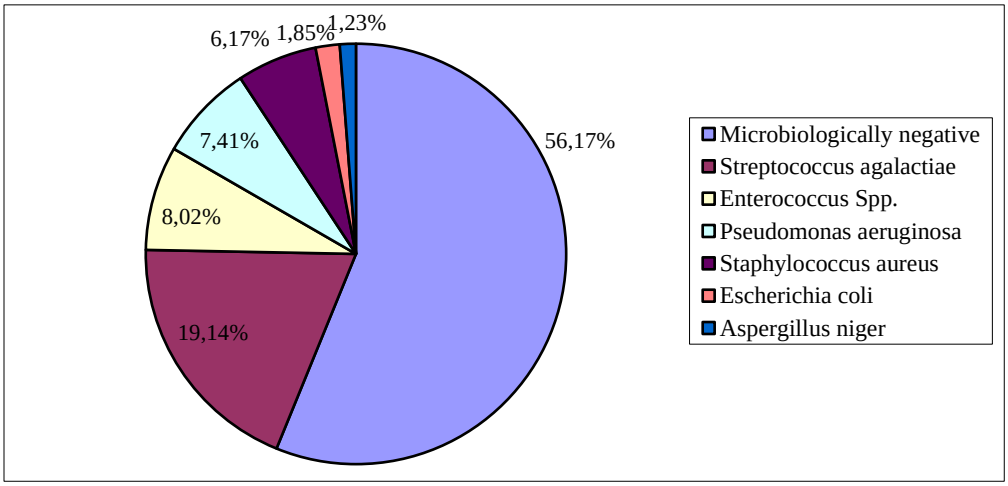
RESULTS AND DISCUSSION

The prevalence of udder health disorders on quarter level, in the observed population of dairy cows in early lactation during the two-year survey is shown in Table 1.

Table 1. The prevalence of udder health disorders in dairy cows on the quarter level in PP of early lactation

LP	**Total**	**CMT(-)**		**CMT(+)**		**MSD**		**IMI**	
	n	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
21	844	765	90,64	79	9,36	45	5,33	34	4,03
42	844	761	90,17	83	9,83	46	5,45	37	4,38

Graph 1 represents the distribution of udder pathogens in milk samples from udder quarters with MSD that showed a positive reaction to CMT.



Graph 1. Udder pathogens isolated in milk sample from quarters with positive CMT

Mastitis pathogens were isolated from 43.83% of udder quarters with MSD, and the dominantly isolated microorganisms were grouped as contagious: *Streptococcus agalactiae* (19.14%) and *Staphylococcus aureus* (6.17%) or environmental: *Enterococcus spp.* (8.02%), *Pseudomonas aeruginosa* (7.41%), *Escherichia coli* (1.85%) and *Aspergillus niger* (1.23%).

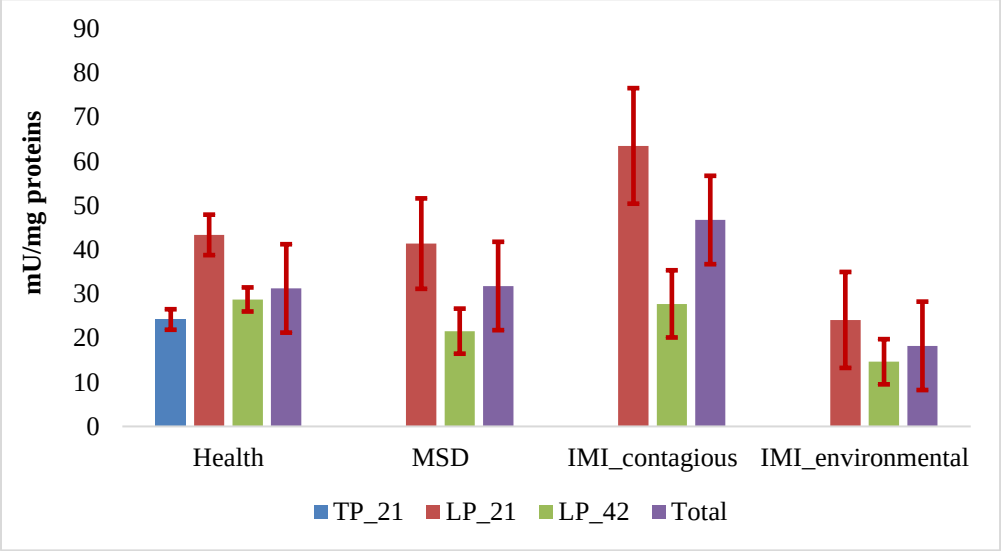
Given that IMIs typically trigger an influx of leukocytes and other macrophages into milk, an elevated SCC has been extensively used as an indicator of mastitis. While higher CMT scores generally indicate increased SCC levels, the accuracy of CMT or SCC in reflecting pathogen-specific IMIs remains uncertain (Dingwell et al., 2003). The concordance between CMT results and bacteriological findings ranges from 70 to 86%, varying with the causative agent (Sanford et al., 2006).

The changes in the activity of antioxidant enzymes SOD and GPX in three PP from late gestation to early lactation, related to udder health status are shown in Graphs 1 and 2.

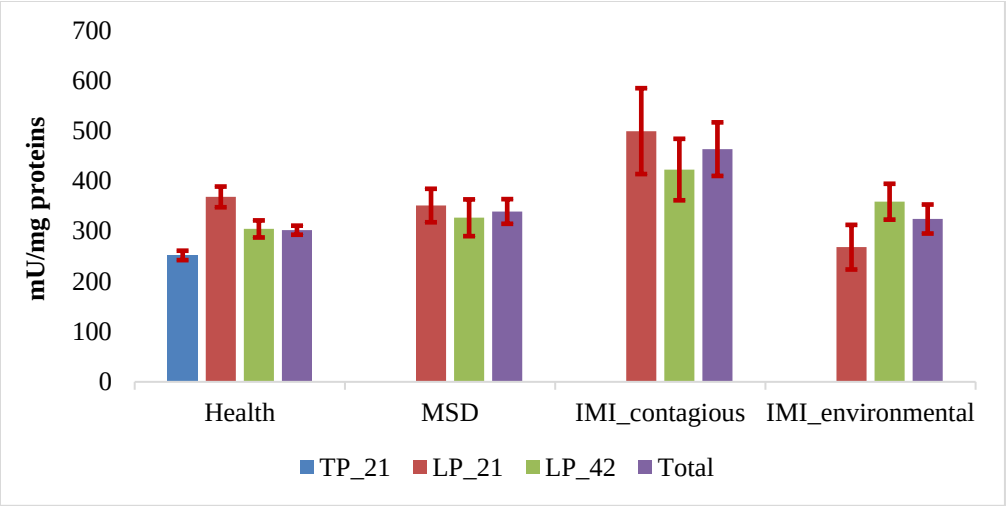
Contagious mastitis pathogens have increased the average activity of SOD in blood, especially in LP_21. Environmental mastitis pathogens did not increase SOD activity in blood compared to mastitis-free cows and those with MSD; instead, SOD activity was reduced.

As shown in Graph 2, the average GPX activity was higher in the blood of cows with IMI caused by contagious mastitis pathogens. The charts indicate that SOD and GPX activity in blood is increasing when IMI caused by major mastitis pathogens is existing.

The escalated activity of SOD and GPX observed in the period after parturition was probably a consequence of the increased synthesis of ROS triggered by the immune response to mastitis pathogens. Previously published paper indicates that the transition period in dairy cows has a statistically significant influence ($p<0.001$) on SOD and GPX activity in blood serum (Nakov et al., 2023). This imbalance in antioxidant enzyme activity increases the risk of oxidative stress during this critical period.



Graph 1. Average activity of SOD (mU/mg proteins) in blood serum related to the health status of cows' mammary gland



Graph 2. Average activity of GPX (mU/mg proteins) in blood serum related to the health status of cows' mammary gland

Table 3. Regression model for the influence of mastitis pathogens on SOD and GPX activity in blood from transition dairy cows

Fixed variable	**df**	**SOD[a]**	**GPX[b]**
Model[a,b]	4	88.001***	365.692***
Mastitis pathogen	4	88.001***	365.692***
Error	629		
Total	633		
[a]R^2 = 0.355; [b]R^2 = 0.697			

***Significant at level p<0.001;

The results indicated that the presence of mastitis pathogens in the milk during the period of early lactation has a statistically significant influence (p<0.001) on the SOD and GPX activity in the blood serum of transition dairy cows. When mastitis afflicts a dairy cow, whether clinical or subclinical, the immune system responds to the invading pathogens (usually bacteria) by releasing various inflammatory molecules and immune cells. Moreover, inflammation and oxidative stress appear to mutually influence each other in the context of mastitis, ultimately fostering a vicious cycle where inflammation leads to increased oxidative stress, which in turn further intensifies inflammation (Turk, 2017). This immune response generates ROS as a part of the body's defence mechanism to eradicate pathogens. Different pathogens may elicit varying responses in terms of oxidative stress and antioxidative enzyme activity (Erisir et al., 2006; Kleczkowski et al., 2008). To counterbalance the potentially harmful effects of ROS, cows have antioxidative defence mechanisms in place. Antioxidative enzymes SOD, GPX and CAT help neutralize ROS and maintain a balanced oxidative state within the body. These enzymes play a crucial role in protecting cells and tissues from oxidative damage. Because SOD activity increases the production of hydrogenated peroxides by dismutation of superoxide, protection against ROS would be achieved only with the increased GPX activity and hydrogenated peroxides reduction (Maurya et al., 2014). The increased activity of antioxidant enzymes in dairy cows that have mastitis in the period of early lactation may indicate that oxidative stress is exacerbated. Elevated somatic cell counts (SCC), which are indicative of mastitis, have also been correlated with increased oxidative stress markers, emphasizing the detrimental effects that oxidative stress has on udder health (She et al., 2023).

CONCLUSIONS

In dairy cattle, oxidative stress is a critical factor during transition periods like parturition and lactation. During these phases, metabolic demands intensify, leading to altered redox status; consequently, imbalances between oxidant and antioxidant systems can predispose animals to metabolic and infectious diseases. The relationship between oxidative stress, anti-oxidative enzymes, and mastitis in dairy cows underscores the importance of managing oxidative levels to prevent mastitis. Nutritional interventions and management practices that bolster antioxidant defences may be an essential strategy to mitigate oxidative damage and improve udder health, dairy production and animal welfare.

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A WEARABLE 3D-GLOVE SYSTEM FOR HYPERSPECTRAL AND CONTACT IMAGING OF ROOT AND RHIZOSPHERE MICROBIOMES : A CONCEPTUAL DESIGN

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ABSTRACT

Monitoring root systems and their associated rhizosphere microbiomes is critical for advancing sustainable agriculture and understanding plant-microbe interactions. Traditional microrhizotron systems rely on rigid transparent tubes and fixed cameras, which restrict flexibility and often limit imaging angles. We propose two novel wearable glove-based designs that address these limitations by enabling non-destructive, in situ root and microbial rhizosphere detection with enhanced mobility and spectral sensing capabilities. The first design integrates a 360° hyperspectral camera controlled by miniaturized geared stepper motors mounted on a wearable glove, allowing dynamic panoramic imaging around root zones. The second design employs Contact Image Sensors (CIS) embedded along the glove's palm and fingers for high-resolution, direct-contact scanning of root surfaces and biofilms. This conceptual design paper presents the architecture, components, expected functionality, and potential agricultural and research applications of these gloves. We also discuss their complementary phenotypic differences, limitations, and future prototyping plans.

INTRODUCTION

Roots and their surrounding rhizosphere host complex microbial communities that profoundly influence plant health and soil fertility. Non-invasive monitoring of these underground interactions is vital for advancing precision agriculture, biofertilizer evaluation, and plant breeding. Microrhizotrons, transparent tubes inserted into soil to observe roots and microbes, have long been used but are constrained by fixed cameras and limited viewing angles. Recent advances in wearable sensing technologies suggest that a **glove-based microrhizotron system** could provide unprecedented flexibility and real-time root phenotyping. Here, we present two independent conceptual designs for such a system: (1) a glove equipped with a geared stepper motor-controlled 360° hyperspectral camera and (2) a glove embedded with Contact Image Sensors (CIS). Both aim to facilitate non-destructive, multi-scale root and rhizosphere imaging.

Related Work

Microrhizotron systems traditionally utilize rigid transparent tubes with fixed or movable cameras to capture root images (Nagel et al., 2012). However, their limited mobility and fixed viewing geometry restrict phenotyping flexibility. Hyperspectral imaging has been applied in root phenotyping to detect biochemical and structural changes (Behmann et al., 2015), but integration into wearable devices remains unexplored.

Contact Image Sensors (CIS) have been used in document scanning and recently adapted for plant surface scanning (Smith et al., 2020). Combining hyperspectral imaging with wearable sensors could

bridge the gap between remote sensing and direct contact phenotyping. Our designs are inspired by stepper motor-driven microrhizotron camera systems (e.g., [PMC7038518](https://pmc.ncbi.nlm.nih.gov/articles/PMC7038518/)) and advances in flexible sensor integration.

Design Concept

Design 1: Geared Stepper Motor-Controlled 360° Hyperspectral Camera Glove

This design features a miniaturized 360° hyperspectral camera mounted on the glove's palm or fingertip. Miniature geared stepper motors housed in the wrist or cuff control rotational motion of the camera probe, enabling dynamic scanning of root zones from multiple angles (figure 1).

(i) Camera Specs: Hyperspectral module covering 400–1000 nm wavelengths, capable of detecting biochemical signatures such as flavonoids, chlorophyll, and microbial metabolites.
(ii) Stepper Motors: Low-torque geared stepper motors provide precise angular control, allowing panoramic scanning.
(iii) Data Handling: Wireless transmission to a handle device or cloud storage for real-time processing.
(iv) Power:Compact rechargeable battery embedded in the glove cuff.

Design 2: Contact Image Sensor (CIS) Embedded Glove

The second design integrates linear CIS arrays along the glove's palm and fingers, enabling direct-contact scanning of root surfaces and microbial biofilms.

(i) Sensors:RGB+NIR CIS arrays similar to document scanners, providing high spatial resolution line scanning.
(ii) Operation:User moves the glove across transparent rhizobox surfaces or accessible root exposures.
(iii) Spectral Range: Limited compared to hyperspectral cameras but sufficient for detecting surface features and pigment changes.
(iv) Data Collection: Continuous scanning with onboard microcontroller; data streamed wirelessly.

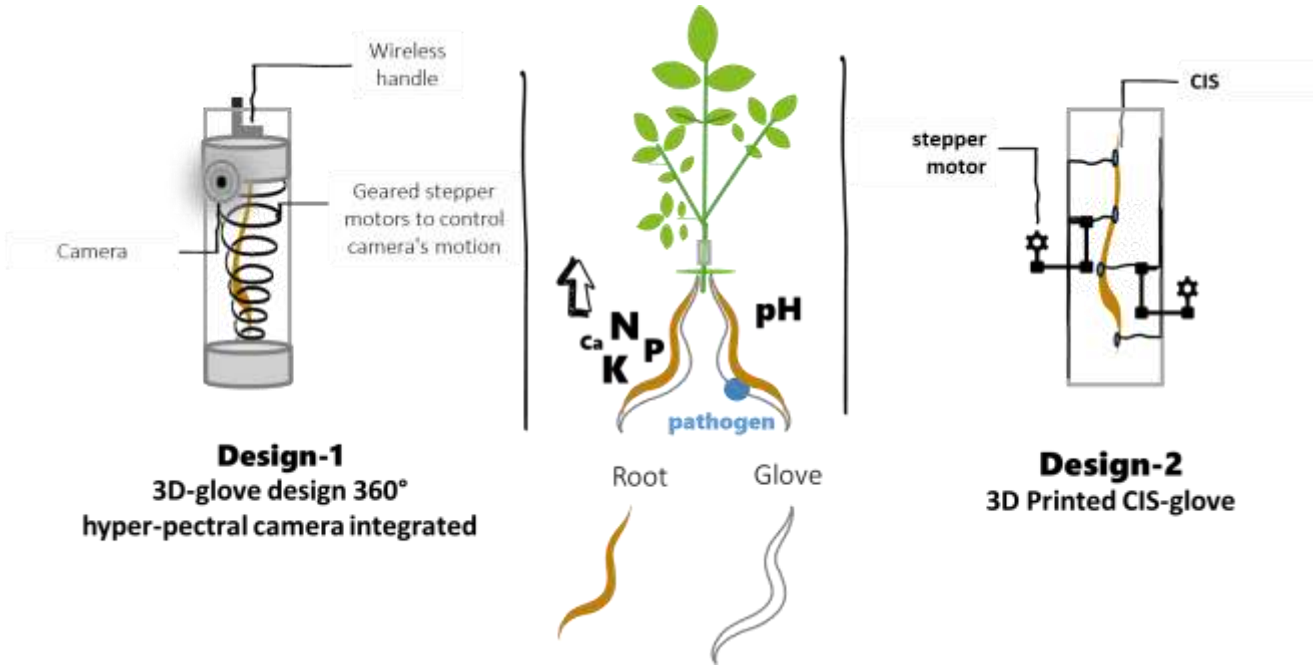

figure 1 : Illustration of two glove-mounted systems for in-situ rhizosphere observation

Technical Details

Component Selection

Unraveling the complexity of root phenotyping demands tools that can capture both spatial and biochemical intricacies with high precision. Design 1 exemplifies this by integrating a motorized system capable of rotating the hyperspectral camera with an angular resolution of approximately 1° and a scan speed of 10° per second, enabling rapid, comprehensive 360° coverage of the rhizosphere. Its spectral resolution of about 5 nm facilitates the detection of subtle physiological and biochemical shifts in root and microbial tissues, offering a three-dimensional hyperspectral map that bridges structural and functional insight (table 1). Such granularity empowers researchers to monitor dynamic microbial colonization and root stress responses with unprecedented detail (Ustin et al., 2004 ; Behmann et al., 2015 ; Walter et al., 2015).

In contrast, Design 2 leverages a Contact Image Sensor with a high spatial resolution of 600 dpi along a linear scan width of roughly 5 cm. Though spectrally limited to broad RGB and near-infrared bands, its simplicity, extended battery life of approximately 3 hours, and portability render it ideal for rapid surface phenotyping and microbial film detection in transparent rhizoboxes. Together, these technologies represent a complementary spectrum of phenotyping tools: one delivering depth through rich spectral and angular resolution, the other breadth through ease of use and extended operational duration. Collectively, they herald new possibilities for scalable, field-adapted root phenotyping that can accelerate advances in plant–microbe research and sustainable agriculture (Fiorani & Schurr, 2013 ; Wasson et al., 2020).

Both designs interface wirelessly with smartphones or tablets running custom apps for real-time visualization, spectral analysis, and data logging. The stepper motor glove supports gesture controls for scanning commands.

Table 1 : Component-level comparison of two wearable root imaging glove designs

Component	Design 1 (Stepper + Camera)	Design 2 (CIS Glove)
Imaging Sensor	Mini hyperspectral camera (400–1000 nm)	RGB + NIR Contact Image Sensor Arrays
Motion Control	Miniature geared stepper motors	Manual hand motion
Processing Unit	ESP32 microcontroller or similar	Low-power MCU for line scan processing
Power Source	Rechargeable Li-ion battery (cuff)	Small rechargeable battery or tethered
Wireless Communication	Wi-Fi or Bluetooth module	Bluetooth Low Energy (BLE)
Lighting	Integrated LED excitation for fluorescence	External or integrated LED strips

Comparative Phenotypic Analysis

To clearly differentiate the two glove designs, Table 1 presents a detailed phenotypic comparison across key parameters including imaging mode, spectral range, resolution, power consumption, and application suitability (table2)

Table 2 : Functional and performance-based comparison of hyperspectral and CIS glove systems for root phenotyping

Category	**Parameter**	**Design 1: Stepper Motor + 360° Hyperspectral Camera**	**Design 2: Contact Image Sensor (CIS) Glove**
Optical Performance	Spectral Range	Broad hyperspectral: 400–1000 nm (visible to near-infrared), ±5 nm spectral resolution [1]	RGB + NIR bands (~400–900 nm), limited spectral resolution (~10 nm) [2]
	Spatial Resolution	~50–100 µm/pixel (depends on optics and sensor size)	High: ~10–20 µm/pixel (scanner-grade linear sensor)
	Field of View (FOV)	Full 360° rotation enabling panoramic scanning of roots	Linear line scan ~5 cm width per pass
	3D Imaging Capability	True 3D surface mapping via motorized rotation	2D imaging only; 3D reconstruction possible by stitching multiple scans
	Fluorescence Imaging Potential	High; excitation LEDs and filters can be integrated	Limited; depends on CIS sensitivity and additional illumination
Mechanical & Power	Mobility & Wearability	Moderate; motors and battery add weight and bulk (~250 g estimate)	High; lightweight (~100 g), low power consumption
	Power Consumption	Higher (~2–3 W continuous operation for motors + camera)	Low (~0.5 W), suitable for extended scanning sessions
	Data Acquisition Speed	Slower; full 360° scan in 30–60 s depending on stepper speed	Fast; real-time line scanning during manual movement
	Operational Environment	Suitable for structured rhizoboxes and controlled soil cores	Best for transparent rhizoboxes or accessible root surfaces
	Durability & Robustness	Moderate; mechanical parts sensitive to dust/moisture, requires casing	High; solid-state sensors easier to protect and maintain
Data & Processing	Data Volume & Complexity	High; hyperspectral data generates large files (100s MB per scan)	Moderate; line scans generate smaller data (~MB scale)
	Processing Requirements	Advanced spectral unmixing and machine learning needed	Simpler image processing; color and NIR analysis

	Calibration Needs	Frequent spectral and motor calibration necessary	Lower; mainly sensor calibration
	Wireless Connectivity	Wi-Fi/Bluetooth; real-time streaming possible	Bluetooth Low Energy (BLE); low latency streaming
User Interaction	Ease of Use	Moderate; requires motor control interface (app or gestures)	High; intuitive manual scanning
	Training Required	Medium; user needs to understand motorized scanning	Low; natural hand movements sufficient
Cost & Scalability	Estimated Cost	High; hyperspectral cameras and motors cost $2000–5000+	Moderate; CIS sensors and microcontrollers ~$500–1000
	Scalability for Field Use	Challenging; power and mechanical complexity	Easy; low power and simple design suited for wide deployment
Application Suitability	Key Strengths	Multi-angle biochemical root phenotyping, microbial metabolite detection	High-resolution root surface imaging, biofilm and exudate detection
	Limitations	Bulkier design, limited soil ruggedness	Limited spectral range, requires accessible surfaces

Use Cases

- Precision Agriculture: Early detection of root pathogens, monitoring biofertilizer colonization, and drought stress via root spectral signatures.-

- Research: Phenotyping root architecture and microbiome dynamics in transparent rhizoboxes and soil cores.

- Education: Hands-on tool for plant biology classes to visualize underground root processes.

- Biofertilizer Development: Tracking mycorrhizal colonization efficiency on roots in situ.

Advantages and Limitations (table 3 below)

Design	Advantages	Limitations
Stepper-Motor + 360° Hyperspectral Camera Glove	• Non-destructive, in-situ imaging of root zones.• Panoramic (360°) scanning around roots for true 3-D mapping.• Captures biochemical/spectral signatures (stress, metabolites).• Custom-configurable scan speed and spectral bands.• Real-time data streaming and AI-ready datasets.• Suited to structured systems (rhizoboxes, root tubes).	• High component cost (hyperspectral sensor + motors).• Bulkier; reduced hand mobility and comfort.• Dust/moisture sensitive; needs protective casing.• Power-hungry; battery life < 2–3 h continuous use.• Requires regular camera/motor calibration.• Large hyperspectral files demand heavy processing/storage.• Less practical in loose, unstructured field soils.
Contact Image Sensor (CIS) Glove	• Lightweight, highly wearable; full finger dexterity.• High-resolution surface scans (≈10–20 µm/pixel).• Low power; extended battery life.• Lower cost; uses off-the-shelf CIS arrays.• Simple to operate; minimal user training.• Immediate visual feedback via phone/tablet.• Ideal for quick phenotyping in transparent rhizoboxes.	• Must touch root or clear surface—no remote imaging.• Limited spectral range (RGB + NIR only).• Ineffective in opaque soil or deep root tubes.• Sensor can foul from soil debris/exudates.• Scan quality depends on steady manual motion.• No inherent 3-D capability (requires post-processing).• Less sensitive to subsurface microbial activity.

CONCLUSION

We present two complementary conceptual designs for wearable 3D gloves capable of hyperspectral and contact imaging of root systems and rhizosphere microbes. By combining advanced sensors with wearable mobility, these devices promise flexible, non-invasive phenotyping tools that overcome limitations of current microrhizotron systems. This work lays the foundation for novel, user-friendly technologies to support sustainable agriculture and plant science research.

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ENVIRONMENTAL IMPACTS OF GREENHOUSE GAS EMISSIONS AND PROPOSED SOLUTIONS FOR THEIR REDUCTION

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ABSTRACT

Greenhouse gas (GHG) emissions are among the primary causes of global warming and climate change. Gases such as carbon dioxide (CO_2), methane (CH_4), and nitrous oxides (N_2O) originate from human-induced activities, including fossil fuel consumption, industrial processes, agriculture, and deforestation. The accumulation of these gases in the atmosphere leads to rising temperatures, glacier melting, sea level rise, and an increase in extreme weather events.

This study explores various solutions to mitigate greenhouse gas emissions. Transitioning to renewable energy sources, such as solar, wind, and hydroelectric power, enhancing energy efficiency, promoting the widespread adoption of electric and hybrid vehicles in transportation, implementing sustainable agricultural practices, and initiating forest conservation projects are among the key strategies. Additionally, carbon capture and storage (CCS) technologies, alongside changes in individual consumption habits such as opting for low-carbon footprint products and increasing recycling efforts play a crucial role in reducing emissions.

Keywords: Greenhouse gas, global warming, industrial process, renewable energy

INTRODUCTION

Greenhouse gas (GHG) emissions represent one of the most critical environmental issues confronting the 21st century. This study investigates the ecological consequences associated with GHG emissions and evaluates a range of mitigation strategies aimed at reducing their impact. Particular focus is directed toward carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O), which constitute the primary anthropogenic greenhouse gases. The paper further discusses sustainable mitigation approaches, such as the deployment of renewable energy sources, enhancement of energy efficiency, implementation of carbon capture technologies, and large-scale reforestation efforts (Chen et al., 2023).

Greenhouse gases contribute to the retention of heat within the Earth's atmosphere, thereby exacerbating the greenhouse effect and elevating global temperatures. Although this natural phenomenon is vital for sustaining life on Earth, anthropogenic emissions have significantly amplified its intensity, culminating in global warming and widespread climate disruption. Key drivers of GHG emissions include industrial processes, deforestation, and agricultural practices (Jensen et al., 2023). The resulting rise in global temperatures has led to an increase in the frequency and severity of heatwaves, as well as accelerated polar ice melt and thermal expansion of ocean waters, collectively contributing to sea level rise and heightened flooding risks (Aghakouchak et al., 2020).

Additionally, coastal marine ecosystems are increasingly threatened by anthropogenic activities, such as industrialization, agriculture, land-sea based aquaculture, and climate change. These pressures have led to coastal eutrophication, which is a major driver of macroalgal blooms (Smetacek and Zingone, 2013).

Macroalgal bloom events—such as green and golden seaweed tides—induce profound shifts in both the physical and biogeochemical characteristics of the water column, thereby exerting considerable influence on greenhouse gas (GHG) emissions. Empirical evidence indicates that microalgal blooms can markedly decrease atmospheric carbon dioxide (CO_2) concentrations as a result of intensified photosynthetic activity. Concurrently, these events often stimulate the production of nitrous oxide (N_2O) and methane (CH_4), primarily through mechanisms involving the degradation of particulate organic matter (POM) and the emergence of hypoxic or anoxic conditions within marine systems (Cai et al., 2024).

The accelerating pace of climate change continues to pose serious threats to both human and ecological health (Romanello et al., 2022). In response to this escalating crisis, achieving the targets outlined in the Paris Climate Agreement and the Kigali Amendment to the Montreal Protocol necessitates a substantial and immediate reduction in global greenhouse gas emissions (Intergovernmental Panel on Climate Change, 2023).

According to estimates by the Food and Agriculture Organization of the United Nations (FAO), emissions from the agricultural sector account for approximately 30% of total global greenhouse gas (GHG) emissions. The predominant greenhouse gases emitted from agricultural soils include carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) (Chataut et al., 2023).

In agricultural systems, both natural factors—such as soil moisture, temperature, and pH—and anthropogenic farming practices exert significant influence over the magnitude of GHG emissions. A wide body of research has investigated various strategies to mitigate these emissions. One promising approach involves the development and cultivation of crop varieties with enhanced capacities for carbon and nitrogen uptake. Such improvements can increase the efficiency of nutrient use, reduce

dependence on synthetic fertilizers, and consequently decrease GHG emissions (Johansson et al., 2023).

Furthermore, optimizing fertilization practices—including the timing, quantity, and type of fertilizers applied—has been demonstrated to be an effective measure for controlling greenhouse gas emissions associated with agricultural activities (Li et al., 2023).**2.**

Environmental Impacts of Greenhouse Gas Emissions

Climate Change and Global Warming

The most direct and severe consequence of GHG emissions is global warming. Average global temperatures have increased significantly since the industrial revolution, resulting in more frequent and intense heatwaves, droughts, and wildfires.

Climate change refers to long-term shifts and alterations in temperature, precipitation patterns, wind, and other elements of the Earth's climate system. Global warming, on the other hand, is the rise in the average surface temperature of the Earth due to increasing concentrations of greenhouse gases in the atmosphere (Koch et al., 2013).

Causes of Global Warming

The primary cause of global warming is human activity, particularly the burning of fossil fuels such as coal, oil, and natural gas. These activities release large amounts of greenhouse gases (especially carbon dioxide – CO_2, methane – CH_4, and nitrous oxide – N_2O) into the atmosphere. Other contributing factors include:

-Deforestation, which reduces the planet's capacity to absorb CO_2.

-Industrial processes that emit large quantities of GHGs.

-Agriculture, especially livestock farming which releases methane.

-Waste management practices that lead to the release of methane and other gases (Holgerson and Raymond, 2016).

Melting Ice Caps and Rising Sea Levels

The warming climate causes glaciers and polar ice caps to melt, contributing to rising sea levels. This threatens coastal ecosystems and human settlements, especially in low-lying countries and island nations.

One of the most visible and alarming consequences of global warming is the accelerated melting of polar ice caps and glaciers. This process directly contributes to rising sea levels, which pose serious threats to both natural ecosystems and human settlements, particularly in coastal and low-lying areas (Ren et al., 2020).

The treason of the melting of the ice caps can be described as; global temperatures rise. This means, Arctic sea ice is shrinking, especially during summer months. Land-based ice sheets and glaciers, such as those in Greenland and Antarctica, are losing mass rapidly. Melting land ice flows into oceans, increasing sea levels. Thermal Expansion means ocean temperatures increase, seawater expands. This thermal expansion accounts for nearly half of the observed sea level rise over the past century. Unlike sea ice, land-based ice adds new water to the ocean when it melts. The vast ice sheets in Greenland and Antarctica are currently the largest contributors to sea level rise from melting ice (Xie et al., 2021).

Current and Future Threats

Coastal Cities at Risk: Major urban areas like New York, Miami, Jakarta, Mumbai, and Istanbul face increased flooding and infrastructure damage. Saltwater intrusion can also contaminate freshwater supplies and damage agriculture.

Small Island Nations in Danger: Countries such as the Maldives, Tuvalu, and Kiribati are threatened with complete submersion if sea levels continue to rise, potentially creating the world's first "climate refugees."

Coastal Erosion and Habitat Loss: Rising seas accelerate erosion, submerge wetlands, and destroy mangroves and other critical coastal ecosystems.

Climate Migration: As coastal regions become uninhabitable, millions may be forced to migrate inland, leading to social, economic, and political challenges (Liu et al., 2021).

Scientific Projections: Over the 20th century, sea levels rose by approximately 15–20 centimeters (6–8 inches).

According to the Intergovernmental Panel on Climate Change (IPCC), sea levels are projected to rise by 0.6 to 1.1 meters (2 to 3.6 feet) by 2100 if greenhouse gas emissions continue at current levels.

Solutions and Adaptation Measures

Reducing greenhouse gas emissions can slow down global warming and ice melt. Coastal protection infrastructure, such as seawalls, flood barriers, and artificial islands, can help shield vulnerable areas. Climate adaptation policies must be developed, including strategic urban planning and relocation strategies for at-risk populations (Sheng et al., 2018).

Ocean Acidification

Excess CO_2 dissolves into oceans, lowering pH levels and disrupting marine ecosystems. Coral reefs, which are home to many marine species, are particularly vulnerable.

Ocean acidification refers to the ongoing decrease in the pH of the Earth's oceans, caused primarily by the uptake of carbon dioxide (CO_2) from the atmosphere. As anthropogenic CO_2 emissions have increased, a significant portion—approximately 30%—has been absorbed by the world's oceans. This

process has altered the chemical composition of seawater, posing severe risks to marine biodiversity, ecosystems, and human societies dependent on oceanic resources (Alcaraz-Rocha et al., 2023).

$CO2(aq)+H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^- \rightleftharpoons 2H^+ + CO_3^{-2}$

This sequence results in the formation of carbonic acid (H_2CO_3), which dissociates to release hydrogen ions (H^+), lowering the pH and increasing ocean acidity. As a consequence, the availability of carbonate ions (CO_3^{2-})—which are essential for marine calcifying organisms to form shells and skeletons—decreases.

Ecological Impacts

Coral Reef Degradation: Coral reefs are particularly sensitive to changes in pH. Ocean acidification weakens coral skeletons and inhibits reef growth, which in turn affects the vast array of marine life that depends on reef ecosystems for habitat and food (Hoegh-Guldberg et al., 2007).

Food Web Disruption: Many planktonic species form the foundation of marine food webs. The decline in their populations due to acidification can have cascading effects on higher trophic levels, including commercially important fish species (Yudhistira et al., 2023).

Impact on Marine Biodiversity and Fisheries: As marine ecosystems become destabilized, biodiversity loss is likely to accelerate. This endangers not only ecological balance but also the livelihoods of millions of people who rely on fisheries and aquaculture (Hollowed et al., 2013).

Socioeconomic Consequences

Food Security Risks: Communities that depend on shellfish and coral reef fisheries are particularly vulnerable to declining stocks and ecosystem collapse (Sovacool and Mukherje, 2011).

Economic Losses: The global seafood industry may face significant economic setbacks. Coral reef tourism, valued at billions of dollars annually, is also at risk (Kuznet, 2019).

Biodiversity Loss

Biodiversity loss refers to the decline or disappearance of biological diversity at the genetic, species, and ecosystem levels. As a result of anthropogenic climate change primarily driven by the emission of greenhouse gases (GHGs) natural habitats are increasingly subjected to environmental stressors that alter ecological balances, disrupt species interactions, and undermine ecosystem resilience. Among these stressors, rising temperatures, altered precipitation patterns, ocean acidification, and habitat degradation are particularly detrimental to biodiversity (Isbell et al., 2013).

Mechanisms of Biodiversity Loss Due to Climate Change

Habitat Alteration and Fragmentation: Shifts in temperature and precipitation can transform ecosystems, rendering them unsuitable for species that are highly specialized or geographically restricted. Alpine, polar, and coral reef ecosystems are especially vulnerable to these changes. Habitat

fragmentation also limits species' ability to migrate in response to changing environmental conditions (Liu et al., 2023).

Consequences for Ecosystem Functioning: Biodiversity plays a critical role in ecosystem stability, productivity, and the provision of essential services such as pollination, water purification, carbon sequestration, and soil fertility. Loss of species richness and genetic diversity can reduce an ecosystem's capacity to respond to environmental changes and recover from disturbances, increasing the risk of ecological collapse (Tong et al., 2019).

Agricultural Disruption

Agricultural disruption refers to the adverse effects of climate change on crop production, livestock systems, soil health, and overall agricultural productivity. As global temperatures rise and climatic variability intensifies, agricultur the foundation of global food security and rural economies faces multifaceted challenges. These include altered growing seasons, increased frequency of extreme weather events, changing precipitation patterns, and the proliferation of pests and diseases. Such disruptions threaten the stability of food systems, particularly in regions already vulnerable to environmental and socio-economic stressors (Rees et al., 2014).

Climatic Stressors Impacting Agriculture

Temperature Increases: Higher average temperatures can accelerate crop maturation, leading to reduced yields. Heat stress also negatively affects livestock health, reproduction, and productivity. For many staple crops such as wheat, rice, and maize, there is a critical threshold beyond which yields decline substantially (Ma et al., 2020).

Extreme Weather Events: More frequent and severe heatwaves, storms, and frosts can cause sudden and severe damage to crops and infrastructure. These events not only reduce agricultural output but also increase production costs and risks for farmers (Wohlfart et al., 2008).

Increased Pest and Disease Incidence: Rising temperatures and humidity create favorable conditions for the spread of pests, fungi, and crop diseases, which were previously confined to specific geographic regions. This increases the vulnerability of food systems and requires greater dependence on chemical inputs (Méndez-Bautista et al., 2009).

Soil Degradation: Changes in temperature and precipitation, along with intensive agricultural practices, contribute to soil degradation, reducing fertility and the capacity to support sustainable farming (Cruz et al., 2020).

Regional Disparities and Vulnerabilities: Developing countries, particularly those in tropical and subtropical regions, are more severely affected due to limited adaptive capacity, high dependence on rain-fed agriculture, and constrained access to technology and infrastructure. Smallholder farmers, who represent a large portion of the global agricultural workforce, face increased livelihood insecurity and food scarcity as a result of climate-induced disruptions (Jia et al., 2018).

Adaptation and Resilience Strategies

Climate-Smart Agriculture (CSA): CSA practices aim to sustainably increase productivity, enhance resilience (adaptation), and reduce greenhouse gas emissions. This includes crop diversification, agroforestry, conservation agriculture, and efficient water management (nazim et al., 2025).

Technological Innovation: The development and dissemination of drought-resistant and heat-tolerant crop varieties, precision farming technologies, and early-warning systems can support adaptation (Lybbert and Sumne, 2012).

Policy and Institutional Support: Governments and international organizations must invest in agricultural research, rural infrastructure, extension services, and risk insurance schemes to support vulnerable farming communities.

Integrated Land and Water Management: Holistic approaches that conserve natural resources and promote sustainable land use are essential to long-term agricultural resilience.

DISCUSSION AND CONCLUSION

Greenhouse gas emissions pose a critical threat to the environment and to human societies. However, through coordinated global action and the implementation of innovative solutions, their negative impacts can be mitigated. Transitioning to a low-carbon future is not only possible but necessary to ensure the health of the planet for future generations.Multiple factors influence the impact of flooding on greenhouse gas emissions, including soil type, water source, and flood duration.

Agricultural disruption is a critical dimension of climate change with profound implications for global food systems, rural livelihoods, and economic stability. Urgent and coordinated adaptation strategies are necessary to ensure the sustainability and resilience of agriculture in a warming world.

Replacing fossil fuels with renewable sources such as solar, wind, and hydropower is one of the most effective ways to reduce GHG emissions. Improving the energy efficiency of buildings, transportation, and industrial processes can significantly cut emissions while reducing energy costs.

CCS technology captures CO_2 from power plants and industrial sources and stores it underground, preventing it from entering the atmosphere. Trees absorb CO_2 through photosynthesis. Large-scale tree planting can act as a natural carbon sink, helping offset emissions. Practices such as crop rotation, reduced tillage, and efficient fertilizer use can lower emissions from agricultural activities, especially methane and nitrous oxide.

Global cooperation through agreements like the Paris Accord is essential. Governments should implement carbon pricing, subsidies for clean energy, and stricter emission regulations.

In conclusion, the reduction of greenhouse gas emissions should not be confined solely to measures taken by governments and large corporations; rather, it should be recognized as a global responsibility

in which individuals must also play an active role. While governments and businesses can make significant contributions by investing in renewable energy, enforcing strict environmental regulations, and implementing policies to limit carbon emissions, individuals can support this process by adopting eco-friendly habits in their daily lives. Key individual actions include conserving energy, opting for sustainable transportation methods, embracing recycling practices, making conscious consumption choices, and preferring products that minimize greenhouse gas emissions. In this context, the adoption of sustainable policies in both production and consumption, the promotion of innovative environmental technologies, and raising public awareness will be crucial in mitigating the devastating effects of climate change.

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THE EFFECTS OF GLOBAL WARMING ON THE WATER QUALITY OF LAKES AND RIVERS

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ABSTRACT

Global warming has emerged as a critical driver of change in freshwater ecosystems, exerting multifaceted influences on the physical, chemical, and biological characteristics of freshwater systems. Rising air temperatures cause to increase in surface water temperatures, which reduces the solubility of dissolved oxygen and intensifies thermal stratification, promoting hypoxic or anoxic conditions in deeper layers.. Enhanced evaporation rates and altered precipitation patterns exacerbate seasonal and annual fluctuations in water levels, concentrating contaminants and reducing the self-purification capacity of lotic systems. Concurrently, elevated runoff during extreme rainfall events transports greater loads of nutrients and associated agrochemicals into water bodies, fueling harmful algal blooms and promoting toxin production by cyanobacteria. Intensified drought periods further increase pollutant concentrations and facilitate the proliferation of invasive or opportunistic species, weakening local biodiversity and destabilizing trophic interactions. These changes collectively threaten aquatic ecosystem services, compromise drinking-water quality, and increase treatment costs, while also posing direct risks to human and animal health through exposure to algal toxins and waterborne pathogens.

This review synthesizes recent findings on the mechanisms by which climate warming is changing freshwater quality and identifies key knowledge gaps. Finally, it outlines adaptive management strategies such as enhanced monitoring networks, watershed-scale nutrient management, restoration of riparian buffers, and controlled flow regimes to mitigate adverse outcomes and enhance the resilience of inland water resources in a warming world.

Keywords: Global warming, lakes, rivers, water quality

INTRODUCTION

Freshwater ecosystems provide drinking water, facilitate food production, and provide the foundation for energy production and biodiversity. Anthropogenic climate change is transforming the hydrology and water quality of lakes and rivers by altering key climatic drivers such as temperature, changes in precipitation patterns, evaporation, snow and glacier melt, and sea level. Intensification of the hydrological cycle (e.g., higher atmospheric humidity and more intense precipitation per degree of warming), earlier snowmelt, and increased terrestrial evaporation are observed with high confidence, along with regionally heterogeneous drying/wetting patterns that shape water quality trajectories through both chronic stresses and extreme events (IPCC WGII). This review integrates mechanistic understanding with recent global syntheses to map how warming alters water temperature regimes, stratification and mixing, oxygen budgets, nutrients, pollutants, and biota, leading to measurable deterioration of water quality indicators and ecosystem services. We highlight the cross-scale interactions between climate forcing, watershed land use, and management (Garrabou et al., 2009; Pershing et. al., 2018; Woolway et al., 2022).

Mechanistic Pathways: From Warming to Water Quality

Thermal Forcing and Lake Stratification

The latest alterations in the global carbon balance and the associated climate tendencies in the thermal and ice dynamics of inland water bodies raise new essential inquiries regarding energy and substance transfer within aquatic systems. Rising air temperatures warm lake surfaces, increase stability, and lengthen stratified periods. Stronger and longer stratification suppresses vertical mixing, reduces oxygen replenishment of bottom waters, and reinforces nutrient and redox stratification. Phenological shifts in onset and breakdown of stratification alter habitat availability and the timing of biogeochemical processes, with cascading effects on primary production and food webs (e.g., trophic mismatches) (Woolway et. al., 2022). Observations and modeling demonstrate earlier stratification onset, prolonged duration, and tendencies toward more frequent and intense lake heatwaves, with clear anthropogenic attribution. Subsurface (metalimnetic/hypolimnetic) heatwaves are also being documented, extending thermal stress below the surface (Woolway et. al., 2021: Kirillin, Shatwell, 2016).

River Thermal Regimes and Hydroclimatic Extremes

Hydroclimatological dynamics shape river discharge (i.e., streamflow). While numerous interacting factors affect the condition of river ecosystems—such as light availability, thermal regime, nutrient supply, spatial connectivity, biodiversity, and human management—discharge remains a fundamental driver in defining the structural framework of river habitats (Laize et al., 2014). In rivers, warming elevates water temperatures, while droughts and heatwaves reduce flows and dilution capacity; floods increase non-point source loads and resuspension of sediments. A synthesis of global case studies shows that river water quality often worsens during droughts and heatwaves due to higher concentrations of nutrients, organic matter (increased BOD/COD), salinity, and pathogens; floods degrade quality via sediment and pollutant pulses. Multidecadal trends in many basins reflect the combined effects of climate change and intensifying extremes (Vliet et.al., 2023)

Oxygen Dynamics and Biogeochemical Feedbacks

Warmer water bodies bind less dissolved oxygen (DO), while increased microbial activity, respiration, mineralization, and sediment oxygen demand further reduce DO. In stratified lakes, bottomwater (hypolimnetic) DO declines are common. In many systems, browning (higher dissolved organic carbon (DOC) increases light attenuation and hypolimnetic respiration, increasing oxygen loss, compressing coldwater habitats, and altering ecosystem metabolism (Jane et. al., 2024).

Nutrients, Internal Loading, and Redox Chemistry

When water oxygen levels decrease or disappear completely, sediments release phosphorus through reductive dissolution of iron oxides, increasing ammonium accumulation; denitrification and anammox alter nitrogen balances. Therefore, stronger and longer stratification with hypolimnetic anoxia increases internal phosphorus load, leading to the risk of eutrophication and harmful algal blooms (HABs) during mixing events and subsequent growing seasons, even when external inputs are reduced (NSTC, 2003; Carpenter, 2005).

Contaminant Mobilization and Salinity Intrusion

Hydroclimatic extremes regulate pollutant pathways: drought concentrates salts and contaminants; floods transport pesticides, pathogens, pharmaceuticals, and microplastics from catchments; sea-level rise promotes salinity intrusion into tidally influenced rivers, threatening drinking-water intakes. The thawing of permafrost and watershed browning mobilize metals and organic carbon, altering acidity and ionic composition in Arctic and mountainous systems (Byl et, al., 2025).

Lakes: Warming, Deoxygenation, and Eutrophication

Lake Heatwaves and Attributed Change

Lake heatwaves—extended intervals of anomalously warm surface water—are becoming more frequent, longer, and more intense. Recorded ecological impacts include mass fish mortalities, shifts in plankton phenology and metabolism, and altered food-web structure. Detection–attribution studies link severe lake heatwaves to anthropogenic warming. Subsurface heatwaves in the metalimnion and hypolimnion are increasingly reported, extending thermal stress into deeper aquatic zones (Woolway et al., 2025).

Widespread Deoxygenation and Habitat Squeeze

Sustained monitoring reveals broad DO decrease in many temperate lakes, driven by warming and stratification effects. Simultaneously, increased terrestrial inputs (browning) intensify hypolimnetic oxygen demand. The net effect is habitat squeeze for cold-water taxa such as salmonids and coregonids as cool, oxygenated waters contract. In many lakes, browning-induced oxygen loss outpaces any surface-cooling benefits for habitat, raising biodiversity risks (Jane et. al., 2024).

Harmful Algal Blooms (HABs) and Toxin Risks

Climate change interacts with nutrient enrichment to increase HAB frequency, duration, and toxicity. Modeling for large reservoirs projects substantial increases in cyanobacterial biomass under plausible warming and stratification scenarios—even with constant nutrient inputs—highlighting the synergism of warming and eutrophication. Treatment plants face challenges related to toxins, taste and odor, and disinfection by-products (Chapra et al., 2017). Climate change and nutrient dynamics influence each other both directly and indirectly. Rising temperatures clearly alter the habitats suitable for harmful algal blooms (HABs). Additionally, climate shifts can drive the expansion of harmful algal species by modifying precipitation patterns—leading in some regions to more frequent droughts, and in others to increased intensity or frequency of storms. Such episodic storm events and broader climate variability impact freshwater flow timing, water residence periods, the scale and timing of nutrient inputs, and the subsequent biological responses (Heisler et al., 2008).

Rivers: Flow Regimes, Extremes, and Quality

Droughts and Heatwaves

During droughts and heat waves, low flows increase residence times, temperatures, and concentrations of nutrients, salts, and pathogens. As dilution decreases, point source contributions increase. Biological oxygen demand increases while DO decreases, stressing the biota and creating treatment challenges for utilities. Observational syntheses indicate that water quality deteriorates under such extremes in the majority of cases (Vilet et al., 2023).

Salinity Intrusion and Heat Stress in Regulated Rivers

In tidal rivers and deltas, sea level rise and drought are advancing salt fronts, threatening water intakes and altering ionic composition. Active reservoir releases and temporary barriers are increasingly being used to control salinity intrusion, demonstrating the interactions of climate risk across water supply and estuary management (Byl et, al., 2025).

Water Quality Constituents and Processes

Temperature and Dissolved Oxygen

Direct thermal effects reduce oxygen solubility. Indirect effects (higher respiration, nitrification and denitrification rates, and sediment oxygen demand) further deplete DO. Under low oxygen levels or anoxia, reduced compounds (NH_4^+, Fe^{2+}, Mn^{2+}, H_2S) accumulate, and emissions of methane (CH_4) and nitrous oxide (N_2O) may increase. (Naqvi et. al., 2010).

Nutrients (Nitrogen and Phosphorus)

Rising temperatures stimulate cyanobacterial blooms and nitrogen-fixing capacity in some species; redox-driven sediment processes increase phosphorus release under oxygen-depleted conditions, while storm events introduce significant external nutrient inputs. Internal nutrient recycling

maintains eutrophic conditions even after external inputs have diminished, highlighting the need for long-term, multifaceted restoration approaches (Nürnberg 2024).

Contaminants and Pathogens

Extreme events mobilize legacy metals, pesticides, and emerging contaminants (pharmaceuticals, PFAS, microplastics), while warm, nutrient-rich waters favor pathogenic bacteria such as Vibrio and E. coli. Permafrost thaw enhances delivery of DOC and metals to high-latitude rivers and lakes, modifying acidity and redox state with visible ecosystem consequences. The distribution of pathogens and the incidence of waterborne disease outbreaks are closely linked to environmental and climatic conditions. Global climate change will affect the hydrological cycle in many different ways that will influence waterborne pathogen survival, infectivity and distribution (Nicholas, 2014).

Ecological and Health Consequences

Thermal habitat loss and hypoxia reduce niche availability for coldwater and stenothermic species, restructure plankton communities, and increase fish mortality risks during heatwaves and turnover events. Browning and deoxygenation compress vertical habitat, leading to mismatches in predator-prey dynamics (Jane et al., 2024).

HAB toxins (microcystins, anatoxins, cylindrical spermopsin) pose a threat to drinking water safety and recreation; storm-related increases in microbial indicators and pathogens complicate treatment. Warmer waters extend seasonal ranges for some pathogens, requiring more intensive monitoring and risk communication (Elidrissi et al., 2024).

CONCLUSION

Global warming is degrading the water quality of lakes and rivers through thermal, hydrological, biogeochemical, and microbiological pathways. Lakes are experiencing increasingly severe and prolonged stratification, heat waves, and bottom-water anoxic conditions, which increase nutrient loading and the risk of HABs, threatening the health of organisms. Rivers are experiencing quality degradation reflecting changes in pollutant dilution, mobilization, and transformation during droughts, heat waves, and floods. These dynamics threaten ecosystems, human health, and economies; however, targeted adaptation (load reduction, nature-based solutions, and dynamic operations) and robust monitoring and forecasting can significantly reduce these risks. Aligning climate change mitigation with water quality management is crucial for maintaining freshwater security throughout the 21st century.

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EFFECT OF SOME ELICITORS ON TAXOL PRODUCTION IN HAZELNUT CELL SUSPENSION CULTURE

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ABSTRACT

Hazelnut cell suspension culture is an effective method for the production of secondary metabolites and increased productivity in biochemical and biotechnological research. This technology allows for precise control of growth conditions, reduced costs, and mass production of pharmaceutical compounds such as Taxol. The present study was conducted to investigate the effects of methyl jasmonate and H2O2 on the levels of secondary metabolites of hazelnut during hazelnut cell suspension culture. Treatments included methyl jasmonate at four concentrations of 100, 150, 250 and 350 mg/L and hydrogen peroxide at concentrations of 0.5 and 1 ml/L along with the control treatment. After the experiment, traits such as fresh weight, dry weight, total phenol and flavonoid content and taxol were determined. The results showed that the use of methyl jasmonate and H2O2 had a significant effect on the amount of metabolites studied in hazelnut cell suspension. The use of medium concentration of methyl jasmonate had a better effect on the content of total phenols and flavonoids than different concentrations of H2O2. The content of phenol (14.88 mg/g dry weight) and total flavonoids (2.65 mg/g dry weight) was observed in cell suspension samples treated with methyl jasmonate at a concentration of 250 ppm. The results also showed that the highest amount of taxol (308.79 ppm) was observed in samples treated with H2O2 at a concentration of 0.5 ml.

Keywords: Taxol, plant growth regulator, cell suspension, *Corylus avellana* L.

INTRODUCTION

Hazelnut (*Corylus avellana* L.) is an economically and medicinally valuable plant, traditionally known as a rich source of proteins (~19%), oils (>60%), vitamins, and minerals (Rezvani et al., 2016). In recent years, growing attention has been directed toward its secondary metabolites, particularly paclitaxel, a diterpenoid alkaloid from the taxane family. Paclitaxel is one of the most important anticancer agents, widely applied in the treatment of breast, ovarian, and lung cancers (Rahpeyma et al., 2015).

Since natural paclitaxel production from yew (Taxus spp.) trees is limited and unsustainable, alternative strategies for large-scale and cost-effective production are essential. Plant tissue culture, particularly cell suspension culture, has been recognized as a promising method for producing secondary metabolites, offering advantages such as controlled growth conditions, reproducibility, and scalability (Sobhani Zadeh et al., 2025).

Among the key factors influencing secondary metabolite biosynthesis are culture medium composition, callus age, carbohydrate source, and the use of elicitors. Elicitors such as methyl jasmonate, salicylic acid, chitosan, and hydrogen peroxide can trigger signaling pathways that enhance secondary metabolite accumulation (Zhang et al., 2001; Jiasari et al., 2023). In this study, we investigated the effects of MeJA and H_2O_2 on the growth characteristics and secondary metabolite profiles of hazelnut cell suspension cultures, with emphasis on paclitaxel production.

MATERIALS AND METHODS

Mature hazelnut seeds were collected from the Rudsar Research Station. After surface sterilization with ethanol and sodium hypochlorite, seeds were cultured on MS medium supplemented with 2,4-D, BA, and NAA for callus induction. Friable and whitish calli were transferred to liquid MS medium to establish suspension cultures.

Treatments consisted of four concentrations of MeJA (100, 150, 250, and 350 ppm) and two concentrations of H_2O_2 (0.5 and 1 mM), along with a control. The experiment was arranged in a completely randomized design with five replicates. At the appropriate growth stage, cultures were harvested, and fresh weight (FW), dry weight (DW), TPC, TFC, antioxidant capacity, and paclitaxel content were measured. TPC and TFC were determined using Folin–Ciocalteu and aluminum chloride methods, respectively, whereas paclitaxel was quantified by HPLC (Jakabová et al., 2012; Mita et al., 1997; Pourmorad et al., 2006; Meda et al., 2004; Miliauskas et al., 2004).

RESULTS AND DISCUSSION

Fresh and dry weight

Both MeJA and H_2O_2 treatments significantly influenced biomass accumulation. Increasing concentrations of elicitors led to a decline in FW and DW. The highest biomass values were recorded under 150 ppm MeJA, with 74.46% and 75.01% increases in FW and DW, respectively, compared with the control. These results confirm the dual role of H_2O_2: acting as a signaling molecule at lower concentrations while exerting cytotoxic effects at higher levels (Hu et al., 2016).

Total phenolic and flavonoid contents

MeJA at 250 ppm resulted in the highest increases in TPC and TFC (39.75% and 71.69% higher than the control, respectively). At higher concentrations, both traits declined significantly. Similarly, H_2O_2 enhanced phenolic and flavonoid production by activating enzymes such as phenylalanine ammonia-lyase (PAL) (Barba-Espín et al., 2021). These findings emphasize the importance of optimizing elicitor concentration to maximize metabolite accumulation (Fan et al., 2024).

Total antioxidant capacity

Antioxidant activity increased in response to both elicitors, with the highest level observed under 250 ppm MeJA. This effect is attributed to the activation of enzymes such as chalcone synthase (CHS) and stilbene synthase (STS), leading to enhanced antioxidant compound biosynthesis (Ghanati et al., 2011; Goktep-Atilgan et al., 2023).

Paclitaxel content

HPLC analysis revealed that both elicitors significantly enhanced paclitaxel production. The maximum paclitaxel concentration (308.79 ppm) was obtained with 0.5 mM H_2O_2, representing a 65.95% increase compared with the control. MeJA also promoted paclitaxel accumulation, with the most pronounced effect at 250 ppm. These results corroborate previous studies highlighting the positive effects of oxidative and hormonal elicitors on taxane biosynthesis (Kim et al., 2017; Yan et al., 2020; Liao et al., 2024).

Correlation analysis

Correlation analysis indicated a negative relationship between paclitaxel accumulation and biomass (FW and DW), but a positive correlation with TPC. This suggests a trade-off between primary growth and secondary metabolism. A strong positive correlation between TFC and antioxidant activity further supports the complementary role of flavonoids in plant defense responses to stress and elicitors (Ramachandra and Ravishankar, 2002; Panche et al., 2016).

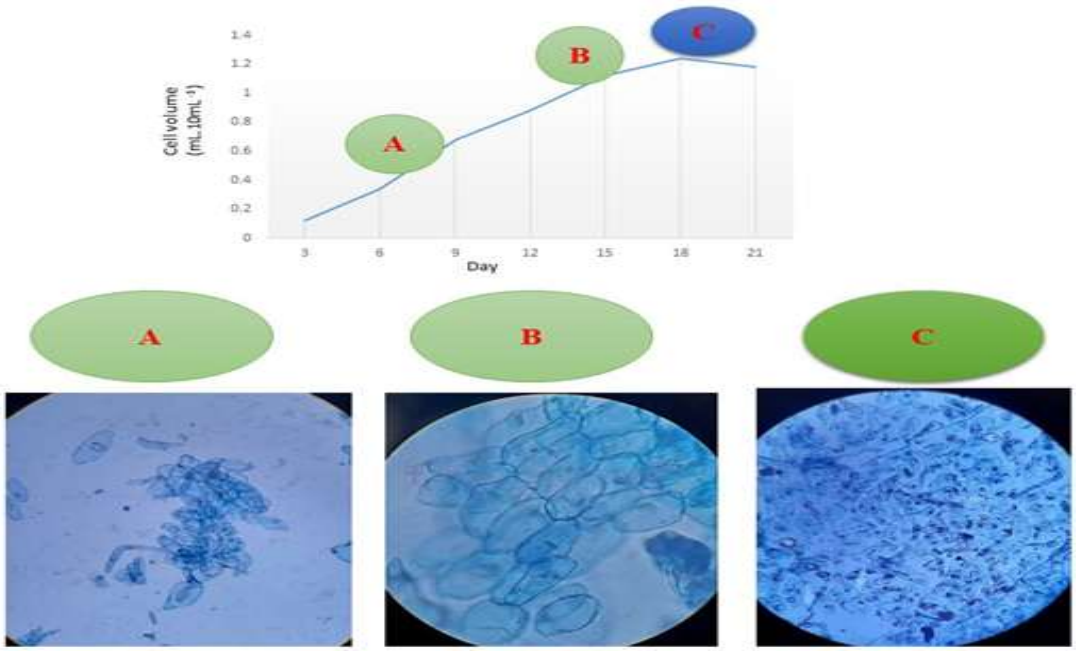

Figure 1. Growth curve of hazelnut cell suspension cultures

CONCLUSION

The present study demonstrates that both methyl jasmonate and hydrogen peroxide are effective elicitors for stimulating secondary metabolism in hazelnut suspension cultures. Although biomass decreased with higher elicitor concentrations, secondary metabolites, particularly phenolics, flavonoids, antioxidants, and paclitaxel, increased significantly. Optimal results were obtained at 250 ppm MeJA (for phenolics and flavonoids) and 0.5 mM H_2O_2 (for paclitaxel).These findings underscore the significance of optimizing elicitor type and concentration to achieve balanced growth and metabolite production. The observed correlations also highlight the metabolic trade-offs between cell growth and secondary metabolite biosynthesis. Future work should focus on refining elicitation strategies, including timing, combination treatments, and scale-up approaches, to further enhance paclitaxel production in hazelnut cell cultures for potential industrial applications.

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PRESENCE OF *merA* GENE AND MERCURY RESISTANCE AMONG PATHOGENIC BACTERIA

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ABSTRACT

Pathogenic bacterial microorganisms cause an important threat to human health and can result in infectious diseases. These bacteria can spread through various environments such as food and water. Mercury is a heavy metal that is highly persistent in the environment and has extremely strong toxic effects. Therefore, this research aimed to determine the mercury resistance of eight distinct pathogenic reference strains using the broth microdilution method and to identify the presence of the *merA* gene using the PCR method. Out of the eight pathogenic reference strains, five (62.5%) strains were resistant to mercury. The highest minimum inhibitory concentration (MIC) value, with 16 µg/mL, was observed in *Pseudomonas aeruginosa* ATCC 27853 and *Enterobacter cloacae* NCIMB 13597 strains. Mercury-sensitive bacteria such as *Salmonella* Typhimurium ATCC 13311, *Staphylococcus aureus* ATCC 29213, and *Escherichia coli* ATCC 25922 had a minimum inhibitory concentration (MIC) of 1 µg/mL. All mercury-resistant strains were also positive for the *merA* gene, except for *Enterococcus faecalis* ATCC 51299. Mercury-sensitive strains did not carry the *merA* gene. The findings demonstrate an important correlation between the existence of the *merA* gene and mercury resistance and emphasize the need for detecting heavy metal resistance in pathogenic bacteria for public health.

Keywords: Pathogenic bacteria, mercury resistance, *merA* gene, MIC

INTRODUCTION

Human health is significantly affected by bacteria, especially pathogenic bacteria, which are responsible for millions of deaths each year (Mocan et al., 2017; Fan et al., 2019). These bacteria can be transmitted through food, water, and airborne transmission to the human body via multiple pathways, causing diseases that pose a significant threat to human health. Pathogenic infections cause many diseases, including septicemia, recurrent intestinal inflammation, and soft tissue infections (Mi et al., 2022).

Contamination of both surface and groundwater by heavy metals is a worldwide concern. For humans and other living things, heavy metals can pose acute and long-term health risks. Exposure to heavy metals can result in health problems including depression, cancer, renal problems, headaches, nausea, and skin irritation (Li et al., 2019; Imron et al., 2021). The release of hazardous heavy metal-containing industrial wastewater and the resulting accumulation of these metals in the environment can be extremely dangerous for human, animal, and plant health (Nanda et al., 2019). Mercury (Hg)

is a persistent environmental pollutant with significant toxicity, and elemental gaseous mercury can travel great distances within the soil and plant (Guo et al., 2024).

Mercury is a hazardous non-essential metal that has serious physiological consequences even in small concentrations (Kiyono et al., 2013). Even at very low levels, mercury can be harmful, particularly to the developing fetus and child. Importantly, the toxicokinetic of Hg vary depending on the mode of exposure (inhalation, oral, or cutaneous) and the type of mercury to which one is exposed (Miklavčič et al., 2013). The fundamental mechanism of cellular damage is the interaction of mercury with decreased sulfhydryl groups on significant proteins. These macromolecules are bound by mercury, which disrupts with their usual biological functions and changes the metabolism of cells. Histological studies have confirmed that mercury toxicity can cause atrophy of the central nervous system (Kiyono et al., 2013).

Mercury due to its toxic effect causes the development of various resistance mechanisms in bacteria (Barkay et al., 2003). The *merA* gene, which plays an important role in resistance, encodes the mercuric reductase enzyme (Christakis et al., 2021). Mercury also leads to the spread of antimicrobial resistance because genes encoding the *mer* system often move on mobile genetic elements containing antimicrobial resistance genes (Lloyd et al., 2016). Furthermore, the emergence of resistant strains, which are more difficult to treat and are linked with increased rates of morbidity and mortality, can be prevented by the rapid dissemination of resistance genes by horizontal gene transfer (Hembach et al., 2022).

Salmonella is a zoonotic pathogen that can cause food poisoning, which can result in gastroenteritis, sepsis, enteric fever, and typhoid fever (Tan et al., 2022; Yang et al., 2025). *Escherichia coli*, an *Enterobacteriaceae* family member, is found in both human and animal gut microbiota (Levinson, 2016). Typically, they are released into the environment, and they can contaminate foods, soil, and water (Bhunia, 2008). *Pseudomonas aeruginosa* is a flagellated, Gram-negative bacterium that readily adapts to novel environments. Bacteria are considered an opportunistic pathogen, causing pneumonia in immunocompromised patients (Goncalves-de-Albuquerque et al., 2016). The *Enterobacteriaceae* family includes *Klebsiella pneumoniae*, a Gram-negative rod-shaped bacterium. It is also an opportunistic pathogen that can be found in the nasal and oral cavities, skin, and intestines as well as in hospitals and clinics tools. This pathogen causes a number of common infections, such as bacteremia, pneumonia, wound infections, and urinary tract infections (UTIs) (Li et al., 2014; Li and Ni, 2023). *Enterobacter cloacae* is commonly found in soil, water, and plants. It is a common hospital-acquired disease and can be an opportunistic pathogen, particularly in immuno-compromised individuals (Izdebski et al., 2015). *Staphylococcus aureus* can cause many human diseases, such as pneumonia, meningitis, impetigo, mastitis, endocarditis, toxic shock syndrome osteomyelitis, food poisoning, and gastrointestinal tract infections (Bhunia, 2008; Tong et al., 2015; Özdemir, 2022). *Enterococcus faecalis* is a potentially pathogenic bacterium found in the intestine, oral, and reproductive systems of people and animals. It causes various types of infectious illnesses (Xiang et al., 2024).

Mercury is a very toxic and harmful heavy metal that poses a major threat to both human health and the surrounding environment. The objective of this study was to test mercury resistance using the broth microdilution method and to detect the presence of mercury reductase, which is encoded by the *merA* gene, using PCR.

MATERIALS AND METHODS

Bacterial strains

In this study, a total of eight ATCC reference strains were used. Gram-negative bacteria included *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Salmonella* Typhimurium ATCC 13311, *Enterobacter cloacae* NCIMB 13597, *Klebsiella pneumoniae* ATCC 700603, and *Escherichia coli* ATCC 35218. The Gram-positive bacteria were *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 51299. DNA was obtained with the cetyl trimethyl ammonium bromide (CTAB) method and was then kept at -20°C.

Determination of the mercury minimum inhibitory concentration

The mercury chloride ($HgCl_2$) used in this study was supplied by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The broth microdilution method was used to determine the minimum inhibitory concentrations (MICs) of mercury quantitatively on a sterile 96-well microtiter plate (Lp Italiana) (CLSI, 2012; He et al., 2016; Dahanayake et al., 2019). The concentrations tested for mercury (Hg) varied between 64 µg/mL and 0.125 µg/mL. MICs were determined by calculating the lowest concentration, after 18–20 hours of incubation at 37°C, completely inhibited organism development. The optical density at 600 nm wavelength was measured (Thermo Electron Corporation, Finland). Three duplicates of each test were performed. Reference strains tested in this study were accepted as resistant if their MIC values were higher than the MIC result of *Escherichia coli* K-12, which was used as control strain those of the control strain (Dahanayake et al., 2019).

Determination of mercury minimum bactericidal concentration

After the incubation and plate reading, turbidity was observed in the wells. Based on this turbidity, possible minimum bactericidal concentration **(**MBC) value ranges were determined and 10 µL of the first eight dilutions (64, 32, 16, 8, 4, 2, 1, 0.5 µg/mL) were inoculated onto Mueller Hinton agar (Merck, Germany). The plates were incubated for 24 h at 37°C. After agar plates were examined, MBC was determined to be the lowest mercury concentration at which no bacterial growth developed.

Detection of mercury resistance gene by PCR

PCR was used to test for the presence of the *merA* gene, which encodes mercury reductase, to determine Hg resistance. The primers for the *merA* gene were forward primer, 5'-GAGATCTAAAGCACGCTAAGGC-3', and reverse primer, 5'-GGAATCTTGACTGTGATCGGG-3' (Misra et al., 1984). The predicted size was 1011 bp. PCR was performed in a reaction volume of 50 µL containing 5 µL of 10× PCR buffer (Thermo Scientific), 2 mM $MgCl_2$ (Thermo Scientific), 0.2 mM dNTP mix (Thermo Scientific), 0.4 µM primer (Oligomer Biotechnology), 1.5 U Taq DNA polymerase (Thermo Scientific), 5 µL DNA template (50 ng/µL), and 30.7 µL PCR-grade water (AppliChem, Germany). The cycling conditions were carried out with the following setup: initial denaturation (94 °C for 3 min), 35 cycles of denaturation (94 °C for 30 s), annealing (57 °C for 30 s), extension (72 °C for 1 min), final extension (72 °C for 5 min). PCR experiments were performed using a thermal cycler (Bio-Rad T100). Electrophoresis (BioRad) was used to examine amplified PCR products on a 1% agarose gel and UV transilluminator (DNR Minilumi Bioimaging Systems) for visualization. The molecular size of DNA fragments was determined with a 100-bp DNA ladder (Vivantis). *E. coli* ATCC 35218 was a positive control for the *merA* gene.

RESULTS AND DISCUSSION

In this study, mercury ($HgCl_2$) MIC, MBC values and *merA* gene presence were determined for eight different pathogenic reference strains. According to the *E. coli* K-12 MIC value, five of the eight pathogenic reference strains showed resistance to Hg at different MIC values (4-16 µg/mL). Table 1 shows the strains that are resistant and sensitive to mercury. Out of the eight pathogenic references strains, five (62.5%) strains were resistant to Hg. These were *E. faecalis* ATCC 51299 MIC value of 4 µg/mL, *K. pneumoniae* ATCC 700603 and *E. coli* ATCC 35218 MIC value of 8 µg/mL, *P. aeruginosa* ATCC 27853 and *E. cloacae* NCIMB 13597 MIC value of 16 µg/mL. The bacterial type strains including *E. coli* ATCC 25922, *S.* Typhimurium ATCC 13311, and *S. aureus* ATCC 29213 had MIC value of 1 µg/mL. Thus, these strains were considered as mercury-sensitive according to *E. coli* K-12 MIC value. The MBC values of the strains were parallel to their MIC values, except for *E. faecalis* ATCC 51299. Figure 1 shows the MBC results. Interestingly, although all mercury-resistant strains carried the (50%) *merA* gene, *E. faecalis* ATCC 51299 did not also harbor the *merA* gene. The presence of the *merA* resistance gene in the pathogenic reference strains was detected and PCR products were visualized using agar gel electrophoresis as shown in Figure 2.

Table 1. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of mercury and the presence of the *merA* gene for pathogenic reference strains

No	Reference Strains	MIC (µg/mL)	MBC (µg/mL)	*merA* gene
1	*E. coli* ATCC 25922	1	1	-
2	*S.* Typhimurium ATCC 13311	1	1	-
3	*P. aeruginosa* ATCC 27853	16	16	+
4	*S. aureus* ATCC 29213	1	1	-
5	*E. faecalis* ATCC 51299	4	16	-
6	*E. cloacae* NCIMB 13597	16	16	+
7	*K. pneumoniae* ATCC 700603	8	8	+
8	*E. coli* ATCC 35218	8	8	+
Control	*E. coli* K-12*	2	2	-

*Reference strains were considered resistant if their MIC values surpassed those of the control strain.

Zhou et al. (2019) found that *Pseudomonas aeruginosa* strains isolated from a mercury-contaminated river sediment in China had an MIC value of approximately 10 mg/L against $HgCl_2$. The high MIC values obtained in this study indicated that environmental *P. aeruginosa* strains can develop significant resistance mechanisms under chronic heavy metal exposure. In a study by Skurnik et al. (2010), MIC values for Hg-resistant *E. coli* isolates were reported as ≥ 64 mM and for susceptible isolates as ≤ 16 mM. Our Hg MIC results in the range of 0.25 to 64 µg/mL obtained in pathogenic reference strains are parallel to both the studies conducted in environmental *E. coli* strains (de Luca Rebello et al., 2013) and the values reported by Skurnik et al. (2010) in clinical isolates. The MIC value of $HgCl_2$ against industrially sourced *Enterobacter* sp. AZ-15 isolates was reported as 20 µg/mL (Amin et al., 2017), which was consistent with our Hg MIC value in this study for pathogenic *Enterobacter cloacae* NCIMB 13597.

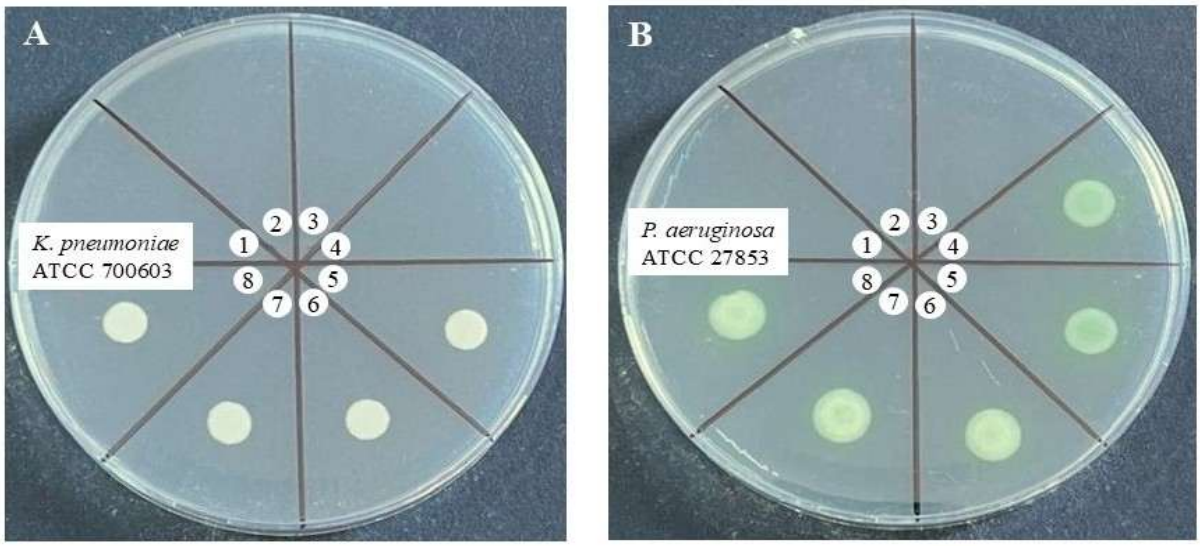

Figure 1. MBC plates of mercury-resistant (A-B) strains. A. *K. pneumoniae* ATCC 706003, B. *P. aeruginosa* ATCC 27853. Mercury concentrations for numbers 1, 2, 3, 4, 5, 6, 7, and 8 are 64, 32, 16, 8, 4, 2, 1, and 0.5 µg/mL, respectively.

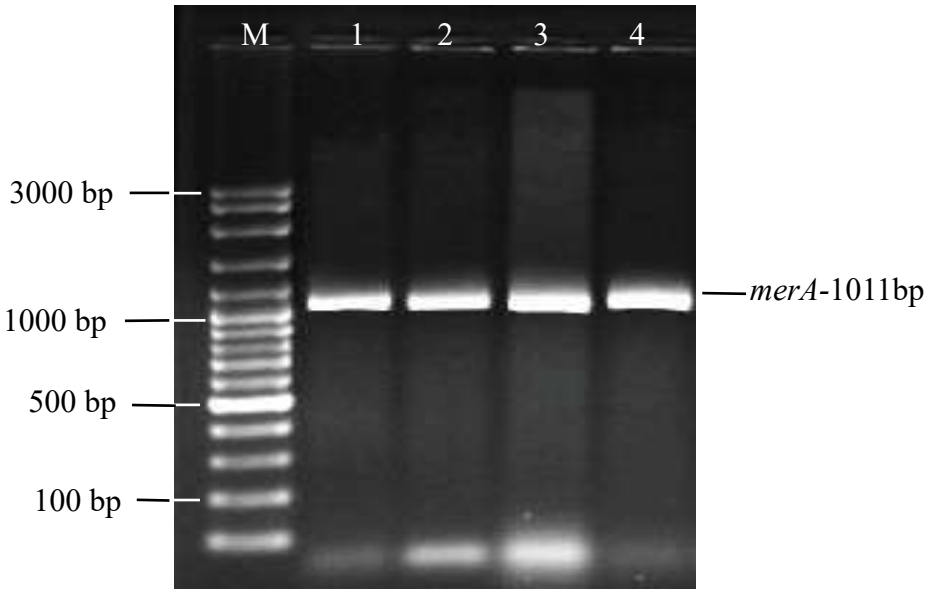

Figure 2. Agarose gel electrophoresis of the *merA* gene (1011 bp). Lane M: 100 bp Marker. Lane 1: *E. coli* ATCC 35218 Lanes 2-4: Positive strains for *merA* gene: *P. aeruginosa* ATCC 27853, *E. faecalis* NCIMB 13597, and *K. pneumoniae* ATCC 700603, respectively.

Several studies have found that different bacterial strains have developed resistance to mercury and other heavy metals. In Shanghai, He et al. (2016) discovered that *Vibrio parahaemolyticus* isolates from fresh shrimp from fish markets had Hg MIC values ranging from 6.25 to 50 µg/mL. In contrast, mercury resistance was not detected in *Aeromonas* spp. isolates from Pacific abalone samples collected in Korea (Wickramanayake et al., 2020). Furthermore, Singh et al. (2013) reported that all *Bacillus* isolates examined in their study exhibited high mercury resistance, with MICs ranging from 125 to 180 µg/mL, indicating that Gram-positive bacteria may also possess strong heavy metal tolerance mechanisms. In contrast, the low MIC value reported in our study and the lack of the *merA* gene are not consistent with this finding. In contrast to our results (50%), Skurnik et al. (2010) detected the *merA* gene in all mercury-resistant isolates. Yang et al. (2020) found a low occurrence of the *merA* gene in the isolates of *E. coli* (2.5%) and *Salmonella* (11.4%) from chicken broiler farms and retail meat, indicating genotypic Hg resistance. In Korea, 17 (47%) *Aeromonas* strains from the Manila clam carried the *merA* gene. Cufaoglu et al. (2022) found that the *merA* gene was more common (50%) in the isolates of *E. coli* from sheep, cattle, and chickens.

CONCLUSIONS

This study revealed the resistance of different pathogen reference strains to the heavy metal mercury with their MIC and MBC values. Most of Hg-resistant strains harbored the *merA* gene associated with mercury resistance. A relationship between mercury resistance and the presence of the *merA* gene was observed. As a result, determining the occurrence of mercury-resistant bacteria may be crucial due to their harmfulness to human health.

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PHENOTYPIC AND GENOTYPIC DETECTION OF BIOFILM PRODUCTION OF *STAPHYLOCOCCUS AUREUS* FROM CHICKEN MEAT

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ABSTRACT

Staphylococcus aureus, a Gram-positive and coagulase-positive bacterium, serves as both a commensal and opportunistic pathogen in mammals. It can potentially cause a wide range of severe and fatal diseases. Biofilm production is an essential virulence factor in pathogenic bacteria including *S. aureus.* Biofilms have a major role in their pathogenesis and persistence. *S. aureus* biofilm is mediated by the *icaABCD* gene cluster, which regulates intercellular adhesion and exopolysaccharide matrix production, facilitating biofilm development and long-term bacterial survival. A total of 11 *S. aureus* isolates from chicken meat were tested for biofilm formation using microtiter plates and the presence of *icaABCD* genes using PCR. All isolates were biofilm producers. Three isolates (27.2%) were classified as weak, four isolates (36.6%) as moderate, and four isolates (36.6%) as strong biofilm producers. All the isolates were positive for *icaAD* genes. In addition, 10 isolates (90.6%) were positive for *icaB* whereas eight isolates (54.5%) were positive for *icaC.* In this study, *S. aureus* isolates from chicken meat demonstrated the biofilm formation ability by the quantitative microtiter plate method and the presence of *icaABCD* biofilm forming genes by the PCR method, emphasizing the risk of food contamination and public health concerns.

Keywords: Biofilm, *icaABCD*, chicken meat, *Staphylococcus aureus*

INTRODUCTION

Staphylococcus aureus, one of the Gram-positive bacteria, is the most prevalent pathogen in the *Staphylococcus* genus (Götz et al., 2006; Bhunia, 2008). In the human body, *S. aureus* lives as commensal bacteria (Wang et al., 2023). *S. aureus* grow at temperatures between 15°C and 45°C, with NaCl concentrations up to 15% (Wu et al., 2018). One of the most significant organisms that causes nosocomial and community-acquired illnesses (Mirzaee et al., 2015). *S. aureus* can cause many diseases, such as soft tissue infections, endocarditis, and pneumonia (Wang et al., 2023). *S. aureus* can secrete a variety of virulence factors, including enzymes, exotoxins, and surface protein adhesins, which help to initiate adhesion, inhibition or evasion of the immune system of host (Mirzaee et al., 2015) *S. aureus* biofilm production promotes the establishment of antimicrobial-resistant bacteria, such as methicillin-resistant and vancomycin-resistant *S. aureus* (Ahn et al., 2018). Furthermore, its ability to produce biofilms on both biotic and abiotic surfaces increases its pathogenicity and persistence. (Zhao et al., 2017).

Biofilms are organized communities of microorganisms that grow and adhere to surfaces that are embedded in extracellular polymeric substances, which are made up of polysaccharides, lipids,

proteins, and nucleic acids (Reungvisesh et al., 2023). Biofilm production in the food industry increases bacterial resistance to environmental difficulties such as dehydration, washing, and sanitation because the extracellular polymeric substance that covers microbial cells serve as a barrier to movement (Rather et al., 2021). Biofilm consists of four stages, attachment, colonization, biofilm maturation and dispersal respectively (Lister and Horswill, 2014). Numerous factors influence bacterial attachment and biofilm formation, including the type of surface material, ambient conditions (such as pH and temperature), the kind of medium, the species of bacteria in the biofilm, exposure duration, and the kind of cleaning and disinfection chemical used (Lake et al., 2024). Multiple factors, including the growth medium, surface characteristics, and other environmental conditions, influence the biofilm formation ability of microorganisms such as *S. aureus* (Jeronimo et al., 2012; Zhao et al., 2017).

Another important component needed for biofilm production and maturation is the polysaccharide intercellular adhesin (PIA) (Mirzaee et al., 2015) The *ica* operon, which includes the *icaABCD* genes and the regulatory gene *icaR*, encodes both PIA synthesis and transcriptional control (Levinson, 2016; Özdemir et al., 2021). Biofilm development requires the products of the locus *icaABCD* genes, and *icaA* and *icaD* gene products are essential for this process (Király et al., 2024). When the *ica* locus in *S. aureus* is deleted, the production of PIA is stopped, and the ability of the bacteria to generate biofilm is also lost (Götz et al., 2006). *Staphylococcus* biofilm formation has been most extensively studied with the *icaABCD* gene cluster. The *icaABCD* genes indicate that the bacteria can produce biofilms (Pizauro et al., 2021). Since the identification and characterization of genes that regulate biofilm formation, there has been an increasing interest in the role of bacterial biofilms in clinical illnesses (Mirzaee et al., 2015).

Contamination with bacteria has been discovered throughout the manufacturing chain, from farm to fork (Klaharn et al., 2023). It has been determined to happen predominantly during the slaughter process, with plucking, cutting, and freezing being the most crucial procedures (Klaharn et al., 2023; Beshiru et al., 2023). *S. aureus* can contaminate chicken meat during processing, with multidrug-resistant and enterotoxigenic strains frequently isolated from retail poultry products (Osman et al., 2016).

During processing, handling, and storage, *S. aureus* can contaminate chicken meat through contact with unhygienic surfaces, equipment, or human skin. Biofilms pose a major problem in the food industry because of their capacity to develop biofilms on encounter food surfaces (Reungvisesh et al., 2023). Therefore, the aim of this study was to determine biofilm formation in *S. aureus* isolates from chicken meat and the presence of biofilm-associated *icaABCD* genes.

MATERIALS AND METHODS

Bacterial isolates

In this study, 11 *S. aureus* isolates from chicken meat, identified and confirmed by both biochemical testing and the species-specific Sa442 DNA fragment, were used. All isolates were revived from stock culture. Isolates were plated on tryptic soy agar (Merck, Germany) and incubated at 37°C for 24 h. For future research, these cultures were preserved tryptic soy broth with glycerol (20%) and kept at -20ºC.

Detection of biofilm formation

The isolates were grown in tryptic soy broth (TSB) (Merck) at 37°C for 24 h. A plate containing 200 µL of cell suspension per well was incubated for 24 hours. After incubation, the wells of the microtiter plate were washed with sterile phosphate buffered saline (PBS). The remaining adhered bacteria were fixed with 200 µL of 99% methanol (Merck) for 15 min. Next, 200 µL of 0.1% crystal violet was applied to each well to stain the biofilm. The stained biofilm was dissolved using a solution of 33% glacial acetic acid (Merck) in 160 µL. Finally, the biofilm formation activity was measured at 570 nanometers by the microplate reader (Thermo Electron Corporation, Finland). Three duplicates of each experiment were performed. Only sterile TSB was present in negative control wells. Isolates as were categorized as strong, moderate, weak and nonbiofilm producers (Stepanovic et al., 2000; Stepanovic et al., 2007).

Detection of the *icaABCD* genes

The DNA of the isolates was extracted using the cetyl trimethyl ammonium bromide (CTAB) technique (Ausubel et al., 1991). The PCR assays were conducted using the primers listed in Table 1. The PCR mixture (50 µL) included 0.3 mM of forward and reverse primers (Biomers, Germany), 5 µL DNA template, 5 µL 10 X PCR buffer (Thermo Scientific), 200 µM dNTP mix (Thermo Scientific), 4 mM $MgCl_2$ (Thermo Fisher Scientific), 1.5 U Taq DNA polymerase (Thermo Scientific), and 31.7 µL of PCR-grade water (Applichem, Germany). The cycling process includes initial denaturation (94 °C /5 min), 30 cycles of denaturation (94°C /1 min), annealing (52 °C /30 s), extension (72 °C /1 min 30 s), and final extension (72 °C /10 min). All amplifications of DNA were performed out using a thermal cycler (Bio-Rad T100) The PCR product was seen using a UV transilluminator (DNR Minilumi Bio-imaging Systems, Israel). *S. aureus* ATCC 25923 as positive control was used.

Table 1. Oligonucleotide primers used for the detection of the *icaABCD* genes

Target gene	Primer	Primer sequence (5'- 3')	Product size (bp)	Reference
icaA	icaA-F icaA-R	ACACTTGCTGGCGCAGTCAA TCTGGAACCAACATCCAACA	188	(Rohde et al., 2001)
icaB	icaB-F icaB-R	AGAATCGTGAAGTATAGAAAATT TCTAATCTTTTTCATGGAATCCGT	900	(Kiem et al., 2004)
icaC	icaC-F icaC-R	ATGGGACGGATTCCATGAAAAAGA TAATAAGCATTAATGTTCAATT	1100	(Kiem et al., 2004)
icaD	icaD-F icaD-R	ATGGTCAAGCCCAGACAGAG AGTATTTTCAATGTTTAAAGCAA	198	(Rohde et al., 2001)

RESULTS AND DISCUSSION

S. aureus is a biofilm-producing bacterium and can sustain contamination in chicken meat processing and on contact surfaces (Igbinosa et al., 2023). In the current study, biofilm formation capacity of 11 *S. aureus* isolates isolated from chicken meat was determined using the microtiter plate method. Figure 1 shows the biofilm-forming ability of *S. aureus* isolates from chicken meat. All *S. aureus* isolates demonstrated biofilm-producing capability; among them, three isolates (27.2%) were classified as weak four isolates (36.3%) as moderate and four isolates (36.3%) as strong biofilm producers. Figure 2 illustrates the classification of biofilm formation among the chicken meat *S. aureus* isolates. Our results are consistent with the study reported by (Bissong and Ateba, 2020), in which 90.9% of *S. aureus* from milk produced biofilm and 94.3% exhibited a strong phenotype. In addition, Dai et al. (2019) showed that all *S. aureus* strains isolated from pasteurized milk formed biofilm and 66.7% of them exhibited strong phenotypes.

The intracellular adhesion (*ica*) locus, which is the main factor promoting adhesive contacts between bacterial cells, encodes the genes required for the manufacture of PIA. The *ica* promotes adhesion between biofilm-producing bacterial cells. It also encodes the genes required for PIA production (Király al., 2024). In this study all *S. aureus* isolates carried the *icaA* and *icaD* genes, but 10 (90.9%) of them had the *icaB* and six (54.5%) had the *icaC* gene. Representative agarose gel photograph of the PCR products of the *icaA* and *icaD* gene is presented in Figure 3. Table 2 shows the degree of biofilm formation capacity and the presence of *icaABCD* genes involved in biofilm formation of the *S. aureus* isolates. Alibegli et al. (2025) found similarly high rates in clinical isolates. Chen et al. (2020) demonstrated that all *S. aureus* from various foods were positive for the *icaADBC* genes, which is consistent with our findings. On the contrary, some studies have documented lower prevalence of *icaA* and *icaD* genes in cheese (76.5% and 58.8%) and milk (25%) samples (Falaki and Mahdavi, 2017; Suvajdzic et al., 2017). In contrast, 52.3% of *S. aureus* isolates from food contact surfaces had *icaADBC* genes (Avila-Novoa et al., 2018). The results of many studies suggest that gene prevalence may vary in isolates of different origins from different geographical regions (Tahmasebi et al., 2019; Parastan et al., 2020; Deepak et al., 2024)

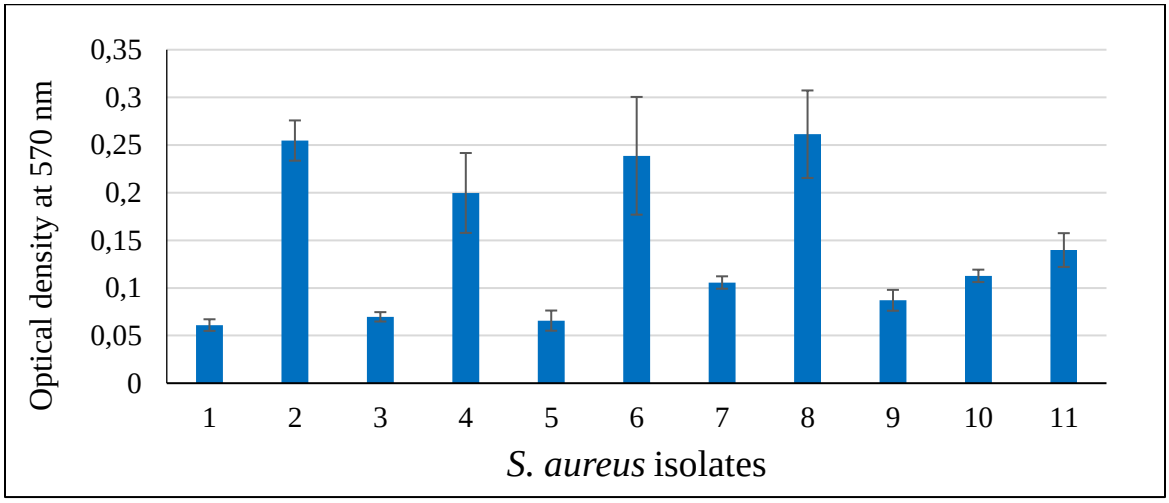

Figure 1. Biofilm-forming ability of *S. aureus* isolates from chicken meat.

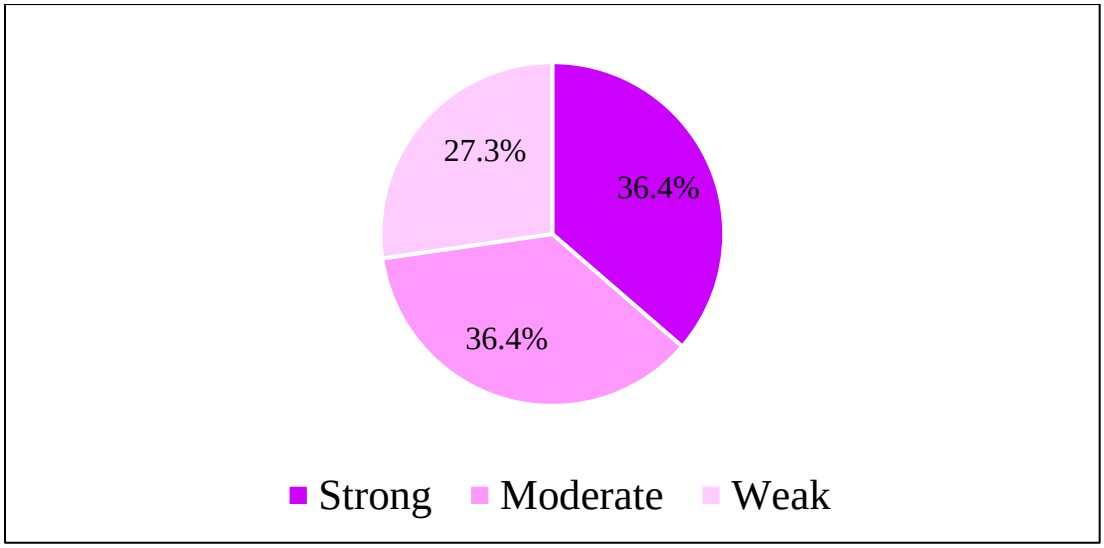

Figure 2. Classification of biofilm formation among the chicken meat *S. aureus* isolates.

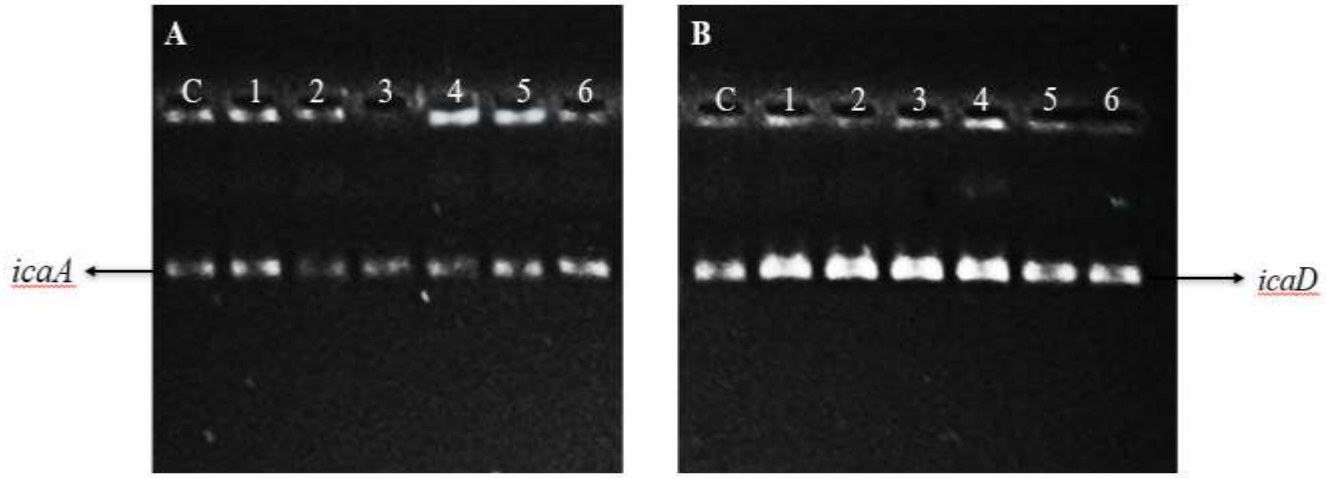

Figure 3. Representative agarose gel electrophoresis illustrating PCR products for *icaA* (188 bp) and *icaD* (198 bp) genes. Picture A: C: positive control (*S. aureus* ATCC 25923) for *icaA* gene. Lanes 1-6: *icaA* positive isolates in this study. Picture B: C: positive control (*S. aureus* ATCC 25923) for *icaD* gene. Lanes 1-6: *icaD* positive isolates in this study.

Table 2. The classification of biofilm formation capacity and presence of *icaABCD* genes in *S. aureus* isolates from chicken meat

Isolate	Biofilm formation	Biofilm-forming genes			
		icaA	*icaB*	*icaC*	*icaD*
1	Weak	+	+	-	+
2	Strong	+	+	+	+
3	Weak	+	+	+	+
4	Strong	+	+	-	+
5	Weak	+	+	-	+
6	Strong	+	+	+	+
7	Moderate	+	+	+	+
8	Strong	+	+	+	+
9	Moderate	+	+	-	+
10	Moderate	+	+	+	+
11	Moderate	+	-	+	+

CONCLUSIONS

The present study demonstrated that all *S. aureus* isolates were positive for biofilm production. In addition, these isolates from chicken meat had at least one or more of the *icaABCD* genes. It was determined that there might be a link between biofilm formation and the presence of the *icaABCD* genes. Biofilm formation by pathogenic bacteria such as *S. aureus* in food processing environments is the main risk for persistent food contamination that occurs at any stage from production to consumption. As a consequence, prevention of biofilm is necessary since it poses a significant threat to human health, food safety, food quality, and economic loss.

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APPROACHES TO OBTAINING OPTIMUM BENEFIT FROM MICROBIAL PRODUCTS USED IN PLANT PRODUCTION

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ABSTRACT

It is clear that in order to achieve higher and better-quality yields per unit area, it is not enough to only focus on plant breeding; effective plant nutrition through adequate watering and proper fertilization (based on the plant's phenological stage and in appropriate amounts) is crucial for obtaining the desired results. Growing healthy, high-yielding, and high-quality crops primarily involves proper soil management. In this context, a plant grown in soil with low organic matter levels (below 2%) and consequently reduced microbial diversity will struggle to cope with disease agents (especially soil-borne ones), have low vegetative and generative organ development, and ultimately may not benefit significantly from the minerals present in the soil or added through fertilizers. In addressing this common issue, commercial products containing microorganisms with functional benefits for soil and plants (such as bacteria, fungi, algae, etc.) which can aid in plant nutrition, plant protection, and plant growth regulation represent a significant alternative to chemical inputs (such as chemical fertilizers, pesticides, and synthetic growth regulators). However, to maximize the benefits of these products, it is essential to determine not only their capabilities under laboratory conditions (such as nitrogen fixation, phosphorus solubilization, siderophore production, auxin production, etc.) but also their abilities to colonize plant roots, proliferate in the rhizosphere, and compete in various plant and soil types under greenhouse or field conditions. Moreover, factors such as the type of plant production area (field, garden, greenhouse), production model (conventional, organic, ecological, etc.), plant type (cereal, vegetable, fruit), irrigation method (surface irrigation, pressurized irrigation), and especially soil characteristics (texture, organic matter, lime, pH, etc.) must be considered when selecting the appropriate product(s).

Keywords: Biofertilizer, Biological agent, Biostimulant

INTRODUCTION

Fertilizer is one of the inputs used to ensure the continuity of food and agricultural products necessary to meet basic needs such as nutrition and shelter, which have increased due to the growing population and steadily rising living standards. Ensuring the productivity and sustainability of agricultural lands is possible by returning the plant nutrients lost from the soil due to various reasons back to the soil (TAGEM 2018).

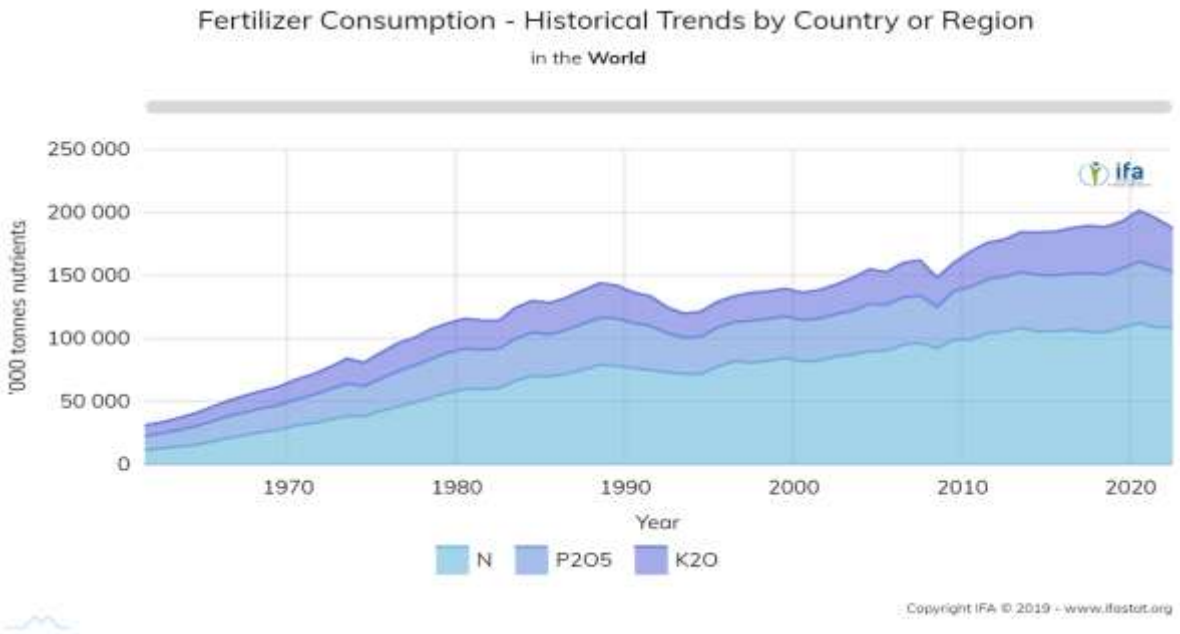
Fertilizers used to increase agricultural production and product quality are generally classified into two categories in the agricultural sector: chemical fertilizers and organic fertilizers (Akmermer

2022). The nutrient content of organic fertilizers is significantly lower compared to chemical fertilizers and is not in a form that plants can directly benefit from. For this reason, organic fertilizers are used for soil regulator purposes rather than meeting the nutritional needs of plants (Yetgin 2010). Chemical fertilizers, on the other hand, typically contain plant nutrients in a form that can be readily absorbed by plants and in high concentrations, allowing nutrient deficiencies to be addressed quickly and easily, with lower costs and labor (Evni ve Kan 2024).

Chemical fertilizers, generally produced as nitrogenous, phosphorous, and potassium-based, play a significant role in increasing crop productivity. However, the intensive use of chemical fertilizers not only enhances crop yields but also brings certain disadvantages. Chemical fertilizers alter the physical, chemical, and biological properties of the soil. Continuous use of chemical fertilizers leads to a decline in soil quality, including a reduction in soil organic matter (SOM) content. Excessive use of chemical fertilizers hardens the soil, reduces soil fertility, pollutes the air, water, and soil, and significantly decreases the availability of soil minerals, thereby posing environmental hazards. The continuous application of chemical fertilizers changes the soil pH, potentially increasing acidity. This can lead to a reduction in beneficial organisms, hinder plant growth and even contribute to an increase in greenhouse gas emissions due to the decrease in organic matter (Pahalvi vd. 2021).

In 2021, nitrogenous fertilizers accounted for most of the global fertilizer production, with a volume of 118.55 million tons, representing 55.53% of the total 213.46 million tons produced worldwide (FAO 2024). Regarding consumption, as shown in Figure 1, it was reported that by 2022, 109.29 million tons of nitrogenous fertilizers, 44.1 million tons of phosphorous fertilizers, and 34.66 million tons of potassium fertilizers were used worldwide (IFASTAT 2024).

Figure 1. Global consumption amounts of N, P_2O_5, and K_2O fertilizers by year (IFASTAT 2024)



Fertilizer production worldwide is shaped regionally according to consumption volumes and raw material resources. Countries with large populations, such as the USA, China, India, Canada and Russia also have extensive agricultural lands, giving them a significant share in fertilizer usage (Figure 2). These countries are also major fertilizer producers (Kocagöz 2022).

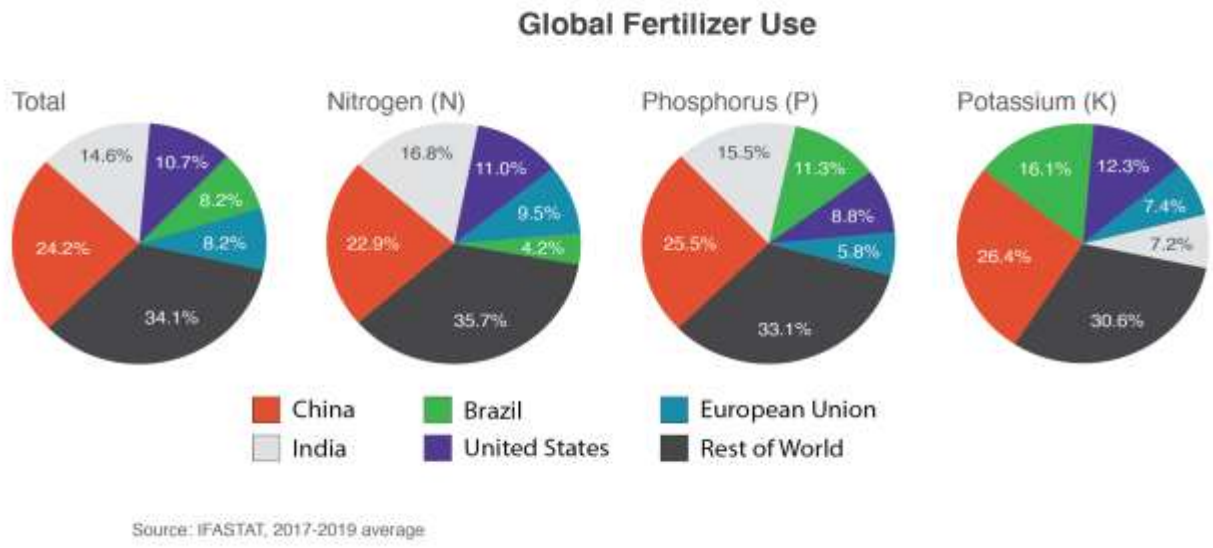


Figure 2. Fertilizer usage percentages in various countries worldwide

One of the most significant issues in the fertilizer sector is the dependence on imports for countries lacking raw materials. The supply of essential inputs, such as natural gas and phosphate rock is largely reliant on external sources. This situation makes fertilizer production companies heavily dependent on imports, which can lead to price fluctuations and increases, as well as challenges in ensuring a continuous supply of high-quality raw materials. Additionally, this dependency, coupled with factors such as raw material-producing countries entering the fertilizer production market, intensifies competition. As a result, the global fertilizer market is dominated by a few countries, creating an oligopolistic market structure (TAGEM 2018).

APPLICATION OF MICROBIAL PRODUCTS IN PLANT PRODUCTION

Given the environmental threats associated with the use of chemical fertilizers and considering the high consumption volumes and associated costs for countries lacking raw materials, there is a clear need for alternative solutions due to the economic disadvantages. One of the primary alternative solutions is microbial products, which are directly related to soil organic matter.

With changing climate and ecosystems, biological approaches aimed at improving plant production continue to gain strong support among engineers and academics focused on sustainability in agriculture. In this context, there is a growing global momentum in exploring a wide variety of rhizobacteria with new properties, such as heavy metal detoxification potential (Ma et al. 2011; Wani and Khan 2010). The rhizosphere, the soil region surrounding plant roots, represents the area of intense

microbial activity in the soil (Cantó et al. 2020). Biological fertilizers, also known as bio-fertilizers, are materials composed of living microorganisms that can colonize the rhizosphere and/or penetrate plant tissues. When applied to the plant surface or soil, they fix free nitrogen, process mineral elements from organic and inorganic sources to increase their availability and uptake by plants, and/or stimulate optimal plant growth through the production of secondary metabolites (Çakmakçı 2005). Both prokaryotes (archaea, bacteria and viruses) and eukaryotes (fungi, oomycetes, nematodes, protozoa, algae and arthropods) colonize the rhizosphere. Among these, bacteria and fungi are the most prevalent in terms of population (Buee et al. 2009).

Soil-dwelling microorganisms that enhance plant growth are bacteria living on or around the root surface. They contribute to plant growth and development directly or indirectly by producing and secreting various regulatory chemicals in the rhizosphere. Beneficial microorganisms can help reduce global reliance on chemical fertilizers in agriculture (Ahemad and Kibret 2014). They increase the availability of nutrients such as phosphorus (P), potassium (K), zinc (Zn), selenium (Se), and iron (Fe) in the soil through biochemical processes like dissolution, chelation, mineralization, oxidation, and reduction reactions. Additionally, microorganisms are known to secrete phytohormones, including auxins, cytokinins, abscisic acid, ethylene, brassinosteroids, jasmonic acid, salicylic acid, strigolactones, and gibberellins, which act as plant growth regulators and stress mitigators (Koskey et al. 2021). Various studies have shown that applying rhizobacteria, which promote plant growth under both normal and stressful conditions such as salinity, drought, acidity, or alkalinity, can improve the health and productivity of different plant species (Ahemad and Kibret 2014).

Indirect effects of microbial products include enhancing plant health by suppressing phytopathogens and other harmful microorganisms through parasitism, creating competitive environments for nutrients in the rhizosphere, and producing antagonistic substances such as siderophores, antibiotics, and antimicrobial metabolites. Additionally, these microorganisms generate lytic enzymes like chitinase, glucanase, and protease (Figure 3). Some bacterial genera have been shown to protect plants from fungal and bacterial pathogens. These bacteria employ various mechanisms to inhibit the development of plant pathogens. These mechanisms include colonizing infection sites, competitively excluding pathogens, engaging in antagonistic activities through the secretion of antibiotics and lytic enzymes, and enhancing plant resistance (Bonaterra et al. 2022).

Benefits of PGPR Inoculation to Plants	PGPR Strain(s)	Tested Plant(s)
Tolerance to drought stress	*Pseudomonas fluorescens* DR11, *Enterobacter hormaechei* DR16, *Pseudomonas migulae* DR35, *Bacillus subtilis*, *Achromobacter piechaudii* ARV8, *Phyllobacterium brassicacearum*, *Paenibacillus polymyxa*, *Rhizobium tropici*, *Azospirillum brasilense*	Foxtail millet (*Setaria italica* L.), Maize (*Zea mays* L.), Bean (*Phaseolus vulgaris* L.), *Arabidopsis thaliana*, Tomato (*Lycopersicum esculentum* Mill cv. F144), Pepper (*Capsicum annuum* L. cv. Maor), Wheat (*Triticum aestivum* L.)
Tolerance to salinity stress	*Bacillus pumilus*, *Exiguobacterium oxidotolerans*, *Bacillus megaterium*, *Azospirillum* sp., *Achromobacter piechaudii*, *Eneterobacter* sp. PR14	Brahmi (*Bacopa monnieri* L.), Maize (*Zea mays* L.), Lettuce (*Lactuca sativa* L.), Tomato (*Lycopersicum esculentum* Mill.), Rice (*Oryza sativa* cv. Sahbhagi), Sorghum (*Sorghum bicolor*), Finger Millets (*Eleusine coracana*)
Tolerance to biotic stress (biocontrol)	*Paenibacillus xylanexedens*, *Bacillus amyloliquefaciens*, *Streptomyces* sp., *Ochrobacttrum intermedium*, *Paenibacillus lentimorbus*, *Pseudomonas* spp.	Wheat (*Triticum aestivum* L.), Rice (*Oryza sativa*), Pine (*Pinus taeda* L.), Tomato (*Lycopersicum esculentum* Mill.)
Increased nutrient absorption	*Pantoea* sp. S32, *Paenibacillus polymyxa*	Rice (*Oryza sativa* L.), Habanero pepper (*Capsicum chinense*)
Seed germination enhancement	*Serratia marcences*, *Pseudomonas fluorescens*, *Azospirillum lipoferum*, *Pseudomonas putida*, *Bacillus subtilis*, *Providencia* sp., *Brevundimonas diminuta*	Maize (*Zea mays* L.), Wheat (*Triticum aestivum* L.)
Biostimulation by phytohormone(s) production	*Azospirillum lipoferum*, *Bacillus subtilis*, *Arthrobacter protophormiae*, *Dietzia natronolimnaea*, *Bacillus* sp.	Rice (*Oryza sativa* L.), Tomato (*Solanum lycopersicum* L.), Wheat (*Triticum aestivum* L.)
Soil fertility enhancement	*Bacillus subtilis*, *Bacillus cereus*, *Rhizobium* spp.	Poplar (*Populus* sp.), Mung bean (*Vigna radiata* L.)
Bioremediation of heavy metals and pollutants	*Ochrobactrum* sp., *Bacillus* spp., *Pseudomonas* spp., *Pseudomonas fluorescens*, *Bacillus cereus*, *Alcaligenes feacalis* RZS2, *Pseudomonas aeruginosa* RZS3, *Enterobacter* sp. RZS5	Rice (*Oryza sativa* L.), Groundnut (*Arachis hypogaea*), Maize (*Zea mays* L.), Ashwagandha (*Withania somnifera*)
Modulation of plant secondary metabolites	*Bacillus subtilis*, *Azotobacter chroococcum*, *Pseudomonas putida*, *Bacillus pumilus*, *Exiguobacterium oxidotolerans*	Basil (*Ocimum basilicum*), Brahmi (*Bacopa monnieri* L.)

Figure 3. An overview of the mechanisms of microorganisms in inoculated plants (Basu et al., 2021).

NEW APPROACHES IN THE USE OF MICROBIAL PRODUCTS

While live microorganisms aim to provide beneficial plant nutrition and health properties, it is important to consider the factors that may limit these benefits. Microorganisms that exhibit positive characteristics under laboratory conditions may not always deliver the desired benefits in natural environments due to various environmental factors. Especially, when dealing with disease agents, it is crucial to thoroughly analyze the relationships between plants and pathogens, as well as the prevalent environmental factors in a specific area, before implementing biological control measures. Instead of relying on a single microorganism, applying a population of microorganisms with cooperative traits is

more beneficial, as it creates a stable rhizosphere that provides more effective control against pathogens. In addition to microbial applications, the use of other plant products, such as extracts, biofertilizers and biopesticides, which are natural enemies of pests and/or disease-causing pathogens, also contributes to the success of biological control (Pandit et al. 2022).

In soil, only about 5% of the necessary nutrients are available compared to those in laboratory conditions. Additionally, there is ongoing competition for essential nutrients like nitrogen and carbon, meaning only microorganisms with strong competitive abilities can survive (Shah et al. 2021). Climate change is contributing to a steady increase in soil temperatures (Zhang et al. 2019). This rise impacts microorganisms closely associated with plants, as their optimal growth, diversity, and physiological functions depend on specific temperature ranges (Wu et al. 2010). Deviations from these optimal temperatures can lead to varied outcomes in microorganism-plant interactions. Soil warming notably increases microbial respiration and mortality rates (Wu et al. 2010; Schindlbacher et al. 2011). Moreover, excessive water evaporation and the accumulation of chloride salts, such as sodium chloride (NaCl) and magnesium chloride ($MgCl_2$), raise soil salinity. This increase in salt concentration adversely affects agricultural microbial biomass, seed germination, and plant development by disrupting osmotic potential and ion exchanges (Sindhu et al. 2022).

Host plant and microorganism interactions often determine host specificity, which can lead to a decrease in microbial benefit rates in many crop plant species. In soils with microbial diversity, the lack of specificity in plant species can lead to high competition among microorganism populations (Figueiredo et al. 2011). Additionally, any changes in biochemical compounds produced by plant roots or signaling molecules produced by microorganisms can disrupt potential symbiotic relationships (Chagas et al. 2018). Another important factor is soil pH. The availability of nutrients in the soil solution, for both plants and microorganisms, is greatly influenced by soil pH. The pH is a measure of the hydrogen ion concentration in the colloidal soil solution and affects most chemical reactions in the soil. Acidic soils are dominated by *Acidobacteria*, while alkaline soils are dominated by *Actinobacteria*, causing shifts in microbial populations with changes in soil pH. This can lead to the failure of the desired population to perform its intended function in microbial product use (Shah et al. 2021). Many rhizobacteria exhibit optimal population and efficacy at a pH level of 6.0 (Roe et al. 1998).

The commercialization and application of biological fertilizers involving live microorganisms begins with a technical procedure to isolate ectophytic or endophytic microorganisms from the rhizosphere and plant tissues, especially the roots. This process starts with bioresearch, where plant or soil samples are collected from diverse environments and subjected to microbial isolation in the laboratory. Once rhizobacteria are isolated, they undergo in vitro screening to evaluate their potential for promoting plant growth or serving as biocontrol agents against phytopathogens. Promising strains demonstrating effective plant growth promotion or biocontrol properties are then tested under controlled environmental conditions to assess their impact on plant development. Effective strains are selected based on these tests and further evaluated under field conditions to determine their ability to enhance crop growth. After successful repeated trials, companies typically proceed to commercialize these biological strains as biological fertilizers and/or biocontrol products, following formulation of the final product (Nakkeeran et al. 2005).

An unconventional approach in the field of microbial products that has emerged due to advances in genetics involves enhancing microorganism species and their efficacy, as well as developing new variants. This approach involves intentionally modifying the genetic material of microorganisms, specifically DNA to improve the beneficial traits of microbial strains. Scientists can alter bacterial DNA using techniques such as CRISPR-Cas9, which allows for specific modifications. This genetic manipulation can help bacteria produce chemicals that assist in their proliferation, growth, stress resistance or more efficient nutrient utilization (Bernela et al. 2021; Hasan et al. 2024).

In recent years, leading global agricultural firms have increasingly focused their investments on bio-based product research. Figure 4 lists the revenues and profits of some international companies in the plant protection sector for the second quarter of 2024. Additionally, Figure 5 shows the targeted revenues from bio-based products and the budgets allocated for research and development (R&D) by these same firms for the year 2024. Comparing the data in both figures reveals that firms leading the world in pesticide production are allocating a significant portion of their current revenues to R&D projects aimed at generating revenues from bio-based products over the next decade.

Figure 4. Revenue Table of Some International Firms in the Plant Protection Sector (UPSTREAM 2024)

Q2 2024 CROP PROTECTION COMPARISON

COMPANY	REVENUE	REVENUE CHANGE YOY (%)	EBITDA	EBITDA MARGIN (%)
BAYER	$5.42 Billion	1.1%	$572 million**	10.5%
Syngenta Group	NA	NA	NA	NA
CORTEVA	$6.11 Billion	1%	$1.92 Billion	29%
BASF We create chemistry	$2.11 Billion	13.2%	$147 million**	6.9%
FMC	$1.04 Billion	2%	$202 million	20%
UPL	$1.09 Billion	1%	$137.4 million	12.6%

2024 Q2 Numbers with USD conversion at 1.09 (UPL Q1 24/25)
**Before Special Items
Sources: Company Quarterly Reports
Red indicates lower than 2023

Upstream

2024 AGRIBUSINESS BIO-BASED REVENUE COMPARISON

COMPANY	CURRENT BIO-BASED REVENUE	FORECASTED REVENUE	FORECASTED CAGR THROUGH PERIOD	2023 R&D EXPENDITURE*	PARTNERSHIPS, INVESTMENTS & ACQUISITIONS****
BAYER	$215 million (2022)	$1.61 billion by 2035	17%	$2.5 billion***	Sound, JOYN BIO, kimitec
syngenta	$400 million (2023)	NA	NA	$1.4 billion	Valagro, VESTARIEN, biotalys, arotic, lavie bio, Sound
CORTEVA	$420 million (2023)	$2 billion by 2035	17%	$1.34 billion	Symborg, Stoller, elemental enzymes, lavie bio
FMC	$180 million (2023)	$2 billion by 2033	20%	$329 million	AgroSpheres, RiePhore, micropep, novonesis
UPL	~$400 million (2022/23)**	NA**	14% through 2027	$195 million	BIOLASTA BIO, IMPETUS AG, Arysta

*2023 Numbers with USD conversion, except UPL 2022/23
**UPL has not release its 2023/24 results in detail as of June 27th 2024. UPL *NPP Business Unit not entirely made up of biologicals. Given destocking challenges, UPL numbers likely significantly down in 23/24 so refraining from calculating the CAGR from 22/23 numbers
*** Bayer Annual Report cite $1.9 Billion USD, but have a "special items" EBITDA adjustment
**** Not all encompassing
Note: BASF has not publicly shared any biological numbers so they have been excluded
Sources: Company Annual Reports

Upstream

Figure 5. Targeted Revenue from Bio-Based Products and R&D Budget Allocation of Some International Firms (UPSTREAM 2024)

CONCLUSIONS

In today's rapidly evolving climate conditions and the anticipated impacts in the coming years, factors such as rising temperatures and drought, excessive use of chemical fertilizers, salinity from intense evaporation, global warming and reduced water resources due to unregulated water consumption are critical issues affecting agricultural production. Additionally, changing ecosystems due to climate change, the evolution of harmful pathogens and pests and the resulting resistance changes in nature due to pest control measures are pressing and unresolved problems in agriculture. To address these widespread issues and enhance the sustainability of agricultural production, microbial products containing bacteria, fungi, algae and other microorganisms offer a promising alternative. These products provide functional benefits for soil and plants, including plant nutrition, plant protection, and growth regulation, as alternatives to chemical fertilizers and plant protection products.

Some technical details regarding the use of these products emerge as factors determining their effectiveness. Research and advancements in the field aimed at eliminating environmental factors that limit the usefulness of microorganisms are promising. Similar to breeding efforts for plants to achieve higher yields, resistance to diseases and pests and meeting market quality criteria such as color, shape, brightness and taste, the production of live microbial fertilizers and microbial breeding through new technologies that minimize the impact of environmental conditions on microorganisms have opened up new possibilities in the field. It is encouraging to see international pesticide-producing firms

investing in scientific research in this area. Additionally, the progress made by some plant nutrition and protection companies in Turkey, which are exploring bio-based product research and commercial applications represents a positive development in agricultural practices.

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ASSESSMENT OF HABITAT HEALTH IN ÇARDAK LAGOON *USING CARCINUS AESTUARII* NARDO, 1847 AS A BIOINDICATOR

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ABSTRACT

Carcinus aestuarii Nardo, 1847 is a portunid crab species native to the Mediterranean Sea. It is commonly found in lagoon systems and is considered a potential bioindicator due to its biological responses to environmental pressures. This study analysed *C. aestuarii* individuals living in different benthic habitats (sand, mud, rock and macroalgae) in Çardak Lagoon, based on morphological and behavioural indicators (e.g. shell change, chela loss and habitat selection). A total of 632 individuals were collected from eight stations during fieldwork conducted between May 2024 and April 2025, and the condition of their shells and the presence of regeneration were recorded for each individual. The findings showed that 67% of individuals in the moulting phase were found in muddy and algal habitats, while the claw loss rate in sandy and rocky habitats increased to 45%. The highest number of individuals (n = 230) was recorded at BS6 station, which has fine sediment with a high organic matter content. The highest shell change rate (21%) was observed at BS1 station, which is muddy and low-energy. The direct effect of habitat structure on crab life strategies (molting, defence and reproduction) was statistically significant ($p<0.05$). These results imply that the habitat preferences and morphological condition of *C. aestuarii* can serve as biological indicators for evaluating the health of the lagoon. In particular, monitoring molting behaviour provides valuable information for the early detection of habitat pressure in ecological management.

Keywords: *Carcinus aestuarii*, lagoon ecosystems, habitat health, bioindicator, molting, Çardak Lagoon

INTRODUCTION

Coastal lagoons are dynamic transition zones between land and sea that play a crucial role in maintaining biodiversity, supporting fisheries, and providing essential ecosystem services. Due to their semi-enclosed nature, lagoons are highly sensitive to anthropogenic pressures such as pollution, eutrophication, and habitat degradation. Therefore, effective monitoring tools are required to assess their ecological health.

One of the most promising approaches in recent years has been the use of bioindicator species, which reflect environmental changes through their physiological, morphological, or behavioral responses. Among benthic crustaceans, the Mediterranean green crab Carcinus aestuarii Nardo, 1847 is a widely distributed portunid species inhabiting lagoons, estuaries, and shallow coastal habitats. Its tolerance to variable salinity and temperature regimes, combined with visible biological responses such as molting frequency, chela loss, and regeneration, make it a strong candidate for bioindicator studies.

Previous research in Mediterranean and Adriatic lagoons has demonstrated that C. aestuarii exhibits habitat-dependent variations in abundance, reproduction, and molting cycles (Kevrekidis, 2004; Glamuzina et al., 2017). However, studies directly linking its morphological and behavioral traits to habitat quality remain limited. Assessing these traits in different benthic environments (muddy, sandy, rocky, and algal substrates) provides valuable insights into habitat suitability and ecosystem resilience.

Building on this perspective, the present study aims to evaluate the potential of C. aestuarii as a bioindicator of habitat health in Çardak Lagoon (Turkish Straits System). Specifically, it investigates the relationships between habitat structure and key biological parameters—molting stage, chela loss, and regeneration—in order to establish reliable biological indicators that complement traditional physicochemical monitoring. Unlike previous studies that mainly focused on the population structure, reproduction, or feeding ecology of C. aestuarii, this study uniquely links its morphological and behavioral responses directly to habitat quality, thereby providing a novel bioindicator-based approach for assessing lagoon ecosystem health.

MATERIAL AND METHOD

This study was carried out in Çardak Lagoon, a semi-enclosed coastal ecosystem located in the Turkish Straits System. The lagoon hosts a variety of benthic habitats, including muddy, sandy, rocky, and algal-dominated areas, each representing distinct environmental conditions. To reflect this heterogeneity, eight sampling stations (BS1–BS7) were selected across the lagoon. BS1 and BS6 were dominated by fine-grained muddy substrates with high organic matter accumulation, BS2 and BS5 represented sandy and rocky areas, BS3 and BS4 consisted of mud with partial algal coverage, while BS7 was situated at the intersection of rocky and muddy grounds, acting as a transitional zone.

Field sampling was conducted during May 2024 and April 2025. Individuals of Carcinus aestuarii were collected manually by hand nets and with baited traps during low tide conditions to ensure adequate representation across habitat types. In total, 632 crabs were recorded, with their distribution varying considerably among the stations.

For each captured individual, several biological parameters were examined. The molting stage (intermolt, premolt, and postmolt) was determined by assessing the condition of the exoskeleton, while chela loss and evidence of regeneration were recorded as indicators of physical stress. Sex determination was performed based on abdominal morphology, and the carapace width of each specimen was measured to the nearest 0.1 mm using a digital caliper.

In addition to biological data, physicochemical parameters of the water column were measured in situ at each station using a YSI 6600 multiparameter probe. These included temperature (°C), salinity (‰), dissolved oxygen (mg L^{-1}), and pH. Sediment type and habitat structure were documented visually and verified through sediment grab samples to ensure accurate habitat classification.

The collected data were analyzed both descriptively and statistically. The distribution of individuals among different habitats and stations was compared using the Chi-square test in order to detect significant differences ($p < 0.05$). Frequencies of molting, chela loss, and regeneration were calculated as percentages relative to habitat type. Environmental variables were expressed as mean values with standard deviations, while spatial differences in crab abundance were illustrated with comparative figures and maps. This integrative methodological approach enabled the evaluation of both biological and environmental indicators in assessing habitat health within the lagoon.

RESULT AND DISCUSSION

A total of 632 Carcinus aestuarii individuals were collected from eight sampling stations in Çardak Lagoon between May 2024 and April 2025. Their distribution across stations showed marked differences. The highest abundance was recorded at BS6 (230 individuals), followed by BS4 (176 individuals). Moderate counts were observed at BS5 (96 individuals) and BS7 (58 individuals), while BS1, BS2, and BS3 yielded the lowest values with 28, 30, and 15 individuals respectively (Figure 1).

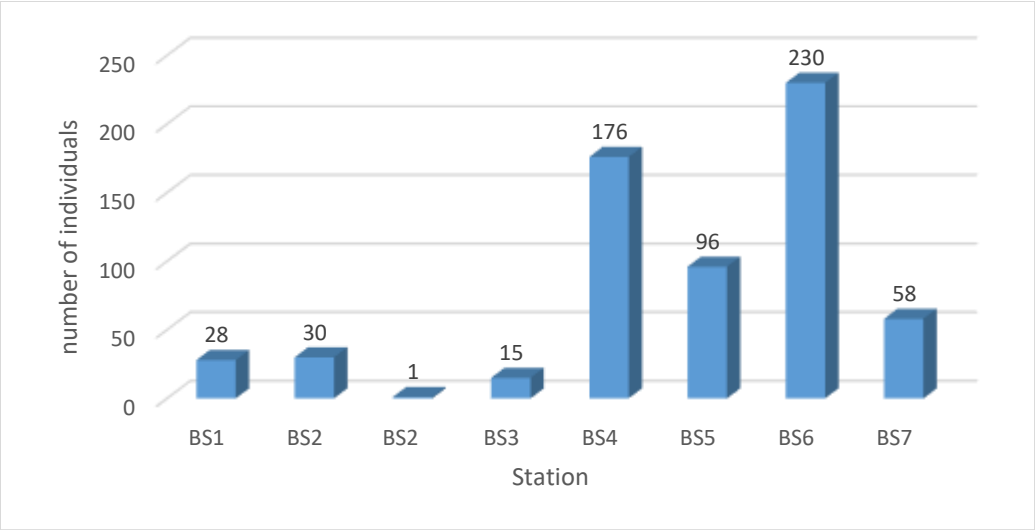

Figure 1. Spatial distribution of *Carcinus aestuarii* individuals across sampling stations (BS1–BS7) in Çardak Lagoon

Seasonal variation was evident in the abundance of C. aestuarii. The maximum number of individuals was observed in May (124), followed by July (83) and June (75). Intermediate values were recorded in October (68), August (55), and November (49), while the lowest abundances occurred in February (8) and January (27) (Figure 2).

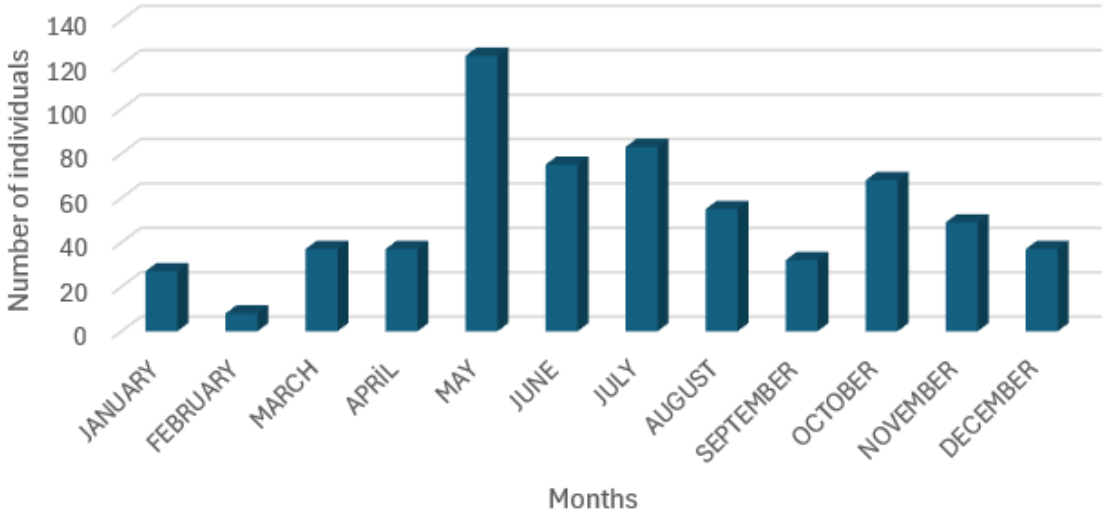

Figure 2. Monthly variation in the abundance of *Carcinus aestuarii* individuals recorded in Çardak Lagoon

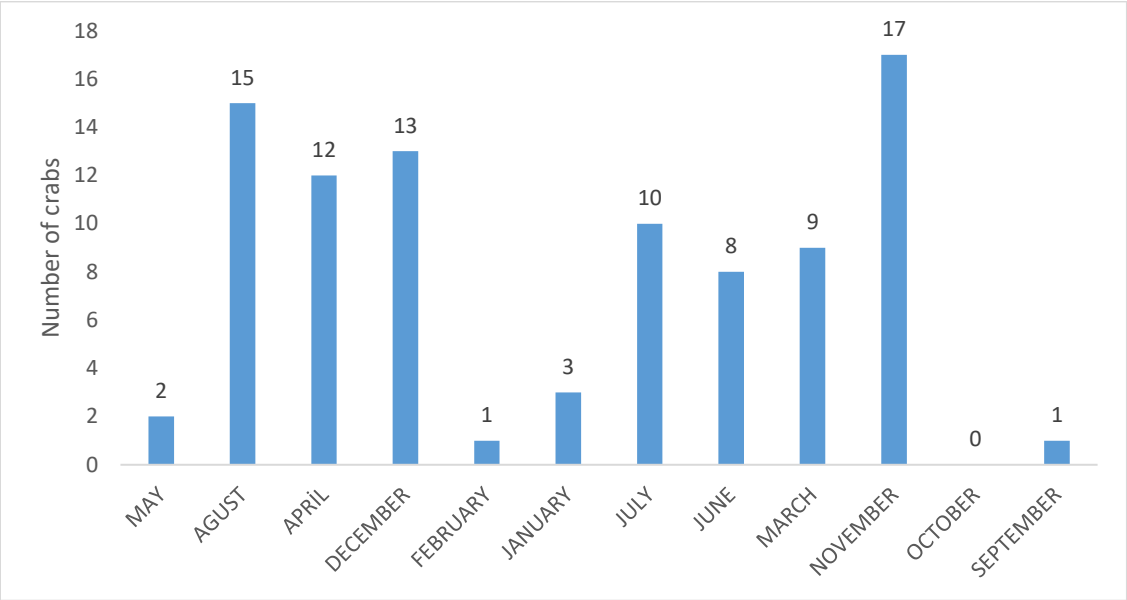

Figure 3. Monthly variation in molting frequency of *Carcinus aestuarii* in Çardak Lagoon

Molting frequency fluctuated throughout the year. The highest number of molting individuals was observed in November (17), followed by August (15), December (13), and April (12). Moderate levels were recorded in March, June, and July, whereas low molting frequencies were noted in January, February, May, and September. No molting individuals were detected in October (Figure 3).

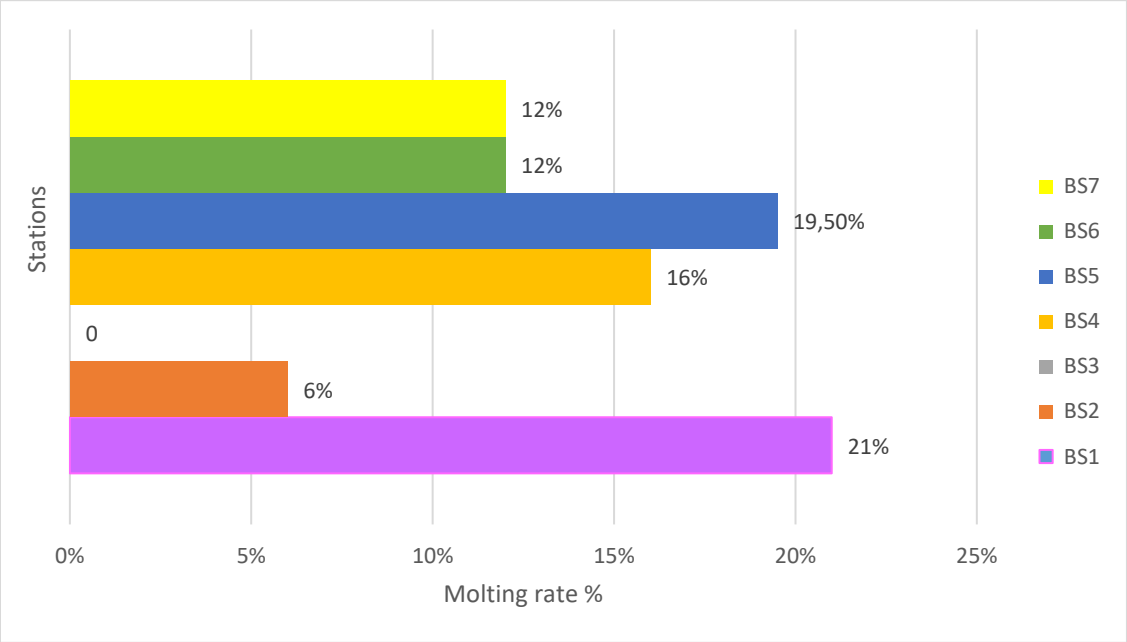

Figure 4. Station-based variation in molting ratio of *Carcinus aestuarii* in Çardak Lagoon

Spatial differences in molting ratios among stations were also evident. The highest molting proportion was observed at BS1 (21%), followed by BS5 (19.5%) and BS4 (16%). Moderate levels were detected at BS6 and BS7 (12% each), while BS2 showed a low proportion (6%), and no molting was recorded at BS3 (0%) (Figure 4).

Chela loss and regeneration varied among habitat types. In sandy and rocky habitats, up to 45% of individuals exhibited chela loss, whereas regeneration was more frequently recorded in muddy and algal stations. Statistical analysis confirmed that these differences were significant (Chi-square test, $p < 0.05$). Finally, the spatial distribution of benthic habitat types in Çardak Lagoon was mapped. Sandy and algal substrates were concentrated in the western lagoon, while muddy and rocky substrates were dominant in the eastern part (Figure 5). This heterogeneity in substrate composition provided a basis for analyzing the relationship between habitat structure and the biological responses of *C. aestuarii*.

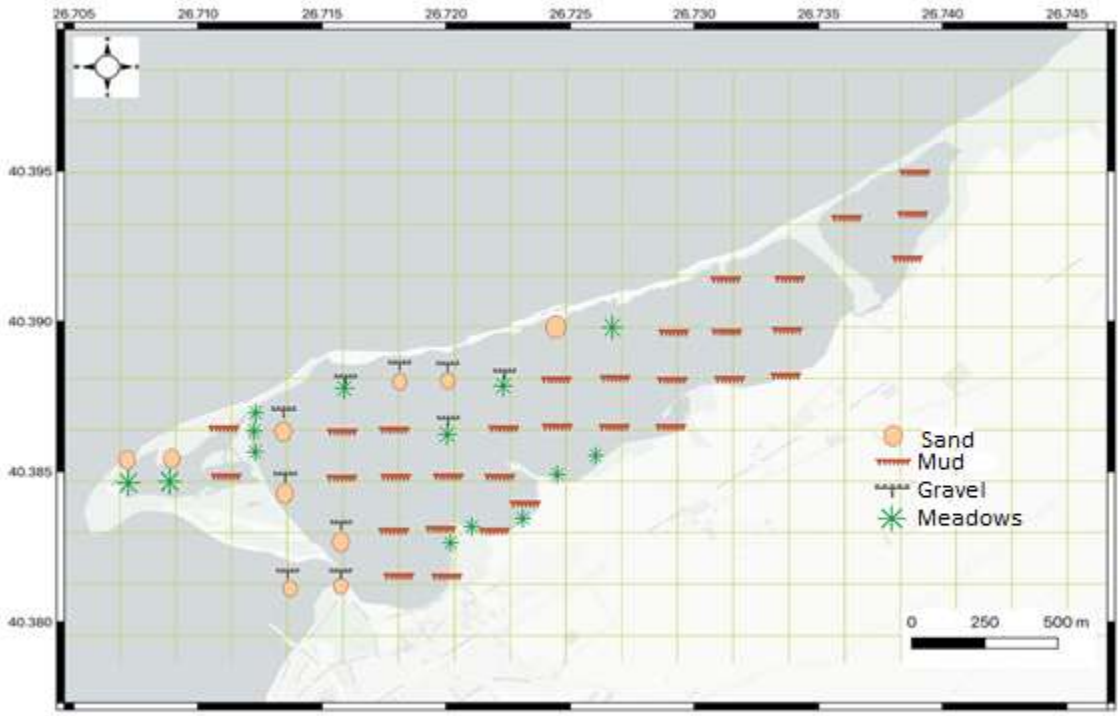

Figure 5. Spatial distribution of benthic habitat types (sand, mud, rocky, and algal substrates) in Çardak Lagoon

The moulting phase is a period during which soft-shelled individuals are more vulnerable to predation. Consequently, they have been observed seeking out more sheltered microhabitats. The rate of chela loss in open areas suggests greater environmental pressure and competition. These observations support the idea that the habitat selection and morphological posture of C. aestuarii may act as biological indicators of lagoon health. Our study evaluated the distribution and morphological responses of C. aestuarii across different habitats (mud, sand, rock and algae) within the Çardak lagoon. The results were then compared with those of other habitat-based studies. The results showed that the species was particularly concentrated in muddy and algal areas, and that it tended towards more sheltered habitats during the moulting phase. These results are consistent with those reported in studies conducted in the Venice Lagoon and other Mediterranean lagoons, which indicated that structurally complex habitats, such as seagrass beds, rocky areas and mussel beds, are preferred for

moulting and sheltering behaviour (Mancinelli et al., 2013; Glamuzina et al., 2017). Therefore, C. aestuarii's tendency to select sheltered areas during vulnerable periods is consistent with our study and previous research. On the other hand, this study highlights lagoon-specific conditions that may influence the distribution and life history characteristics of crabs by revealing differences between stations. For example, the particularly high moulting rate at BS1 suggests that environmental factors such as oxygen regime and nutrient cycling in low-energy muddy areas may play a significant role in triggering moulting. Conversely, the absence of moulting individuals at BS3 indicates that habitat quality in this area is not optimal. The moulting cycle of Carcinus aestuarii, a key species in many lagoon ecosystems, is affected by various environmental factors, including temperature, salinity and food availability. Seasonal changes play a significant role in determining the moulting timing of this species of crab, particularly in Mediterranean lagoons, where it is commonly found. Studies have shown that C. aestuarii generally exhibits higher activity and abundance during the warmer months (May to October), which is associated with higher water temperatures that increase metabolic rates and feeding in crustaceans. A study conducted in Monolimni Lagoon reported that the C. aestuarii population peaked in May and June, indicating a direct relationship between increased temperature and biological activity. This could affect moulting frequency and success (Kevrekidis, 2004). Similarly, Acar and Ateş emphasised that environmental conditions such as temperature and salinity directly affect the growth and reproductive characteristics of C. aestuarii (Acar & Ateş, 2020), implying that moulting times differ in different lagoons. Furthermore, salinity levels are also crucial. As a species inhabiting both rivers and lagoons, C. aestuarii can adapt significantly to fluctuating salinity levels, which can also influence moulting behaviour. Salinity fluctuations can cause stress responses that may delay the moulting process, indicating that changing lagoon conditions in heterogeneous environments can lead to different moulting programmes (Acar & Ateş, 2021; Rivera-Ingraham et al., 2015). Additionally, high summer temperatures not only accelerate metabolic processes but also require the organism to effectively manage its osmoregulation. This is particularly important during moulting, as any environmental stress can jeopardise this sensitive phase (Glamuzina et al., 2017). The economic importance of soft-shell crabs, particularly in the fishing industry, reflects their annual moulting cycles. The seafood industry in the Venetian Lagoon highlights the economic significance of harvesting crabs during their moulting period, when they are most vulnerable yet most valuable (Cilenti et al., 2024). The economic perspective under discussion here emphasises the necessity of understanding and accurately predicting moulting cycles in order to optimise sustainable harvesting practices. Research studies have also examined the biochemical and physiological responses during moulting under changing environmental conditions. Research has demonstrated that crabs undergo fluctuations in metabolic markers that are associated with the moulting cycle. This suggests that environmental stresses have the capacity to alter moulting times (Cogo et al., 2018; Matozzo et al., 2011). For instance, the maintenance of suitable temperature and salinity ranges is imperative to optimise the moulting period, a process that exerts a substantial influence on population dynamics within lagoon ecosystems. In concluding this study, it is evident that the moulting time of Carcinus aestuarii in lagoon ecosystems is a multifaceted interaction of seasonal climatic effects, environmental salinity and temperature changes, which significantly affects its life cycle and economic viability. In order to make accurate predictions about these cycles, further integrated studies are required to examine the physiological adaptations and responses of C. aestuarii to its dynamic habitat.

CONCLUSIONS

This study demonstrated that *Carcinus aestuarii* can serve as a valuable bioindicator species for assessing habitat health in lagoon ecosystems. The results revealed clear spatial and temporal

variations in the abundance and biological responses of the species within Çardak Lagoon. Individual density was highest in muddy and organic matter-rich habitats, particularly at stations BS6 and BS4, while sandy and rocky substrates supported fewer individuals. Seasonal patterns showed that population abundance increased notably during late spring and summer, corresponding with favorable environmental conditions and potential reproductive activity. Molting frequency and chela loss provided further insights into habitat quality. Molting individuals were predominantly observed in sheltered habitats such as muddy and algal areas, whereas chela loss was more common in exposed sandy and rocky habitats, indicating higher physical stress and predation risk. These findings confirm that the morphological and behavioral traits of C. aestuarii reflect habitat characteristics and environmental pressures.

In view of these findings it is recommended that Carcinus aestuarii be integrated into long-term lagoon biomonitoring programs as a practical and cost-effective bioindicator species. Standardizing biological responses such as molting frequency, chela loss, and regeneration alongside physicochemical parameters would provide a more holistic understanding of habitat health. Conservation strategies should particularly emphasize the protection of muddy and algal habitats, which support critical life stages of the species and serve as ecological hotspots within lagoon systems. Furthermore, future research should adopt multivariate analytical approaches to strengthen the link between environmental drivers and biological responses, thereby improving predictive capacity in ecosystem assessments. Given the ecological and economic relevance of C. aestuarii, especially in the context of soft-shell crab fisheries, incorporating such biological indicators into management frameworks could also support sustainable resource use and enhance the resilience of lagoon ecosystems under increasing anthropogenic and climatic pressures.

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LIGHT-EMITTING DIODE LIGHT QUALITY INFLUENCES GERMINATION, GROWTH AND BIOCHEMICAL INDICES OF *ARNEBIA EUCHROMA* L.

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ABSTRACT

Arnebia euchroma (is locally known as *Hava Chobeh*) is a native plant of Iran and has a valuable place in traditional Iranian medicine. A. *euchroma* is used to treat many problems such as inflammation, joint pain, uterine infections, fungal and bacterial infections, and wound healing and burns. One of the limitations in cultivating this valuable plant is its low germination percentage and uneven germination. In recent years, the use of LED light sources has increased the germination of medicinal plants. Therefore, in this study, LED light treatments were used to improve the germination and growth characteristics of the A. *euchroma* plant. The present study was conducted at the Faculty of Agriculture and Natural Resources, University of Mohaghegh Ardabili (IRAN) in 2025. The experiment was conducted in a completely randomized design with 4 treatments and 4 replications. Four light treatments were used as follows: 100% white light, 100% blue light, 100% red light, and a combination of blue and red light (50:50). Germination indices (germination percentage and germination rate), morphological (seedling dry weight, root length, and stem length), and biochemical (photosynthetic pigments, total carbohydrates, and total protein) were examined. The findings indicated that red light enhanced various germination and growth metrics of the A. *euchroma* plant. When analyzing the biochemical traits of 30-day-old seedlings of the A. *euchroma* plant, it was observed that combining blue and red light significantly elevated the levels of photosynthetic pigments, total carbohydrates, and total protein. Therefore, the use of red LED during seedling stages was considered an effective treatment to improve germination and growth characteristics. Additionally, according to the findings, the A. *euchroma* plant exhibited different reactions to light wavelengths during later growth stages, with the blue and red light combination promoting higher rates of photosynthesis and increasing carbohydrate and protein levels.

Keywords: Germination percentage, Germination rate, Photosynthetic pigments, Total carbohydrates, Total protein

INTRODUCTION

Medicinal plants, as an important part of natural and biological diversity, have played a significant role in the traditional medicine of human societies and in improving human health (Buso et al., 2020). As a rich source of bioactive compounds, these plants possess numerous therapeutic properties that have been beneficial in the prevention and treatment of various diseases. One of the valuable medicinal species in traditional Iranian medicine is the plant *Hava Chobeh* or *Hochoe*, which is known as Abu Khalsa in the book of Abu Ali Sina's Canon (Mizani et al., 2021).

Hava Chobeh is known scientifically as *Arnebia euchroma* L. and belongs to the *Boraginaceae* family. A. *euchroma* is a hairy and perennial plant that is found mainly in the temperate and sub-alpine regions of the western Himalayas, the subtropical regions of North Africa, and parts of Central Asia, especially Iran, Afghanistan, and India (Kumar et al., 2021).

The leaves and roots of this plant have been widely used in the traditional systems of various communities as an antimicrobial and anti-inflammatory medicine in the treatment of chronic diseases (Chawla et al., 2021). Phytochemical studies have shown that the roots of this plant (Fig. 1) contain a natural phytochemical compound called naphthoquinone, such as shikonin, phenolic compounds, and their derivatives, which act as an anticancer, antibacterial, antifungal, and antioxidant drug (Kazybekov et al., 2024).

Population ecology reports have shown that overexploitation of this plant has placed it in the group of endangered species (Kumar et al., 2021). To address the crisis of the extinction of medicinal plants, the use of cultivation techniques for endangered plants can help preserve and restore these valuable resources (Catana et al., 2020).

Previous studies have indicated that the germination of many medicinal plants is related to the spectrum (quality or color) of light, and the combination of specific wavelengths of light has had a significant effect on increasing germination and growth of some species (Sena et al., 2024). Today, LEDs (light-emitting diodes) have replaced traditional light sources as a source of various colored light with many advantages. With LEDs, the quality of light can be easily adjusted, and growers and researchers can achieve different physiological responses in plants according to their specific goals (Wang et al., 2021).

Recent studies have revealed that a high proportion of red light is essential for seed germination and seedling establishment of *Matricaria chamomilla* L. (Bączek-Kwinta and Michałek, 2025). Also, the application of red light to pepper seeds (*Capsicum annuum* L.) has improved the germination rate, emergence and growth of radicles (Zaghdoud et al., 2023). Several studies have shown the importance of light spectra on the germination of plant species, however, it has been found that the germination and growth responses of plants to color spectra depend on the species (Soufi et al., 2025). Therefore, considering the medicinal importance of the A. *euchroma* plant and its uncontrolled harvesting from nature, the present study aimed to use different light spectra to improve the germination and growth of this valuable species.

Figure 1. The roots of *Arnebia euchroma* L. plant collected from mountainous areas of Ardebil, Iran

MATERIAL AND METHOD

The present study was conducted at the Faculty of Agriculture and Natural Resources, University of Mohaghegh Ardabili (Iran) in 2025. The experimental treatments included 4 light treatments (4 light chambers) as follows: 100% white LED light or W (wavelength 420-680 nm), 100% blue LED light or B (wavelength 460-470 nm), 100% red LED light or R (wavelength 620-630 nm), and a combination of blue and red LED light or BR (with a ratio of 50:50). Day and night temperatures were set at 20°C and 16°C, respectively. The relative humidity of the light chambers was set at 60% and the photoperiod in all light chambers was 16 hours of light. The photosynthetic photon flux density (PPFD) measured above all Petri dishes and plants was 20 μmol/m^2 s. LED lamps (16 W, 1200 × 24 mm) were purchased from Pars Shahab Company, Iran. Each panel contained 96 LED lamps. The voltage, current, and frequency were AC 85-220 V, 100 mA, and 60.50 Hz, respectively.

The seeds of *Arnebia euchroma* L. were collected from the mountainous areas of Ardabil, Iran. Seeds were disinfected using sodium hypochlorite solution (2%) for 5 minutes and placed in a Petri dish containing wet filter paper. For each treatment, 4 Petri dishes were considered, and 25 seeds were placed in each Petri dish (a total of 100 seeds for each treatment). Then the petri dishes were transferred to light chambers.

Germination indices were evaluated by radicle emergence (2 mm in length) for 21 days based on the following formulas:

$$\text{Germination Percentage (GP)} = \frac{\text{Total number of germinated seeds}}{\text{Total number of seeds per assay}} \times 100$$

$$\text{Speed of Germination (SG)} = \frac{n_1}{d_1} + \frac{n_2}{d_2} + \frac{n_3}{d_3} + \cdots \frac{n_i}{d_i}$$

In this formula, n is the number of germinated seeds and d is the number of days.

Morphological indices were measured in 7-day-old seedlings. In each experimental replicate, 10 seedlings were evaluated. Morphological traits included seedling dry weight (DW), stem length (SL), and root length (RL). To measure dry weight (g), a digital laboratory balance (Semi-Micro Analytical Balances GR-200 model, A&D, Japan, with an accuracy of 0.0001 g) and an oven (at 40°C for 48 hours) were used. A ruler was utilized to measure stem length (cm) and root length (cm).

Then, the seedlings were grown in plastic pots containing peat moss and perlite (1:1) and placed in the same light chambers. The A. *euchroma* plants were evaluated for biochemical indices after 4 weeks. Photosynthetic pigments were measured at 470, 645, and 662 nm using a spectrophotometer (model SP-UV 200, Spectrum Instruments Limited, Australia). The concentration of photosynthetic pigments (µg/g fresh weight) was calculated using the following formulas (Lichtenthaler and Buschmann, 2005):

$$\text{Chlorophyll a} = C_a\ (\mu g/ml) = 11.24 \times A_{661.6} - 2.04 \times A_{644.8}$$

$$\text{Chlorophyll b} = C_b\ (\mu g/ml) = 20.13 \times A_{644.8} - 4.19 \times A_{661.2}$$

$$\text{Total carotenoids} = C_{(x+c)}\ (\mu g/ml) = \frac{(1000 \times A_{470} - 1.90C_a - 63.14C_b)}{214}$$

Total carbohydrate content was measured using anthrone reagent at a wavelength of 630 nm. A glucose standard curve was used to calculate glucose equivalents (mg/g fresh weight) in each sample (Omokolo Doumou et al., 1996). Coomassie Brilliant Blue reagent was used to measure total protein content. The absorbance of the samples was read at a wavelength of 595 nm using a spectrophotometer. The total soluble protein content was calculated in mg/g fresh weight using a Bowen serum albumin (BSA) standard curve (Bradford, 1976).

This experiment was conducted in a completely randomized design with 4 replications. Data analysis was performed using SAS software version 9.1. Means were compared using Duncan's multiple range test at a 5% probability level. Graphs were drawn using Excel software.

RESULTS AND DISCUSSION

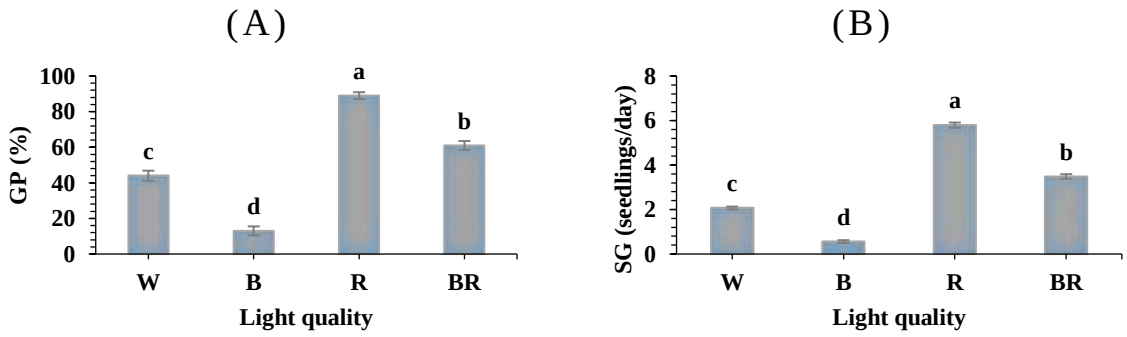
According to the results of the present study, light quality significantly affected the germination percentage and speed of germination. Comparison of the mean data showed that blue light treatment (B) reduced seed germination. In contrast, red LED light (R) significantly increased the GP and SG indices compared to other treatments (Fig. 2A and 2B).

Previous studies have indicated that increasing the germination percentage and germination speed are two important factors in improving the initial growth of seedlings, achieving uniform coverage in fields, increasing the ability to compete with weeds, etc. Accordingly, the application of treatments to improve the germination of plant seeds, especially in medicinal plants, can be fruitful with the aforementioned goals. Medicinal plants have been able to ensure their survival for many years by having a dormancy mechanism in their seeds. However, the presence of dormancy in medicinal plant seeds often led to great problems for researchers and breeders (Jamnani et al., 2023). Light not only provides energy for photosynthesis but also regulates the morphological development of plants. Some plant growth and development processes, such as germination, seedling development, and flowering induction, are affected by light. In the present study, although the B light treatment reduced the germination percentage, the application of R light enhanced germination. Furthermore, these results indicate that the seeds of the A. *euchroma* plant respond differently to light quality. According to the present study, in the experiments of Wang et al., the application of red light produced a similar result in the medicinal plant *Momordica charantia* L. (Wang et al., 2021). Furthermore, in the study of Pardo et al. (Paniagua Pardo et al., 2015), red light significantly increased the germination speed in *Brassica oleracea* L.

The results revealed that the growth characteristics (seedling dry weight, stem length, and root length) of the A. *euchroma* were significantly affected by LED treatments. Based on the results of the comparison of the mean data, the red light treatment (R) significantly increased the seedling dry weight (0.0899 g), stem length (4.125 cm), and root length (6.45 cm) compared to other light treatments. Also, the weakest growth characteristics were observed in the blue light treatment (B). In the stem length index, the combination of blue and red light (BR) was similar to the W light (Fig. 2C, 2D, and 2E).

Changes in light quality and quantity can affect the efficiency of photosynthesis and indirectly the availability of carbohydrates and energy for growth. Accordingly, the dry weight of the seedling of the A, *euchroma* increased under the influence of red light (R) at the seedling stage, as previously observed in *Lactuca sativa* L.(Sharma et al., 2024) and *Triticum aestivum* L. (Li et al., 2022). On the other hand, red light often causes elongation of the stems and roots. These effects have been observed in some plant species. It seems that the application of red light can facilitate shoot growth by increasing primary root growth and water and nutrient uptake (Solano et al., 2020). In addition, it has been reported that phytochromes regulate primary root length through red light (Li et al., 2022). It appears that shoot elongation in plants can vary significantly based on both the plant species and the specific growth stage. This variation is influenced by the interactions between blue/red light receptors and phytochromes, which can either promote or hinder growth. (Solano et al., 2020).

According to the results, light treatments were significantly effective on the content of photosynthetic pigments, total soluble carbohydrate, and total soluble protein. Blue light treatment (B) significantly reduced the content of chlorophyll a. Two light treatments, white (W) and red (R), showed similar results in the content of photosynthetic pigments. On the other hand, the combined treatment of blue and red light (BR) led to a significant increase in the content of photosynthetic pigments. In the indices of total carbohydrate content (176.36 mg/g fresh weight) and total protein (34.9208 mg/g fresh weight), the combined treatment of blue and red light (BR) indicated a greater effect than the other treatments in increasing the aforementioned indices. In addition, blue light treatment reduced the content of carbohydrate and protein (Fig. 3A, 3B, and 3C).



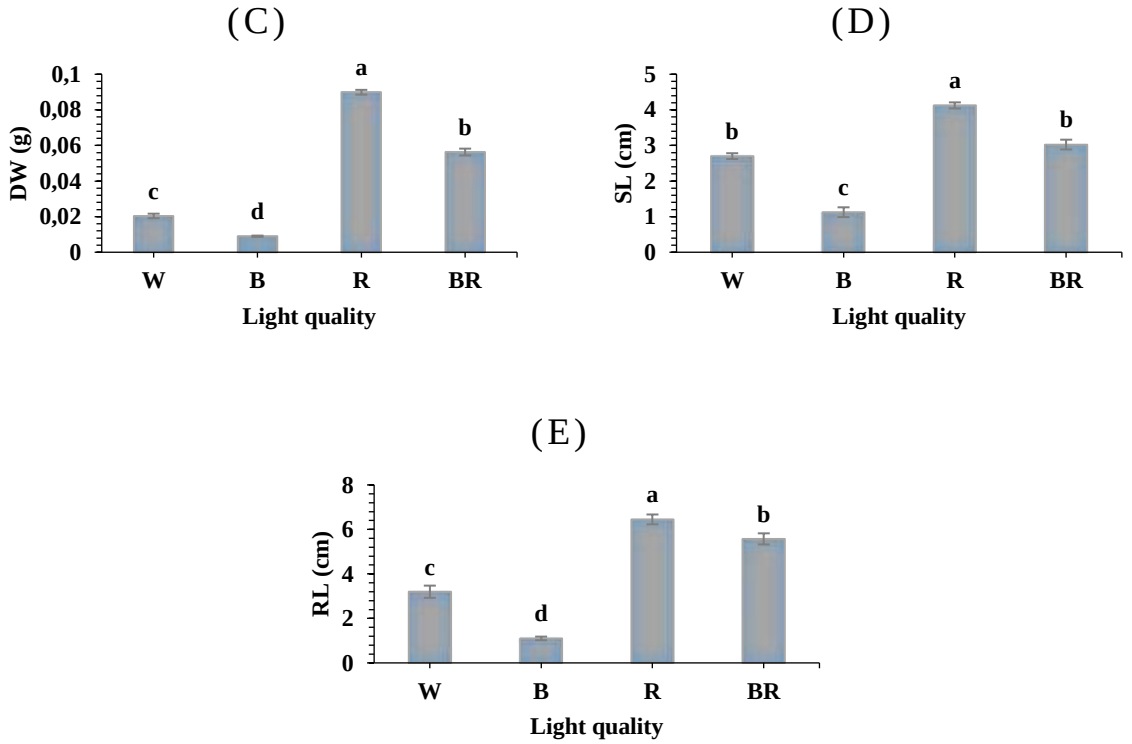


Figure 2. The effect of light qualities on germination and growth characteristics of the *Arnebia euchroma* L. plant. (A), germination percentage (GP), (B), speed of germination (SG), (C), seedling dry weight (DW), (D), stem length (SL), and (E), root length (RL). Different letters indicate significant differences determined using Duncan's multiple range test ($p < 0.05$). Error bars represent ±SE, n= 4

Studies have shown that the effect of spectral changes on growth and biochemical parameters (in addition to plant species) is likely to depend on the growth stage of plants. In the present study, biochemical indices were increased by the application of a combination of blue and red light (BR). Red and blue light constitute the largest share of the total light received by plants and are considered to be the most influential wavelengths. Chlorophyll and carotenoids cause photosynthesis in plants by absorbing the blue and red light spectrum (Li et al., 2022). In addition, blue light is involved in chlorophyll formation and chloroplast development. It has been reported that light quality is an important factor affecting the synthesis and accumulation of primary metabolites such as carbohydrates and proteins (Li et al., 2022). In this study, the content of total carbohydrates and soluble protein under BR light treatment was the highest. In some plant species, such as *Momordica charantia* L.(Wang et al., 2021), an increase in total carbohydrate content under BR light has been reported, which is due to the intervention of red and blue light in improving plant photosynthesis, carbohydrate biosynthesis, and increasing growth (Sharma et al., 2024). Although proteins affect photosynthetic performance, they have been less investigated in light quality studies. Similar to the results of this study, total protein content increased under BR treatment in *Cunninghamia lanceolata*. It has been reported that blue light has higher photon energy and can play a role in the synthesis of macromolecules such as protein. Additionally, blue light increases pyruvate kinase protein in the glycolysis pathway (Wang et al., 2021).

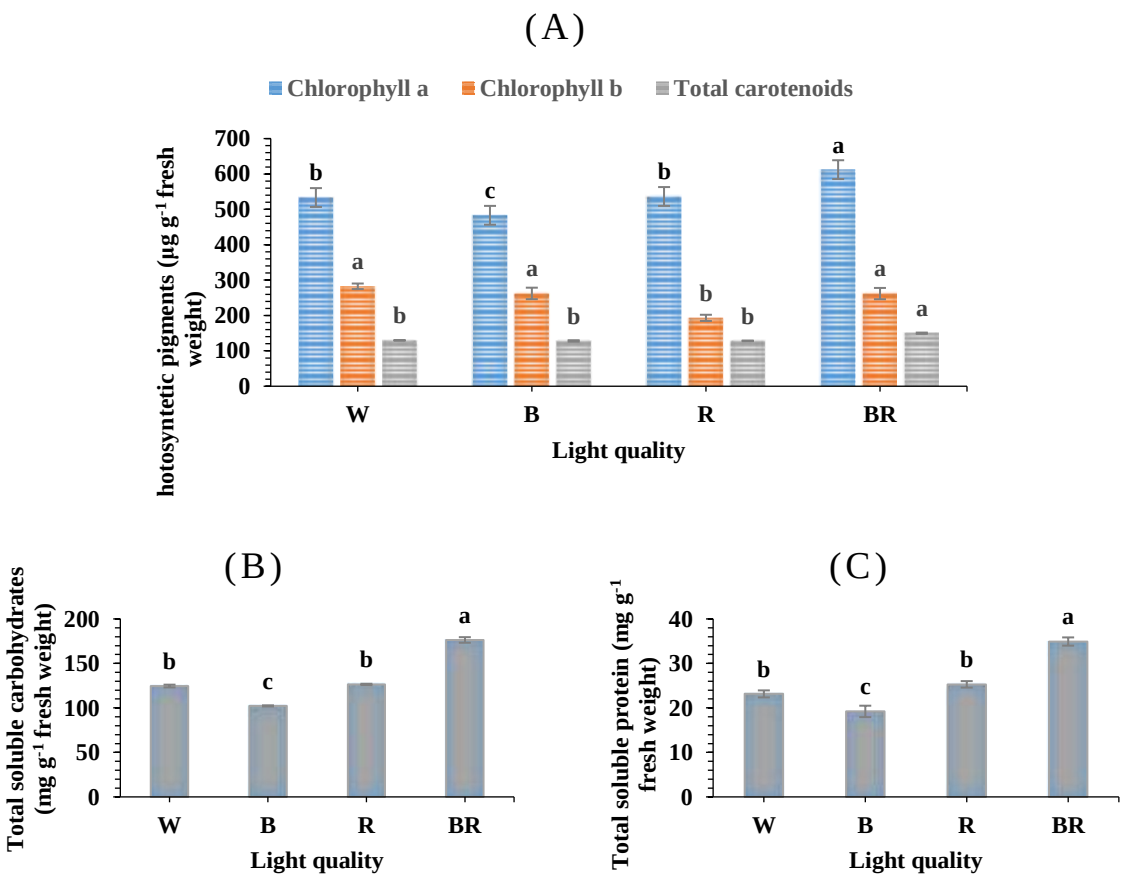


Figure 3. The effect of light qualities on biochemical characteristics of the *Arnebia euchroma* L. plant. (A), photosynthesis pigments, (B), total soluble carbohydrate, (C), total soluble protein. Different letters indicate significant differences determined using Duncan's multiple range test ($p < 0.05$). Error bars represent ±SE, n= 4

CONCLUSION

This study investigated the germination, growth, and biochemical characteristics of the A. *euchroma* plant under LED sources with different light spectra. Treatment of A. *euchroma* seeds with red light (R) enhanced germination. It also increased seedling dry weight, stem length, and root length. In contrast, blue light (B) treatment reduced seed germination and severely affected growth. In the later growth stages, the response of A. *euchroma* plants to light spectra was different from that of the seedling stages. Thus, under BR light treatment, the highest content of photosynthetic pigments, total carbohydrate content, and total protein content was obtained. This study provided a basis for manipulating the light spectrum and selecting the appropriate ratio of blue and red light according to the goals of the breeders.

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IMPACT OF NANOPARTICLES ON MORPHOLOGICAL TRAITS AND SECONDARY METABOLITES OF SNAPDRAGON

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ABSTRACT

This study aimed to investigate the effects of chitosan-coated iron oxide nanoparticles and silver nanoparticles on the morphological traits and secondary metabolites of the ornamental plant snapdragon (*Antirrhinum majus*) under greenhouse conditions. Treatments were arranged in a factorial experiment based on a completely randomized design with four replications, involving four concentration levels (0, 50, 100, and 150 mg/L for silver nanoparticles and µM for chitosan-coated iron oxide nanoparticles). The measured parameters included stem diameter, bud diameter, fresh weight of aerial parts, number of lateral branches, chlorophyll content, total sugars, flavonoids, and antioxidant capacity (DPPH). Analysis of variance revealed that the main effects of nanoparticles on the fresh weight of aerial parts, stem diameter, and number of lateral branches were significant at the 1% probability level, and on bud diameter at the 5% level. The interaction effects of nanoparticles on total sugars, flavonoids, and DPPH were also significant at the 1% level. The highest fresh weight and number of lateral branches were observed in the treatment with 100 µM chitosan-coated iron oxide nanoparticles and 50 mg/L silver nanoparticles, while the highest total sugars, flavonoids, and antioxidant capacity were recorded with 150 mg/L silver nanoparticles and 150 µM chitosan-coated iron oxide nanoparticles. These findings confirm the potential of nanotechnology in enhancing the quality and antioxidant properties of ornamental plants and suggest that future research should focus on optimizing concentrations and evaluating long-term effects.

Keywords: Flavonoids, Snapdragon, Total Sugars, Nanoparticles.

INTRODUCTION

Snapdragon (*Antirrhinum majus*), a perennial plant from the *Scrophulariaceae* family native to the Mediterranean region, is highly valued in the floriculture and ornamental industries due to its colorful flowers and attractive structure. This plant is cultivated as an annual in cold regions and as a biennial in areas with mild winters, with its growing demand in global markets highlighting the need for innovative methods to improve its quality and performance. In recent decades, nanotechnology has emerged as a pioneering field of modern science, opening new horizons in sustainable agriculture. Nanoparticles, with their nanometer-scale size and high surface area, have become key tools for enhancing plant growth, controlling pests, and increasing resistance to environmental stresses. Among these, chitosan-coated iron oxide nanoparticles (Fe_3O_4@Chitosan NPs) and silver nanoparticles (AgNPs) stand out due to their targeted nutrient delivery and strong antimicrobial properties, respectively. The application of nanoparticles as pesticides, protective agents, antimicrobials, and nutritional supplements has revolutionized agriculture. Recent studies (e.g., 2024 research on ornamental plants) have shown that silver nanoparticles at low concentrations (e.g., 60 ppm) can

significantly increase fresh weight, stem length, and anthocyanin production by stimulating antioxidant enzyme activity, particularly in a time-dependent manner (Behzad *et al.*, 2024). Additionally, silver nanoparticles at low concentrations have been found to enhance plant growth and boost the production of secondary metabolites like flavonoids, improving antioxidant properties (Mirzaei, & Kiarostami, 2022), which could benefit snapdragon quality. On the other hand, chitosan-coated iron oxide nanoparticles, due to their biocompatibility and ability to prevent particle aggregation, have wide applications in managing environmental stresses. A study on peppermint (*Mentha piperita*) under drought stress showed that increasing concentrations of chitosan-coated iron oxide nanoparticles improved chlorophyll fluorescence and photosynthetic indices (Giglou *et al.*, 2022). Chitosan, a natural polymer derived from chitin, not only enhances nanoparticle stability but also reduces their potential toxicity, enabling safer use in biological systems. These findings suggest that combining iron and silver nanoparticles with chitosan coating could offer a novel approach to improving snapdragon growth, stress resistance, and its medicinal and ornamental applications. This study examines the synthesis, mechanism of action, and potential of these nanoparticles in enhancing snapdragon performance, with an emphasis on their antimicrobial effects and growth stimulation.

MATERIALS AND METHODS

This research was conducted using snapdragon seeds purchased and cultivated in the greenhouse of the Faculty of Agriculture and Natural Resources, University of Mohaghegh Ardabili. Treatments included two types of nanoparticles; silver nanoparticles and chitosan-coated iron oxide nanoparticles; at four concentration levels: 0 (control), 50, 100, and 150 mg/L for silver nanoparticles, and 0 (control), 50, 100, and 150 µM for chitosan-coated iron oxide nanoparticles. Treatments were applied via foliar spraying in three growth stages of the plants. Seeds were initially sown in seedling trays and, after germination and reaching the four-leaf stage, transplanted into pots containing a 1:1:1 volumetric mixture of organic matter, sand, and garden soil. The experimental design was a factorial arrangement based on a completely randomized design with four replications. Each experimental unit included a sufficient number of plants to ensure reliable statistical data. To assess treatment effects, morphological and growth parameters were measured. The number of lateral branches was recorded using a counting unit. Stem and bud diameters were determined using a caliper with 0.1 mm precision. Fresh weight of aerial parts was measured with a balance accurate to 0.001 g. Total flavonoid content was assessed using the method of Krizek *et al.* (1998) with a spectrophotometer. For total carbohydrate measurement, 0.2 g of green plant tissue was combined with 10 mL of 95% ethanol in a sealed test tube and heated in a water bath at 80°C for 1 hour. After cooling, 1 mL of the sample was mixed with 1 mL of 5% phenol and 5 mL of 98% sulfuric acid, and absorbance was read at 483 nm using a spectrophotometer.

RESULTS

Analysis of variance indicated that the main effects of the treatments (chitosan-coated iron oxide nanoparticles and silver nanoparticles) were significant, except for bud diameter, which was significant at the 5% probability level. The fresh weight of aerial parts, stem diameter, and number of lateral branches were significant at the 1% level (Table 1).

Table 1. Analysis of Variance of the Effects of Silver Nanoparticles and Chitosan-Coated Iron Oxide Nanoparticles at Different Concentrations on Morphological Traits of Snapdragon

Sources of Variation	DF	Stem Diameter	Aerial Parts Fresh Weight	Lateral Branches Number	Bud Diameter
Chitosan coated IONPs	3	8,76**	1957,51 **	24,87**	1,89 **
AgNPs	3	1,45**	13,57 ns	56,70**	0,43 **
Chitosan coated IONPs × AgNPs	9	1,50**	177,33**	31,02**	4,70*
Error	48	0,324	9,94	2,43	0,089
Coefficient of Variation	_	14,25	5,43	86,10	6,36

*, **, and ns indicate significance at the 5%, 1%, and non-significant probability levels, respectively.

Analysis of variance for traits also showed that the effects of chitosan-coated iron oxide nanoparticles and silver nanoparticles, as well as their interaction, were significant at the 1% level for total sugars and flavonoids (Table 2).

Table 2. Analysis of Variance of the Effects of Silver Nanoparticles and Chitosan-Coated Iron Oxide Nanoparticles at Different Concentrations on Biochemical Traits of Snapdragon

Sources of Variation	DF	Total Sugars	Flavonoids	DPPH
Chitosan coated IONPs	3	0,068**	54,11 **	1595,57**
AgNPs	3	0,1163**	4,58**	152,67**
Chitosan coated IONPs x AgNPs	9	0,016**	1,05**	184,104**
Error	48	0,003	0,126	8,48
Coefficient of Variation	_	8,06	8,745	7,265

*, **, and ns indicate significance at the 5%, 1%, and non-significant probability levels, respectively.

FRESH WEIGHT OF AERIAL PARTS

The results revealed that chitosan-coated iron oxide nanoparticles at 100 µM and silver nanoparticles at 50 mg/L had a significant effect on the morphological traits of snapdragon. The highest plant height, number of first-order lateral branches, and fresh weight of aerial parts were observed in plants treated with 100 µM chitosan-coated iron oxide nanoparticles and 50 mg/L silver nanoparticles (Figure 1A).

STEM DIAMETER

The results showed a significant effect of chitosan-coated iron oxide nanoparticles on stem diameter. The highest stem diameter was observed in plants treated with 50 µM chitosan-coated iron oxide nanoparticles and 150 mg/L silver nanoparticles, while the lowest was found in untreated control plants (Figure 1B).

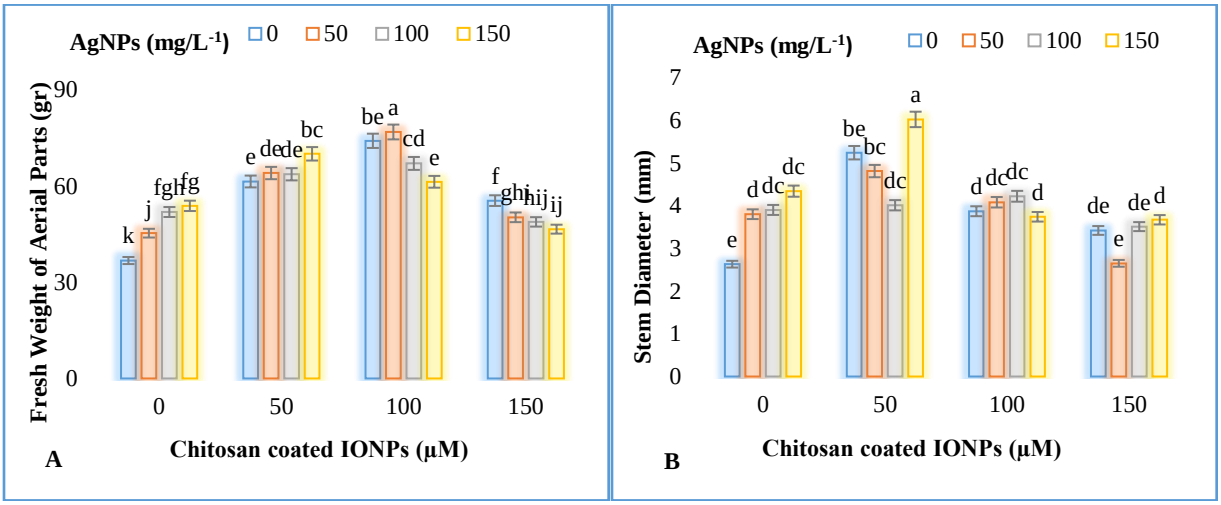

Figure 1. Interaction effects of chitosan Coated IONPs and AgNPs on Fresh Weight of Aerial Parts (A) and Stem Diameter (B) of Snapdragon.

BUD DIAMETER

Comparative analysis of morphological traits indicated that chitosan-coated iron oxide nanoparticles and silver nanoparticles had a significant effect on bud diameter. The highest bud diameter was observed in plants treated with 150 mg/L silver nanoparticles and 100 µM chitosan-coated iron oxide nanoparticles, while the lowest was found in untreated control plants (Figure 2A).

NUMBER OF LATERAL BRANCHES

The analysis of the effects of chitosan-coated iron oxide nanoparticles and silver nanoparticles on the number of lateral branches showed that the highest number was achieved in the treatment with 100 µM chitosan-coated iron oxide nanoparticles and 50 mg/L silver nanoparticles (Figure 2B).

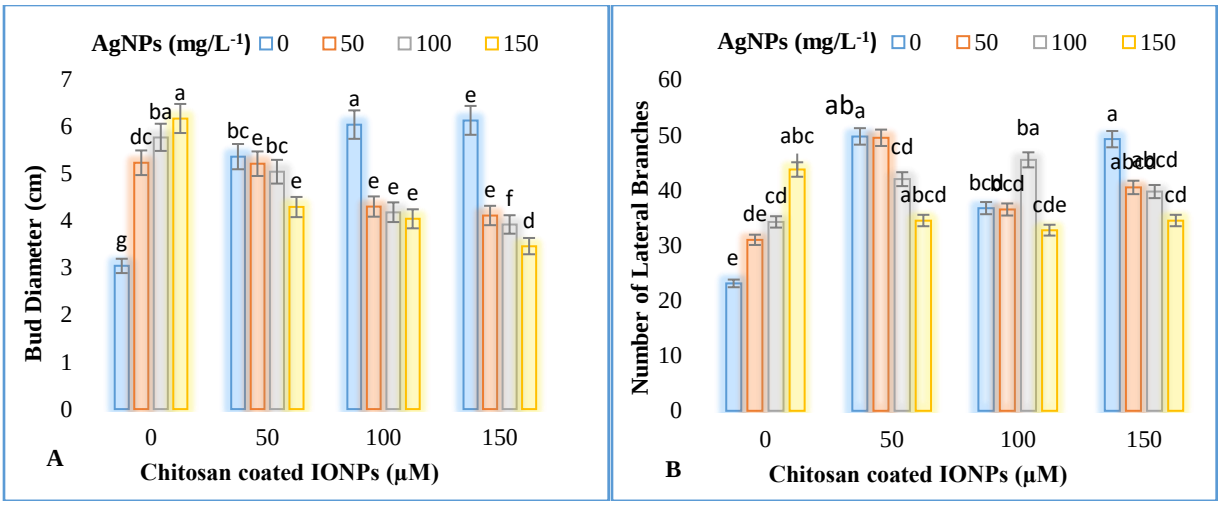

Figure 2. Interaction effects of chitosan Coated IONPs and AgNPs on Bud Diameter (A) Number of Lateral Branches (B) of Snapdragon.

TOTAL SUGARS

The analysis indicated that total sugar content in snapdragon varied with increasing concentrations of silver nanoparticles and chitosan-coated iron oxide nanoparticles. The lowest value was observed in the control treatment (0 mg/L silver nanoparticles and 0 µM chitosan-coated iron oxide nanoparticles), while the highest was recorded with 150 mg/L silver nanoparticles and 150 µM chitosan-coated iron oxide nanoparticles. Intermediate concentrations, such as 50 mg/L silver nanoparticles with 100 µM chitosan-coated iron oxide nanoparticles, also showed an increase in total sugars (Figure 3A).

FLAVONOIDS

The results showed that flavonoid content in snapdragon was influenced by varying nanoparticle concentrations in treatments. The lowest flavonoid content was observed in the control (0 mg/L silver nanoparticles and 0 µM chitosan-coated iron oxide nanoparticles), while the highest was achieved with 150 mg/L silver nanoparticles and 150 µM chitosan-coated iron oxide nanoparticles. Intermediate treatments, such as 50 mg/L silver nanoparticles with 100 µM chitosan-coated iron oxide nanoparticles, exhibited a significant increase in flavonoids, whereas 150 µM chitosan-coated iron oxide nanoparticles without silver nanoparticles resulted in a decrease, highlighting the importance of balanced concentrations (Figure 3B).

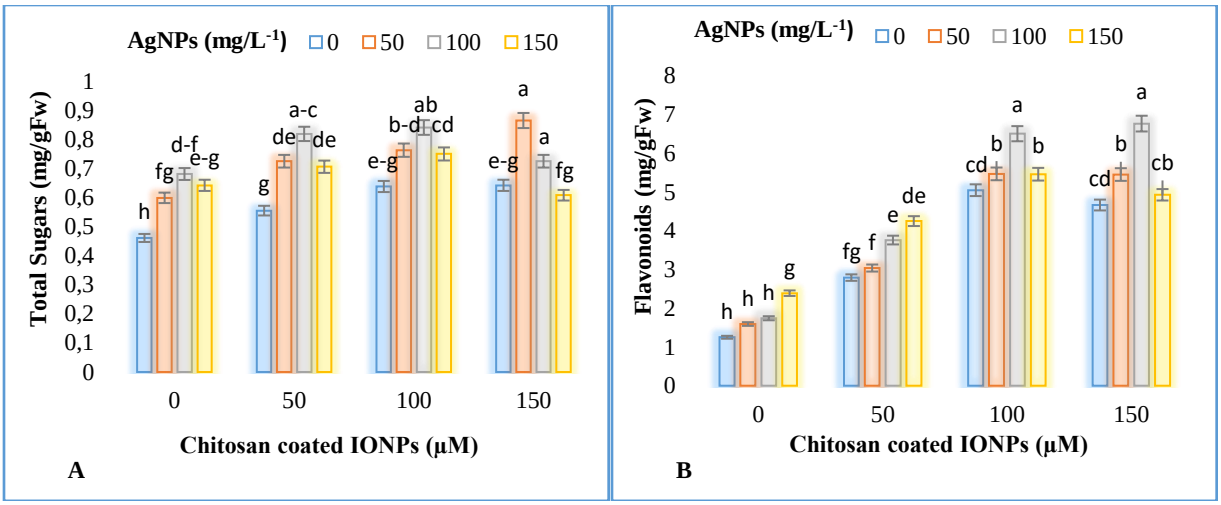

Figure 3. Interaction effects of chitosan Coated IONPs and AgNPs on Total Sugars (A) Flavonoids (B) of Snapdragon.

DPPH

The comparative analysis of mean data revealed that increasing concentrations of chitosan-coated iron oxide nanoparticles generally enhanced antioxidant capacity. The greatest treatment effect was observed from 0 to 150 µM chitosan-coated iron oxide nanoparticles combined with 50 mg/L silver nanoparticles. Additionally, the interaction of both treatments at 100 µM chitosan-coated iron oxide nanoparticles and 100 mg/L silver nanoparticles yielded the highest antioxidant capacity (Figure 4).

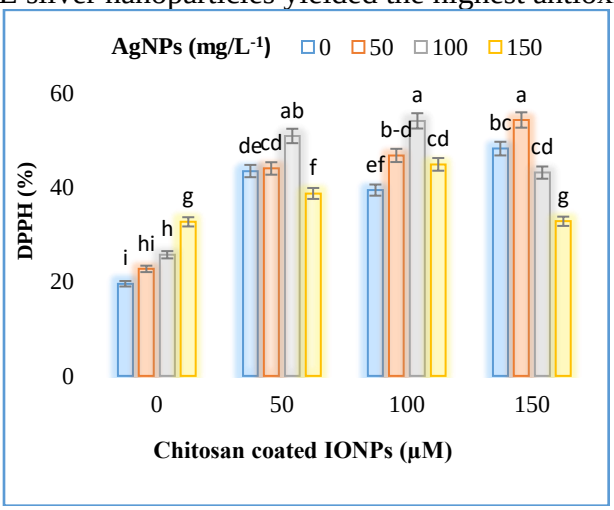

Figure 4. Interaction effects of chitosan Coated IONPs and AgNPs on DPPH.

DISCUSSION

The results of this study demonstrated that the application of chitosan-coated iron oxide nanoparticles and silver nanoparticles can significantly affect the morphological and biochemical traits of snapdragon. These findings align with previous reports on the efficacy of nanoparticles in improving growth characteristics and quality of ornamental plants (Anushi *et al.*, 2023; Behzad *et al.*, 2024). Silver nanoparticles, particularly at low concentrations such as 50 ppm, promoted growth, increased the number of lateral branches, and enhanced the fresh weight of aerial parts, likely due to

their role in stimulating antioxidant enzyme activity and reducing oxidative stress (Liu, *et al.,* 2020). These nanoparticles may also influence plant signaling pathways by regulating the expression of growth-related genes (Rasheed *et al.,* 2022). Conversely, chitosan-coated iron oxide nanoparticles, with their biocompatible properties and gradual iron release, particularly at 100 µM, contributed to increased chlorophyll, stem diameter, and fresh weight. This could be attributed to improved iron availability, a key element in chlorophyll synthesis and plant metabolism (Giglou *et al.,* 2022). The chitosan coating enhances absorption and reduces potential nanoparticle toxicity, playing a significant role in their effectiveness (Shi *et al.,* 2020). The significant increase in total sugars and flavonoids in combined treatments with high nanoparticle concentrations indicates their interactive effect on secondary metabolite pathways. Flavonoids, important defensive compounds in plant systems, are influenced by environmental factors and stimulants like nanoparticles, potentially through the activation of phenylpropanoid pathways or increased activity of PAL and CHS enzymes (Selvakesavan *et al.,* 2023). These results are consistent with findings from another study on snapdragon by Pourbeyrami Hir and Sardari (2025), which reported significant improvements in vegetative growth, flower diameter, number of lateral branches, and total phenol content with combined use of chitosan-coated iron oxide and silver nanoparticles at concentrations of 100-150 µM and 50-150 mg/L, respectively. Overall, these findings not only confirm the potential of nanoparticles in enhancing ornamental plant quality but also open new avenues for sustainable production, reduced chemical fertilizer use, and increased medicinal value of plants. Given the growing adoption of nanotechnology in precision agriculture, future research should focus on targeted, safe, and cost-effective nanoparticle applications.

CONCLUSION

This study demonstrated that the combined application of chitosan-coated iron oxide and silver nanoparticles, particularly at moderate to high concentrations, can effectively improve growth and physiological traits in Snapdragon. These nanoparticles enhanced plant height, branching, and flowering while also increasing chlorophyll fluorescence and phenolic compounds.

These findings support the strategic use of biocompatible nanoparticles in ornamental plant management as a promising approach for boosting performance and resilience, particularly within sustainable and eco-friendly agricultural systems.

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IMPROVING THE EFFICIENCY OF BIO-COAGULATION AND FLOCULATION FOR TURBIDITY CONTROL IN WASTEWATER TREATMENT

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ABSTRACT

The process of coagulation and flocculation is a common method used in the water treatment process. Coagulants used in this study were chemical coagulants i.e. Aluminum Sulfate ($Al_2(SO_4)_3$) as reference of chemical coagulant and biocoagulants (i.e. pineapple peels, Araucaria hyterophylla). The aim of this study was to examine the impact of biocoagulants on their performance, which is marked by the decrease in turbidity, suspended matter (SM), and electrical conductivity (EC). A wide range of coagulant dosage (0.1– 0.4 g/500 mL) and synthetic turbid water at pH 8 were used to evaluate the coagulation process. The results showed that the highest turbidity removal for Araucaria hyterophylla and AS achieved at the dosage of 0.1 g as much as 84.97% and 97.62 % respectively and the optimal dose of pineapple peels was 0.4g.

Keywords: Water treatment, coagulation-flocculation, turbidity removal, biocoagulant.

INTRODUCTION

Because of cost savings, environmental issues of synthetic organic polymers and inorganic chemical products it is of interest to use natural materials. Natural polyelectrolytes are completely free of toxicity, naturally biodegradable and locally accessible. The coagulants are derived from mineral products, micro-organisms, animals, plants, vegetation (Mogbo et al., 2020) Natural polymers cannot always be successful and should, like any other coagulant substance, be investigated on a case-to - case basis.

Extracts made from Moringa Oleifera seeds, for example, reduced turbidity and suspended solids in raw water by 80 to 99.5 percent (Putra, R. S et al., 2020). Moringa Oleifera decreased from water 90 percent to 99 percent of bacteria and 90 percent of cercariae. Nirmali seed can be used as a coagulant to extract turbidity, bacteria, and viruses from water [Ebeling, James M et al., 2003, Vishali, S et al., 2015).

The extracted seed of chestnut and corn species could also be used as natural coagulants to eliminate the turbidity (Hendrawati, Het al.,2013).

Chitosan can be used as a coagulant and the mechanisms for the reduction of turbidity have generally been charged neutralization due to positive amino grouping and bridging (Muralimohan, Net al., 2014).

It was found that cornstarch and cationic waxy cassava can be used as turbidity recovery coagulants and primarily as adsorption and bridge action mechanisms (Okuda, Tetsuji, et al.,2008, Srawaili, N. J. B. I. T. B. 2008) respectively, that cactus extracted polymer can be employed as the primary turbidity recovery coagulant and its efficiency is stated (Aldosky, H. Y. Y et al.,2011).

MATERIALS AND METHODS

Bio Coagulant Preparation

In this study, pineapple peels and the leaves of Araucaria hyterophylla was used as a natural coagulant/flocculent. The leaves of Araucaria hyterophylla were collected from the University of Skikda and ananas comosus were purchase from the town of Skikda, Algeria. To remove dust, impurities, the biomaterials were cleaned with distilled water.

The biomaterials were cut into small pieces, and then crushed using a domestic grinder. The solids were used as a raw coagulant to treat turbid water without any chemical pre-treatment.

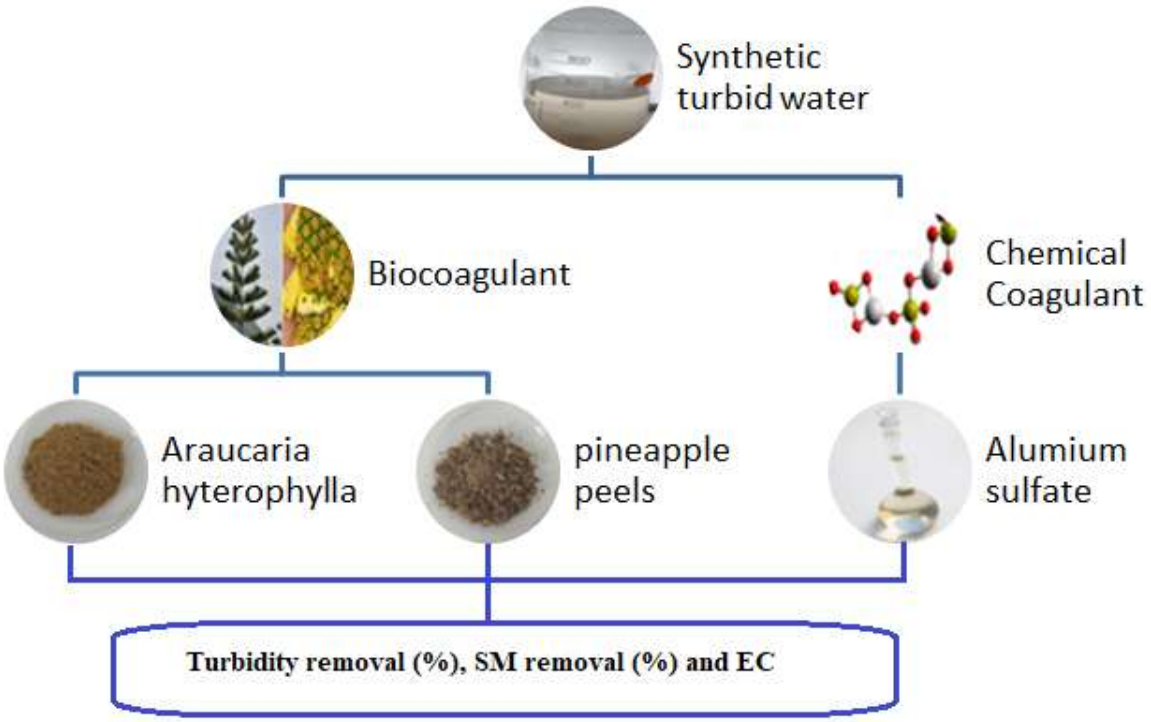
A. Experimental Procedure

Synthetic turbid water was prepared by adding clay into tap water. The suspension was stirred for 1 h to achieve a uniform dispersion of clay particles [7]. The values of turbidity, pH, electrical conductivity (EC) and suspended matter in raw water were shown in table 1.

Table 1. Initial conditions of raw water

Parameters	**Values of turbid water**	
	Biocoagulant	**chemical coagulant**
pH	8	8.2
Turbidity (NTU)	53	55.2
electrical conductivity (µS/cm)	943	900
suspended matter (mg/l)	0.05	0.06

At the start of the experiment, the contents of the tank were mixed to a uniform initial turbidity, temperature, and pH values. All the experiments were carried out at a room temperature of 20 °C. Jar test is the most widely used experimental methods for coagulation-flocculation. The conventional jar-test technique was used. A six-beaker jar-test apparatus (Lobivond Model) was used to simulate the coagulation/flocculation process. The synthetic turbid water was treated using coagulants (i.e. chemical and biocoagulant) at different dosages (i.e. 0.1; 0.2; 0.3 and 0.4 g) in 500 mL solution. The solution and the coagulant were mixed together at 160 rpm for 5 min. followed by slow mixing at 45 rpm about 25 mins and the formed flocs were allowed to settle for 1h. After settling, the performance of biocoagulant was evaluated by using turbidity removal and suspended matter removal.



The Suspended matter removal efficiency (SM %) and turbidity removal efficiency (TUR %) were calculated using Eqs.1 and 2, respectively.

$$SM\ (\%) = \frac{SM_0 - SM_F}{SM_0} \times 100$$

$$R(\%) = \frac{TUR_0\ - TUR_F}{TUR_0} \times 100$$

SM_0 and SM represent the initial and final SM (mg/L) of wastewater, respectively and, similarly, TUR_0 and TUR represent the initial and final supernatant turbidity of turbid water (NTU).

RESULTS AND DISCUSSIONS

The performance test of biocoagulants were prepared by initial artificial turbid water was 53 NTU for pineapple peels and araucaria heterophylla and 55.2 NTU for AS. The final turbidity can be affected by varying amounts of pineapple peels, araucaria heterophylla, and AS added to the solution. Addition of 0.1, 0.2, 0.3 and 0.4g of pineapple peels decreased respectively the turbidity as much as 23, 20, 19,2 and 17,8 NTU and araucaria heterophylla also decreased the turbidity to 8.04, 8.24, 9.26 and 9.50 NTU respectively.

While different results showed when AS was used in similar dosages. The decrease of turbidity achieved were as much as 1.31, 6.96, 10.5 and 13.1 NTU, respectively. Figure 1 shows the turbidity removal when using AS and pineapple peels and araucaria heterophylla as coagulant. The turbidity removal of pineapple peels at 0.1, 0.2, 0.3 and 0.4g were 56.6, 62.26, 63.77 and 66.41 %, respectively. As well as the turbidity removal of araucaria heterophylla were 84.97, 84.59, 82.69 and 83.01 %, respectively.

AS removed turbidity at dosages of 0.1, 0.2, 0.3, and 0.4g respectively with 97.62, 87.39, 80.97, and 76.26%, suggesting that there is a difference in optimal dose for pineapple peels, araucaria heterophylla and AS to remove turbidity. The increase of AS and araucaria heterophylla dosages was followed by the decrease of turbidity removal. On the contrary, the increase of pineapple peels dosage was followed by the increase of turbidity removal.

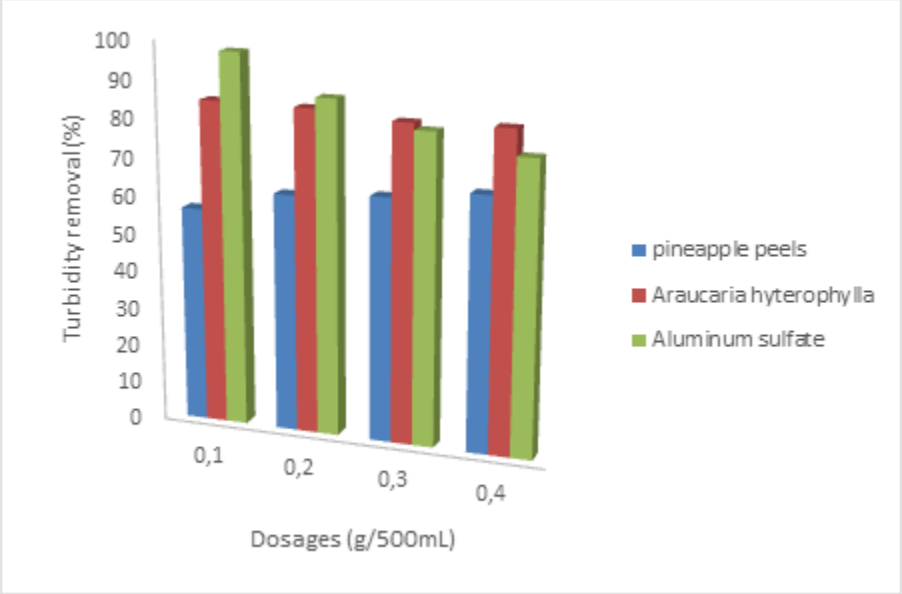


Figure 1. Turbidity removal (%) from synthetic turbid water after treatment.

The excessive dosage of chemical coagulants and araucaria hyterophylla has the effect of decreasing SM removal. Increasing the Pineapple peels dosage increased the SM removal of the solution.

The optimum dosage of AS and araucaria hyterophylla was 0.1 g to increase the SM removal to 85.5 % and 84.4 % respectively. The maximum elimination of suspended matter occurs at low doses of AS and araucaria hyterophylla. On the other hand, the optimal dosage of Pineapple peels to eliminate 78.4 % of suspended matter was 0.4 g.

The coagulation process occurs during rapid mixing. By interacting with all colloidal surface particles, the polymers of those coagulants will ensure that the end chain is not attached, causing the flocculation

process to occur. The process of flocculation occurs during slow stirring. The microflocs which form during the coagulation process bind together to form a larger floc. That was how the coagulants can reduce the suspended mater and the turbidity

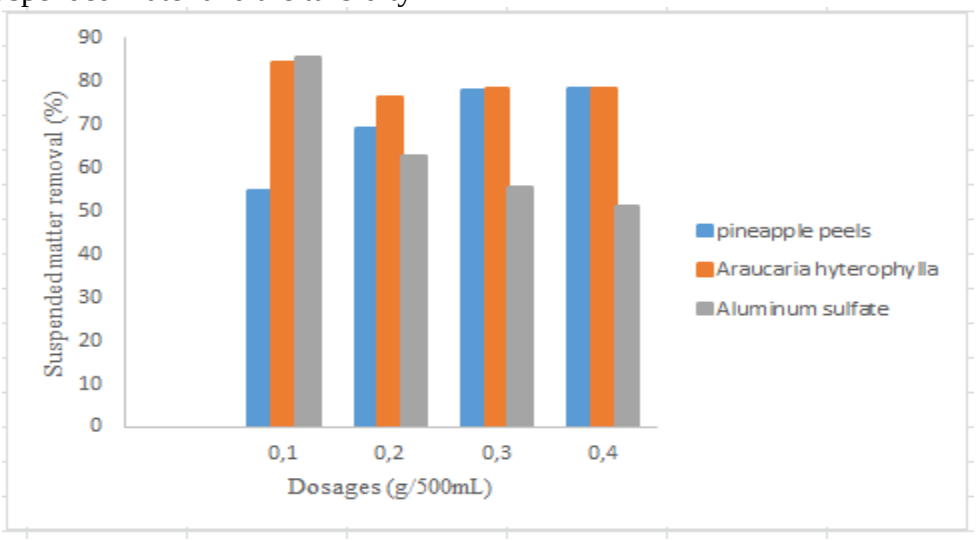


Figure 2. Suspended matter removal of synthetic turbid water after treatment using chemical coagulants and biocoagulants.

Table 2 shows the electrical conductivity (EC) of artificial turbid water after treated by chemical coagulants and biocoagulants. The EC shows how much materials were dissolved in solution. EC was calculated from the measurement of the resistance of an electrolyte where the conductivity and resistance were inversely related (Aldosky, H. Y. Y et al.,2011).

TABLE 2. Electrical conductivity (µS/cm) of synthetic turbid water after treatment using chemical coagulants and biocoagulants.

Dosage (g/500mL)	**Chemical coagulant**	**Biocoagulant**	
	Aliminium sulfate	**pineapple peels**	**araucaria hyterophylla**
0.1	958	945	950
0.2	1116	945	961
0.3	1176	955	972
0.4	1260	963	982

When AS, pineapple peels and araucaria hyterophylla were dissolved in a solution at pH ~8, these materials were expected to produce ions in solution and lead to increase the EC value.

The presence of mineral elements charged macromolecules and other ionic compounds in moringa was dissolved or dissociated into the turbid water caused a high increase of EC (Magaji, U. F et al., 2015)

TABLE 3. Comparison of efficiency of natural coagulant

Natural coagulant	Turbidity removal	Suspended mater removal
pineapple peels	66.41 %	78.4 %
araucaria hyterophylla	84.97 %	84.4 %

Table 3 presents comparative results of two natural coagulants; pineapple peels and araucaria hyterophylla in the reduction of turbidity and suspended mater with their optimum dosage. From the table 3, it is clear that, out of two coagulants araucaria hyterophylla is more efficient in reduction of SM of 84.4% and gave the highest turbidity reduction of 84.97%.

CONCLUSIONS

The removal of turbidity from a synthetic turbid water sample treated with biocoagulants has been evaluated.

The performance of the biocoagulants was also compared with the AS as a reference. The addition of 0.1 g araucaria hyterophylla decreased the turbidity, SM, and EC successfully. The turbidity and suspended mater decreased by up to 84.97 % and 84.4 % respectively. The increasing of EC cannot be avoided while using AS as coagulant due to the presence of organic matter in this material. However, the use of biocoagulant did not bring the toxic effect and was not harmful to human health.

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EVALUATION OF SUGAR, ORGANIC ACID, AND AROMA PROFILE IN BITTER MELON (*MOMORDICA CHARANTIA* L.)

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ABSTRACT

Medicinal plants have played an important role in the treatment of diseases for thousands of years and continue to be a fundamental resource for the pharmaceutical industry today. Secondary metabolites synthesized by plants, such as alkaloids, tannins, flavonoids, and phenolic compounds, are known for their therapeutic effects and are widely used in traditional medicine. In particular, traditional medical systems in countries such as China, India, Japan, and Thailand are largely based on these plants.In this context, bitter melon (*Momordica charantia* L.) of the Cucurbitaceae family stands out not only for its medicinal properties but also for its use as an ornamental plant. Thanks to its fast-growing, twining stems and striking foliage, it is frequently preferred as a decorative climbing plant in landscape architecture. This species is an annual climber that grows in tropical and subtropical regions and is notable for its rich content of vitamins (C, A, B complex), minerals (iron, calcium, phosphorus, potassium, zinc, magnesium), and β-carotene. In this study, the biochemical components and volatile organic compounds (VOCs) of bitter melon fruits grown under the ecological conditions of Adana were determined. The analyses included sugars, organic acids, total phenolic compounds, antioxidant capacity (DPPH) and the volatile compound profile using GC-MS. Glucose (367.53 mg/100 g FW) and fructose (229.43 mg/100 g FW) were identified as the dominant sugars, while succinic acid (221.43 mg/100 g FW) and citric acid (55.78 mg/100 g FW) were the predominant organic acids. The ascorbic acid content was 10.50 mg/100 g FW, total phenolic content was 47.54 mg GAE/100 g FW, and antioxidant capacity was measured as 24.09% DPPH inhibition.Volatile compound analysis revealed that esters (48.18%), alcohols (34.59%), and aldehydes (4.43%) were the most abundant groups. Among the most prominent compounds were ethyl acetate (23.91%), ethanol (11.80%), 5-(hydroxymethyl)-2-furfural (3.39%), benzeneethanol (9.01%), and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (5.36%). These compounds contribute to the characteristic aroma and potential biological activities of the fruit. In conclusion, studies have shown that *M. charantia* possesses antidiabetic, anticancer, antioxidant, and antimicrobial properties. Its fruits, leaves, and seeds are traditionally used in medicine due to active compounds such as charantin, polypeptide-p (a plant insulin-like compound), and steroidal saponins. Furthermore, bitter melon is also regarded as a dual-purpose plant, serving as an ornamental climber in landscape designs: it can be cultivated in pots on balconies or on trellises and arbors in gardens, offering both medicinal and aesthetic value. Under Adana's ecological conditions, bitter melon fruit exhibits a rich biochemical and volatile profile, making it a promising candidate as a functional food, natural aroma source, and ornamental plant with pharmacological and landscape significance.

Keywords: Functional food, GC-MS, Organic acids, Ornamental plant, Sugars, Volatile compounds.

INTRODUCTION

Medicinal plants have long been recognized as crucial sources of therapeutic compounds and remain indispensable in modern pharmaceutical development. Secondary metabolites such as alkaloids, flavonoids, tannins, and phenolic compounds synthesized by plants exhibit diverse biological activities, including antimicrobial, antioxidant, antidiabetic, and anticancer effects. These properties have made medicinal plants a focal point in traditional medicine systems across Asia, particularly in China, India, Japan, and Thailand, and they continue to attract global scientific interest (Grover and Yadav, 2004; Basch et al., 2003). Among these species, bitter melon (*Momordica charantia* L.), a member of the Cucurbitaceae family, holds significant importance due to its rich phytochemical composition and multipurpose applications. *M. charantia* is a tropical and subtropical annual climbing plant characterized by vigorous vine-like growth, deeply lobed leaves, and bright yellow flowers. Traditionally, it has been widely consumed as both food and medicine in tropical and subtropical regions. The fruit contains diverse bioactive compounds such as polyphenols, flavonoids, and saponins, which are largely responsible for its pharmacological properties (Hsiao et al., 2013; Kubola and Siriamornpun, 2008; Shan et al., 2012). For example, aqueous extracts of the fruit are rich in phenolic compounds contributing to strong antioxidant activity (Kubola and Siriamornpun, 2008), while saponins have demonstrated anti-proliferative and hypoglycemic effects (Hsiao et al., 2013). In the food industry, the stability and bioactivity of these phytochemicals are strongly influenced by processing methods (Choi et al., 2012; Jin et al., 2018). Hot-air dehydration, for instance, has been shown to significantly reduce total phenolic content due to prolonged exposure to high temperatures (Jin et al., 2018). Moreover, the inherently bitter taste of *M. charantia* limits consumer acceptance. To mitigate bitterness, techniques such as blanching and the use of additives (sodium chloride, carboxymethyl cellulose, gum Arabic) have been applied, though these methods may compromise bioactive compounds, raise production costs, and reduce health benefits (Rashima et al., 2017). Beyond its medicinal and nutritional importance, *M. charantia* is also valued as an ornamental plant. Its vigorous twining stems and attractive foliage make it suitable for landscape use on trellises, pergolas, balconies, and garden fences, providing both aesthetic and ecological benefits (Kubola and Siriamornpun, 2008). Nutritionally, bitter melon fruits are rich in vitamins (C, A, B-complex), minerals (iron, calcium, phosphorus, potassium, zinc, magnesium), and bioactive compounds such as charantin, polypeptide-p, and steroidal saponins. These compounds play critical roles in regulating blood glucose levels and exhibit anti-inflammatory and anticancer properties (Ray et al., 2010). Additionally, the fruit contains phenolic compounds, flavonoids, and carotenoids that enhance antioxidant capacity and reduce oxidative stress (Horax et al., 2010). Recent studies have also focused on the biochemical composition and volatile organic compounds (VOCs) of *M. charantia*, as these attributes contribute to both its nutritional and aromatic profiles. VOCs, including esters, alcohols, aldehydes, and terpenes, are key determinants of the fruit's aroma. Advanced analytical tools such as gas chromatography–mass spectrometry (GC-MS) have enabled detailed profiling of these compounds, which may influence both sensory perception and bioactivity (Chowdhury et al., 2012). The biosynthesis of bioactive and volatile compounds is strongly affected by environmental factors. Thus, investigating plants under specific ecological conditions is essential. The Adana region, located in the eastern Mediterranean part of Türkiye, provides unique agro-climatic conditions characterized by high temperatures and long sunshine duration, which are favorable for tropical and subtropical crops such as bitter melon. These conditions may significantly influence the phytochemical and volatile compound profiles of the fruit.

The present study was conducted to comprehensively evaluate the biochemical composition and volatile organic compound profile of *M. charantia* cultivated under the ecological conditions of Adana. Specifically, analyses targeted sugars, organic acids, total phenolics, antioxidant capacity, monomeric anthocyanins, and volatile compounds using GC-MS. By integrating nutritional and aroma profiling, this research highlights the potential of bitter melon as a functional food, a natural aroma source, and an ornamental species. Beyond its well-documented medicinal value, this dual perspective emphasizes the role of *M. charantia* in both health-related applications and sustainable landscape design, where multifunctional plants with aesthetic and ecological benefits are increasingly sought.

MATERIALS AND METHODS

Plant Material

Fruit samples of *Momordica charantia* L. were collected from a commercial orchard located in the Sarıçam district of Adana province,

Method

Determination of total phenol

The total phenolic content of pulp was determined colorimetrically using Folin Ciocalteu's reagent (Spanos and Wrolstad, 1990). Results were described as mg of gallic acid equivalent/100 g of weight (mg/ GAE 100 g).

Determination of total antioxidant capacity

Radical scavenging activity of DPPH:(2,2-diphenyl-1-picrylhydrazyl): The procedure was performed as outlined by Brand-Williams (1995) with slight modifications as detailed by Polat et al. (2022).

Total monomeric anthocyanin content determination

For total anthocyanin analysis, the method described by Giusti and Wrolstad, (2001) was used with some modifications. Total anthocyanin determination was read at 510 nm and 700 nm in a spectrophotometer and calculation was made according to the following formula.

Absorbance = X = (510 nm pH 1 - 700 nm pH 1) - (510 nm pH 4.5 - 700 nm pH 4.5)

Anthocyanin content (mg/L) = (absorbance/ 29600 x 1) x 1000 x 445 x 20

Individual sugar and organic acid extraction and determination

Sugars and organic acids were extracted individually following the procedure described by Mikulic-Petkovsek et al. (2016). Homogenized, unfrozen fruit samples were placed in a test tube containing 4 mL of bi-distilled water, at 1 g. The mixture was continuously mixed on a vortex mixer at room temperature for thirty minutes. After extraction, the samples were centrifuged at 9000 x g for ten minutes at 4°C to obtain the supernatant and then filtered into vials using cellulose membranes. The extracts were stored at -20°C for further investigation. The high-performance liquid chromatography (HPLC) system was equipped with a refractive index detector (Shimadzu RID 20A VP, Kyoto, Japan), an in-line degasser, a pump, a controller, and a 20 µL injection volume automatic injector. Chromatographic separation was performed using a reverse-phase Ultrasphere Coregel-87 C analytical column (300 mm × 7.8 mm i.d., 5 µm, Transgenomic) at a flow rate of 0.6 mL/min, heated to 70 °C, and with the mobile phase being ultrapure water. Data acquisition and processing were performed with Shimadzu Class VP chromatography management software (Kyoto, Japan).

Concentrations of individual sugars are reported as percentages of fresh weight (FW) determined using original standards.

Volatile compounds were extracted by solid-phase microextraction (SPME)

Volatile compounds obtained from bitter melon fruit juice were studied using three randomly selected fruits at each stage, both unripe and commercially ripe. For each case, 1 gram of homogenized seed tissue was placed in a headspace vial, and then 1 mL of $CaCl_2$ was added. Following a 30-minute 40 °C treatment, the volatile components were extracted using a solid-phase microextraction (SPME) fiber coated with CAR/PDMS/ DVB (gray fiber). Using the method detailed by Polat and colleagues (2022), the obtained aroma compounds were then analyzed and quantified using a Shimadzu GC-2010 Plus gas chromatography-mass spectrometry (GC/MS) system.).

Statistical Analysis

The experiment was conducted using a completely randomized block design with three replications in Türkiye. A total of four trees were selected, and eight fruits were harvested from each tree at the commercial maturity stage. The collected fruits were traditionally dried using conventional methods and subsequently subjected to biochemical analyses. Data were processed using the SPSS statistical package program (version 23.0; SPSS Inc., Chicago, IL, USA). All results were expressed as mean ± standard error (SE) and evaluated by one-way analysis of variance (ANOVA) following the procedure described by Steel et al. (1997).

RESULTS AND DISCUSSION

The DPPH assay is one of the most widely used methods for evaluating the antioxidant capacity of plant extracts. It is based on the reduction of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical by physiologically active compounds with antioxidant potential, including tocopherols, ascorbates, flavonoids, aromatic amines, Maillard-type browning products, and peptides. Upon accepting an electron or hydrogen atom from these compounds, the deep violet color of DPPH fades, and the degree of discoloration can be quantified spectrophotometrically. This change reflects the hydrogen-donating ability of the sample, which is a direct indicator of its radical scavenging activity (Re et al., 1991).

Among phytochemicals, phenolics and polyphenols have attracted significant attention because of their diverse physiological functions, including antioxidant, antimutagenic, and antitumor activities (Othman et al., 2007). Plant phenolics, abundant in fruits and vegetables, are recognized for their free radical scavenging abilities, which may have important health-promoting implications (Dziedzic & Hudson, 1983; Lopez-Velez et al., 2003; Li et al., 2006; Govindarajan et al., 2007; Imeh and Khokhar, 2002; Ross and Kasum, 2002).Within this group, anthocyanins represent a major class of flavonoids responsible for the cyanic coloration observed in many flowers, fruits, and leaves of angiosperms, with hues ranging from salmon pink to dark blue (Andersen et al., 2006). In recent years, interest in anthocyanins has increased considerably due to their potential role as nutritional supplements. Regular dietary intake of anthocyanins from fruits, vegetables, wines, jams, and preserves has been linked to a reduced risk of chronic diseases such as cancer, cardiovascular disorders, diabetes mellitus, hypertension, cataracts, viral infections, and Alzheimer’s disease. Their strong antioxidant capacity underlies their classification as important nutraceuticals, with a potential preventive role in diseases associated with oxidative stress (Glade, 1997; Middleton et al., 2000).

Total phenolic content (TPC)

Total phenolic content (TPC) was determined using gallic acid as a standard, and the results were expressed as mg GAE/g fruit sample. The TPC value for bitter melon juice extract was **47.54 ± 0.51 mg GAE/g (Table 1;Figure 1).** For fraction extracts, the TPC values ranged from **4.64 to 6.84, 6.03 to 8.94, 5.39 to 7.81**, and **6.07 to 8.90 mg CAE/g**, respectively (Horax et al., 2010).

Previous studies have also reported considerable variation in phenolic content among different bitter melon tissues and extracts. Kubola and Siriamornpun (2008) reported TPC values of **474 ± 0.71 mg GAE/g** in leaves, **324 ± 1.63 mg GAE/g** in green fruits, **259 ± 1.20 mg GAE/g** in stems, and **224 ± 0.86 mg GAE/g** in ripe fruits, all expressed on a dry weight basis. Similarly, Wang et al. (2019) found TPC values ranging from **4.81 to 6.09 mg GAE/g dw**, while Dongmoon (2013) reported that bitter melon extract contained **49.42 ± 1.28 mg GAE/g,** confirming its high phenolic concentration.

The present results are in agreement with these findings, indicating that *M. charantia* fruits are rich sources of polyphenolic compounds. Such high phenolic content is closely associated with the antioxidant activity of bitter melon and supports its potential as a functional food (Kubola and Siriamornpun, 2008; Horax et al., 2010).

Antioxidant activity (DPPH Radical Scavenging Assay)

In the present study, the antioxidant capacity of bitter melon was evaluated using the DPPH radical scavenging assay, and the inhibition value was determined as 24.08% (Table 1;Figure 1). These results are comparable with the findings of Tan et al. (2013), who reported that the percentage of DPPH scavenging activity in bitter melon ranged from 15.18–22.19% in the hydrophilic fraction and 13.96–45.59% in the lipophilic fraction. Among different drying methods, freeze-dried bitter gourd fruit extracts showed the highest radical scavenging activities (hydrophilic: 22.19%; lipophilic: 45.59%), followed by oven-dried samples at 50 °C (hydrophilic: 15.39%; lipophilic: 40.11%). Similarly, Horax et al. (2010) investigated four bitter melon varieties (India green, India white, China green, and China white) and found that the antioxidant activities of methanolic extracts ranged from 81.7–86.5% in the flesh, 78.8–88.4% in the inner tissue, and 78.5–85.4% in the seeds. In another study, Lee et al. (2017) reported that the DPPH and ABTS radical scavenging activities of bitter melon fruits were 61.57% (DPPH, dry material) and 46.99% (ABTS) at 5 days after fertilization, and these activities significantly increased to 222.86% and 122.87%, respectively, at 35 days after fertilization. Taken together, these results highlight the strong antioxidant potential of *M. charantia* fruits, although the extent of radical scavenging activity can vary considerably depending on the extraction solvent, fruit tissue, processing conditions, and maturity stage. The DPPH value obtained in the present study (24.08%) is in line with previously reported ranges and further supports the role of bitter melon as a promising source of natural antioxidants.

Total Anthocyanin Content

In the present study, the total anthocyanin content of bitter melon was determined as **21.18 ± 0.02 mg/L**, expressed as cyanidin-3-glucoside equivalents (Table 1; Figure 1) Güdr (2016) reported that the total anthocyanin contents of bitter melon extracts ranged from **24.48 to 31.04 mg/L** cyanidin-3-glucoside equivalents, which are slightly higher than the values observed in this study. Phytochemicals such as phenolics and flavonoids are well known for their health-promoting properties, and among these, anthocyanins represent one of the most biologically active groups of flavonoids. Their vivid colors and strong antioxidant properties not only contribute to the aesthetic and sensory qualities of plant-derived foods but also play a significant role in reducing oxidative stress and preventing chronic diseases (Kumar et al., 2013).

Table 1. Shows the results of bioactive compound of bitter melon

Total Antioxidant (DPPH)	24.08±3.10
Total Phenolic	47.54±0.51
Monomeric Anthocyanin	21.18±0.02

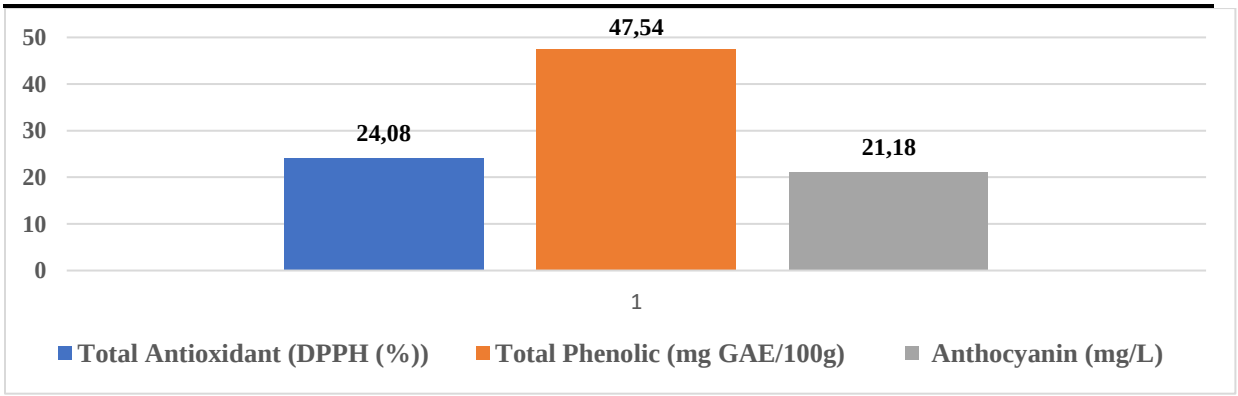

Figure 1. Contents Antioxidant Capacity (DPPH), Total Phenolic and Anthocyanin of bitter melon

Sugars and Organic Acids Composition

In this study, four sugars sucrose, xylose, glucose, and fructose were identified, with glucose and fructose being the predominant components (Table 2; Figure 2). Among them, glucose was the most abundant sugar (367.52 ± 12.32 mg/100 g), followed by fructose (229.43 ± 8.20 mg/100 g), sucrose (88.57 ± 2.28 mg/100 g), and xylose (53.38 ± 2.62 mg/100 g). The total sugar content was determined as 738.91 ± 9.04 mg/100 g.

Organic acids, which play a crucial role in flavor development and several physiological processes in fruits, were also quantified. The sugar–acid balance is considered a key factor in determining fruit taste characteristics. In the bitter melon samples, citric acid was identified as the dominant organic acid (55.78 ± 3.14 mg/100 g), followed by malic acid (20.34 ± 4.33 mg/100 g), and ascorbic acid (10.49 ± 6.15 mg/100 g). The total organic acid content was calculated as 308.06 ± 6.41 mg/100 g (Table 3; Figure 3).

For sugars: Previous studies by *Kubola & Siriamornpun (2008)* also reported that glucose and fructose are the main sugars in bitter melon. This finding supports the results obtained in the present study.

For organic acids: Perez et al. (2019) reported the detection of several organic acids in bitter melon, including succinic, malic, fumaric, and tartaric acids, some of which had not been previously identified in this species. Studies by *Horax et al. (2010)* and *Tan et al. (2013)* indicated that malic and citric acids are the predominant organic acids; however, the dominant acid may vary depending on ecological conditions. The higher succinic acid content observed in the current study may reflect the influence of the specific ecological conditions of Adana.

In study, glucose and fructose were found to be the predominant sugars, and succinic acid was identified as the major organic acid in bitter melon fruits grown under the ecological conditions of Adana. These findings are consistent with previous studies, such as Kubola and Siriamornpun (2008) and Horax et al. (2010), and indicate that the ecological conditions of Adana may influence these compositional characteristics. However, the concentrations measured in Adana-grown fruits are

relatively higher, possibly due to the warmer and sunnier Mediterranean climate, which promotes sugar accumulation.

Table 2. Shows the results of free sugars in bitter melon samples (mg /100 g FW)

Sucrose	88.57±2.28
Glucose	367.52±12.32
Xylose	53.38±2.62
Fruktose	229.43±8.20
Total sugar	738.91±9.04

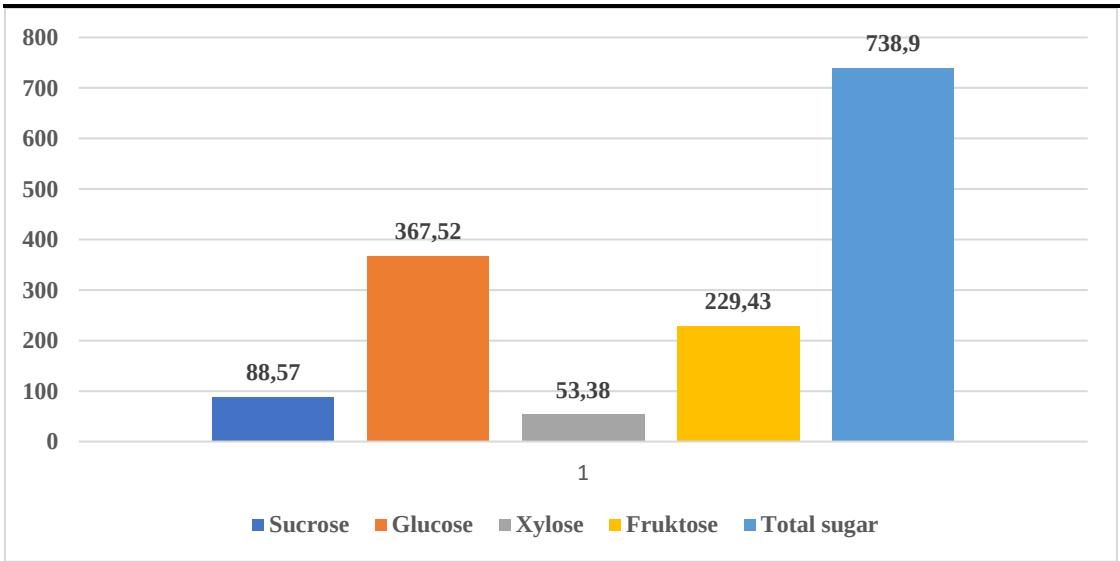

Figure 2. Individual sugars contents in bitter melon

Table 3. Shows the results of organic acid in bitter melon (mg /100 g FW)

Citric Acid	55.78±3.14
Malic Acid	20.34±4.33
Ascorbic Acid	10.49±6.15
Total acid	86.61±4.54

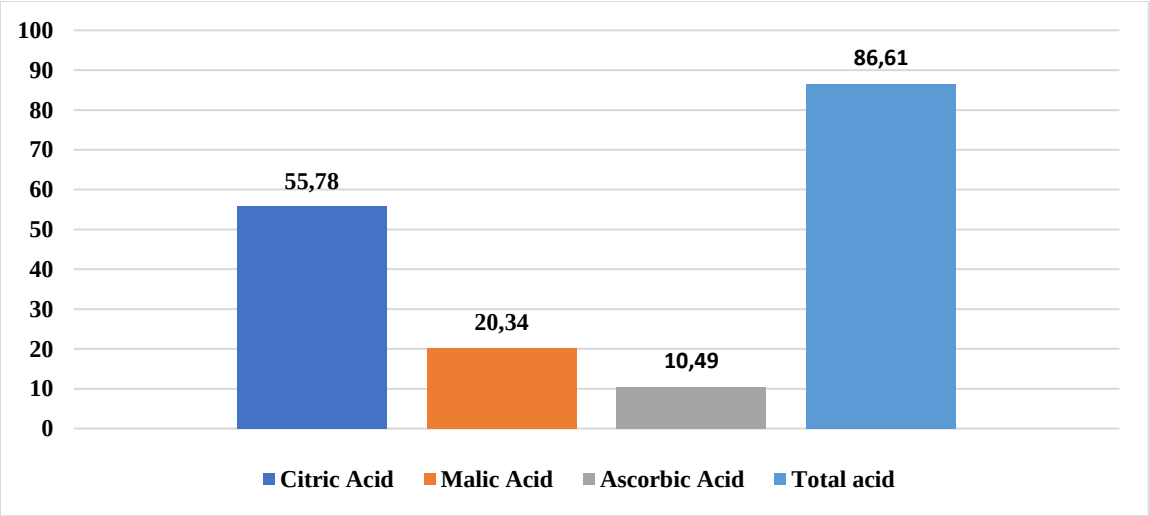

Figure 3. Individual Organic acid contents in bitter melon

Volatile Compounds of Bitter Melon Juice

Headspace Solid Phase Microextraction (HS-SPME) is a widely used technique for the identification and analysis of volatile essential oils and aroma compounds, provided that the target compounds are adequately distributed on the fiber (Toci et al., 2012). HS-SPME allows a rapid, solvent-free extraction of volatiles from the headspace above the sample onto a stationary phase coated on a fused silica fiber (Roberts et al., 2000). Gas chromatography (GC), often coupled with mass spectrometry (GC-MS), is a fundamental analytical technique for analyzing plant metabolites due to its efficiency, sensitivity, and versatility (Sgorbini et al., 2006). In the present study, volatile compounds in bitter melon juice were analyzed using the gray SPME method. The major classes of volatiles were identified as total aldehydes (4.43%), total alcohols (34.59%), total esters (48.18%), total ketones (7.27%), and total acids (5.53%) (Figure 4). A total of 31 individual components were detected, including 10 alcohols, 2 aldehydes, 12 esters, 4 acids, and 2 ketones (Table 4). Among the extraction methods tested, the gray SPME method proved most effective. Alcohols and esters were the predominant compounds in the volatile profile. The most abundant alcohols were **ethanol (11.80%), 2-phenylethanol (benzeneethanol) (9.01%)**, and **(Z)-3-hexen-1-ol (4.39%).** Among esters, **ethyl acetate (23.91%), 2-phenylethyl acetate (5.51%)**, and **ethyl 9-hexadecenoate (5.93%)** were dominant. For ketones**, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (5.36%)** was the main compound. The most abundant acids were **pentadecanoic acid (2.45%)** and **2-oxopentanedioic acid (1.08%) (Table 4).** These results highlight that alcohols and esters are the primary contributors to the aroma profile of bitter melon juice, which may be significant for both sensory and functional properties. GC-MS analysis confirmed that esters and alcohols dominate the VOC profile, consistent with previous reports (Choudhury et al., 2012). The most abundant VOCs included **ethyl acetate, ethanol, 2-phenylethanol,** and **5-(hydroxymethyl)-2-furfural,** which contribute substantially to the characteristic aroma of bitter melon.

Several studies have examined VOCs in different parts of *M. charantia*. Ferreira Almeida et al. (2025) reported 18 and 21 compounds in mature fruits, depending on the extraction method, with **benzaldehyde, linalool,** and **β-cyclocitral** consistently identified, and **linalool** as the major compound. For the flower, Fernando and Grün (2001) found **linalool (5%), 2-aminobenzaldehyde (27%), 1H-indole (33%),** and **methyl anthranilate (32%),** together comprising over 95% of the headspace volatiles. Moronkola et al. (2009) identified 63 compounds (88.8% of the total oil) in the

essential oil, with **(Z)-3-hexenol (34.7%), (E)-2-hexenol (10.1%), phytol (8.3%),** and **linalool (5.8%)** as the major constituents.

Collectively, these studies illustrate the diversity of volatile compounds in bitter melon fruits, flowers, and oils, emphasizing the influence of plant part, extraction method, and environmental conditions on VOC profiles. Notably, the ecological conditions of Adana not only support bioactive compound synthesis but also promote a rich and complex aromatic composition. The relatively higher ester content compared to some Asian studies may be linked to environmental factors, such as heat stress, which can affect metabolic pathways involved in ester biosynthesis.

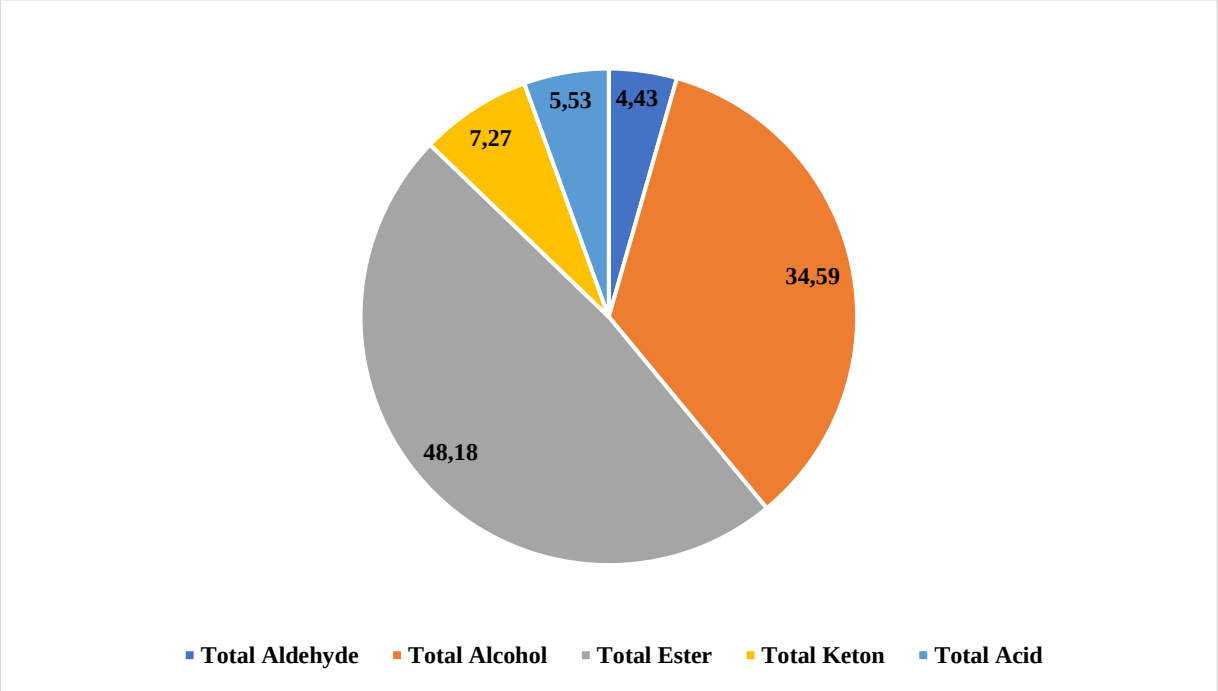

Figure 3.Volatile composition of bitter melon (%)

Table 3. The volatile compounds (%) in fruits of bitter melon

R. Time	Compounds Name	%
	Aldehyde(-al)	
13.892	Benzaldehyde (CAS)	1.04
19.289	2-Furancarboxaldehyde, 5-(hydroxymethyl)- (CAS)	3.39
	Total Aldehyde	**4.43**
	Alcohol(-ol)	
3.93	Ethanol (CAS)	11.8
9.697	2,3-Butanediol (CAS)	2.47
11.245	(Z)- 3-Hexen-1-ol, (CAS)	4.39
11.413	1-Hexanol (CAS)	3.26
14.749	(E)-3-Hexen-1-ol, (CAS)	1.08
15.474	Benzyl alcohol	0.8
16.801	Linalool	0.68
17.219	Benzeneethanol (CAS)	9.01
18.944	Myrtenol	0.66
19.812	Geraniol	0.44
	Total Alcohol	**34.59**
	Ester (-ate)	
5.998	Acetıc acıd ethyl ester	23.91
16.573	2-Furancarboxylic acid, methyl ester (CAS)	0.8
19.953	Acetic acid, 2-phenylethyl ester	5.51
28.17	Tetradecanoic acid, ethyl ester (CAS)	0.91
29.685	(Z)-9-Hexadecenoic acid, methyl ester,	1.21
29.911	Hexadecanoic acid, methyl ester (CAS)	0.79
25.353	Dodecanoic acid, ethyl ester (CAS)	0.47
30.594	Ethyl 9-hexadecenoate	5.93

30.829	Hexadecanoic acid, ethyl ester (CAS)	3.91
32.451	9,12-Octadecadienoic acid (Z,Z)-, methyl ester (CAS)	0.49
33.574	Ethyl linoleate	2.45
33.645	Ethyl octadec-9-enoate	1.8
	Total Ester	**48.18**
	Acid	
5.177	Formic acid (CAS)	0.96
13.434	2-Oxopentanedioic acid	1.08
30.334	Pentadecanoic acid (CAS)	2.45
33.085	(E)-9-Octadecenoic acid,	1.04
	Total Acid	**5.53**
	Keton(-one)	
7.081	2-Propanone, 1-hydroxy- (CAS)	1.06
17.825	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	5.36
24.006	Ionone <(E)-, beta->	0.85
	Total Keton	**7.27**

CONCLUSIONS

This study comprehensively evaluated the biochemical composition and volatile organic compound (VOC) profile of bitter melon (*M. charantia L.*) cultivated under the ecological conditions of Adana, Türkiye. The fruits were rich in bioactive compounds, with glucose and fructose as the predominant sugars and succinic acid as the main organic acid. Total phenolic content, antioxidant activity (DPPH), and total anthocyanins were also notable, highlighting the fruit's functional and nutraceutical potential. VOC analysis revealed that alcohols and esters were the major contributors to the aroma profile, with Acetıc acıd ethyl ester, ethanol, and Benzeneethanol, as the most abundant compounds. Comparative evaluation with previous studies showed that VOC composition and abundance vary depending on plant part, extraction method, and environmental factors. The relatively higher ester content in Adana-grown bitter melon may be linked to high temperatures and prolonged sunlight, which can influence ester biosynthesis pathways.

These findings confirm the multifunctional nature of bitter melon. Its traditional ethnomedicinal uses, including antidiabetic, anti-inflammatory, and antimicrobial applications, are strongly supported by its bioactive composition (Ray et al., 2010; Alam et al., 2016). Moreover, its climbing growth habit, ornamental yellow flowers, and lobed foliage highlight its dual role as both a functional food and an ornamental plant. Adana-grown bitter melon exhibits a rich biochemical composition, moderate antioxidant potential, and a complex volatile profile, making it a promising candidate for functional food applications, bioactive compound extraction, and natural aroma production. Simultaneously, its ornamental characteristics allow its use in garden and urban

landscapes. Future research should aim to optimize cultivation practices under Mediterranean conditions to enhance bioactive compound production and explore breeding programs that combine nutritional, functional, and ornamental traits.

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STUDY OF THE INTERACTIONS OF POLYMERIC BINARY MIXTURES: PHYSICO-CHEMICAL AND RHEOLOGICAL CHARACTERISATION

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ABSTRACT

The aim of this work is to conduct a study on a binary polymer blend in order to combine the characteristics of two polymers, namely sodium alginate and hydroxypropyl methylcellulose. This combination will result in the formation of a viscous blend. Several binary mixtures were prepared at different ratios, for which we studied the effect of pH and ionic strength variation. These mixtures will undergo physico-chemical and rheological characterisation in order to evaluate the effect of pH and ionic strength on the properties of the different polymer mixtures considered. The results revealed that the highest absorbance is observed for sodium thiosulphate, probably due to the dissolution of $Na_2 S_2 O_3$ in water. The study of viscoelastic behaviour in oscillatory modulus revealed gelation, which was recorded for pH values of 2 and 2.5, where phase separation was observed. The concentration of salt added had an effect on the macroscopic and microscopic appearance of the different polymer ratios.

Keywords: Alginate-Na, HPMC, interaction, rheology, ionic strength, pH .

INTRODUCTION

Molecular interactions are ubiquitous and occur in all states of matter. In the field of polymer physical chemistry, much research focuses on the study of so-called weak molecular interactions in soft matter. These non-covalent interactions, which have low energy compared to covalent bonds, are mostly reversible. [1]

Mixtures of polymers in aqueous solution generally lead to three types of phenomena: total miscibility, segregative phase separation when the interaction between the solvent and one of the polymers is strong, or associative phase separation when the interaction between the two polymers is strong. The latter phenomenon is called complex coacervation [2, 3].

This coacervation depends on environmental conditions such as pH, ionic strength, temperature, and polymer concentration [4–10] ...

To this end, two polysaccharides were selected for development: sodium alginate (Alg-Na) and hydroxypropyl methylcellulose (HPMC).

HPMC is a synthetic polymer derived from cellulose, widely used for its gelling, adhesive and drug release control properties. Sodium alginate, on the other hand, is a natural polymer extracted from brown algae, known for its ability to form gels in the presence of calcium [11].

The interactions between HPMC and sodium alginate are mainly due to mechanisms such as electrostatic interactions, hydrogen bonds and hydrophobic interactions.

The aim of our study is to conduct a study on a binary polymer blend in order to combine the interactions between sodium alginate and HPMC and to see the effect of pH variation and different ionic strengths (sodium thiosulphate, sodium chloride and potassium chloride).

To this end, a physico-chemical and rheological characterisation was carried out in order to evaluate the effect of pH and ionic strength on the properties of the different polymer blends studied.

MATERIALS AND METHODS

Medium-grade sodium alginate was used in this study by Sigma Aldrich, HPMC was supplied by Novapharm and acts as an emulsifier. Sodium thiosulphate is an inorganic sodium salt with the formula Na2S2O3 composed of a mixture of sodium and thiosulphate ions. Sodium chloride is an ionic chemical compound with the formula NaCl. Potassium chloride is a mineral chemical compound with the formula KCl. In our study, these three salts are used as ionic forces.

RESULTS AND DISCUSSIONS

Observations and interpretation of macroscopic and microscopic results

For the alginate solution alone, gel formation was observed at pH=3.5 with no precipitate formation, as demonstrated by microscopic examination, which revealed the formation of transparent aggregates. For the other ratios 1/2, 2/1 and 1.5/1.5, visual examination of the test tubes shows cloudiness from pH 3.5 onwards, with an opalescent appearance, confirming the interaction between the polymers, but of a weak nature since no precipitate is observed.

The effect of ionic strength on Alg-Na/HPMC ratios

We represent the range of Alginate/HPMC mixture samples with the effect of salt:

1. **Sodium thiosulphate (0.1M):**

For the alginate solution alone, we observe the formation of a white precipitate at the bottom of the tubes starting at pH 3, which becomes increasingly visible as the pH decreases.

For the other ratios 1/2, 2/1 and 1.5/1.5, we observe that phase separation is even more visible at acidic pH values, namely pH 3.5, 3, 2.5) with the formation of a white precipitate and a transparent supernatant. Under the microscope, we see more or less dense aggregates resulting from the interaction of the two polymers in the presence of salt.

This separation can be explained by the effect of salt in accelerating the neutralisation of the ionic charges present in the polymers. For example, for a 1/2 ratio, a white precipitate appears at a pH of 3, confirmed by microscopy, where dense aggregates are observed. This phenomenon can be explained by the saturation of anionic and cationic sites by the Na+ ions in the thiosulphate, which is justified by the competition between the ions and the micromolecules. In reality, this means that at high ion concentrations in the solution, the charges carried by the biopolymers are reduced by interactions with these ions, which then neutralise the biopolymers, causing them to precipitate.

KCl and NaCl appear to have the same effect on alginate solutions alone and polymer blends, as precipitation occurs with phase separation at acidic pH (from 3 onwards) with aggregates visible under an optical microscope. This confirms the role of salt (K+ and Na+ ions) in neutralising charges by saturating the anionic sites of biopolymers, which contributes to changes in polymer solubility and precipitation.

Variation in the absorbance of Alg-Na/HPMC binary mixtures at 600 nm as a function of pH and ratio

The absorbance variation results at 600 nm obtained for the Alg-Na/HPMC mixture at a total polymer concentration of 3% show that for Alg-Na/HPMC ratios between 1/2, 2/1 and 1.5/1.5, all curves show maximum absorbance at pH 2.5 to 2. We also estimate that for a given pH value, a maximum variation in the absorbance of the mixture is linked to the formation of a maximum number of particles and therefore maximum interaction between the two macromolecules [80].

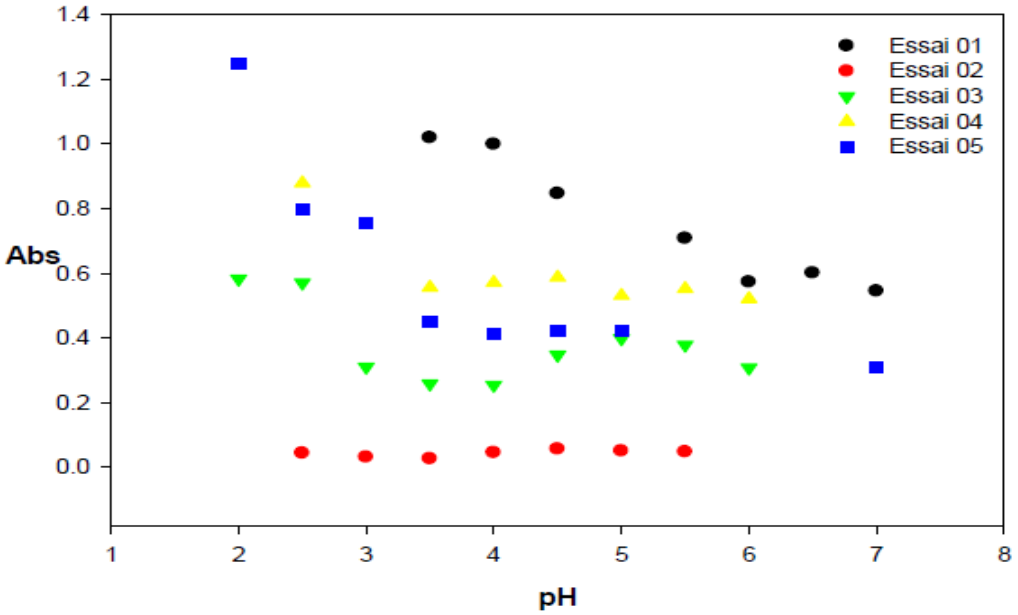

Figure . 1: Variation in absorbance as a function of pH for different Alg-Na/HPMC ratios without ionic strength

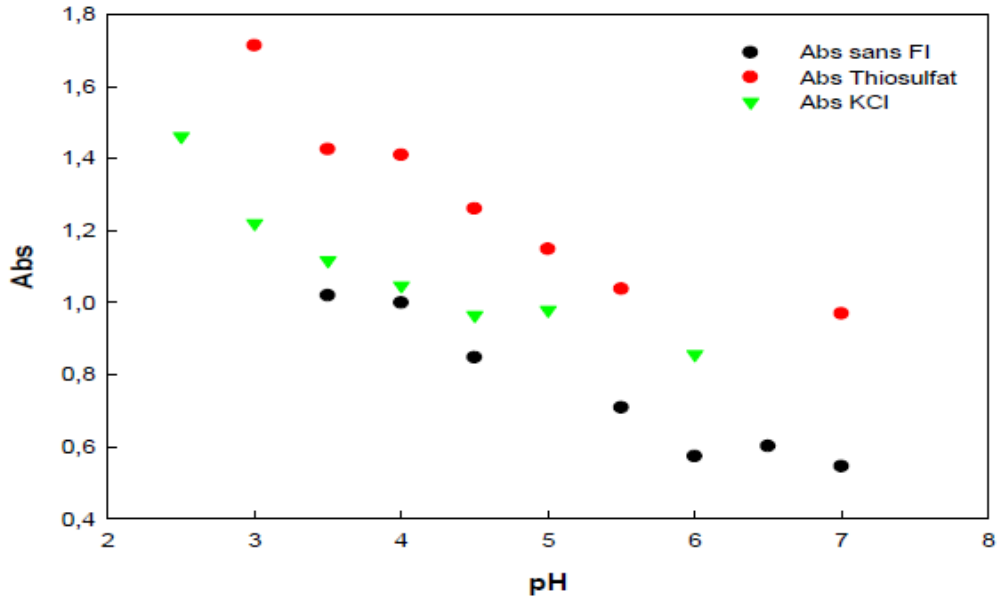

Figure 2: Variation in absorbance as a function of pH for the Alg-Na/HPMC 3/0 ratio (KCl, $Na_2S_2O_3$ 0.1M)

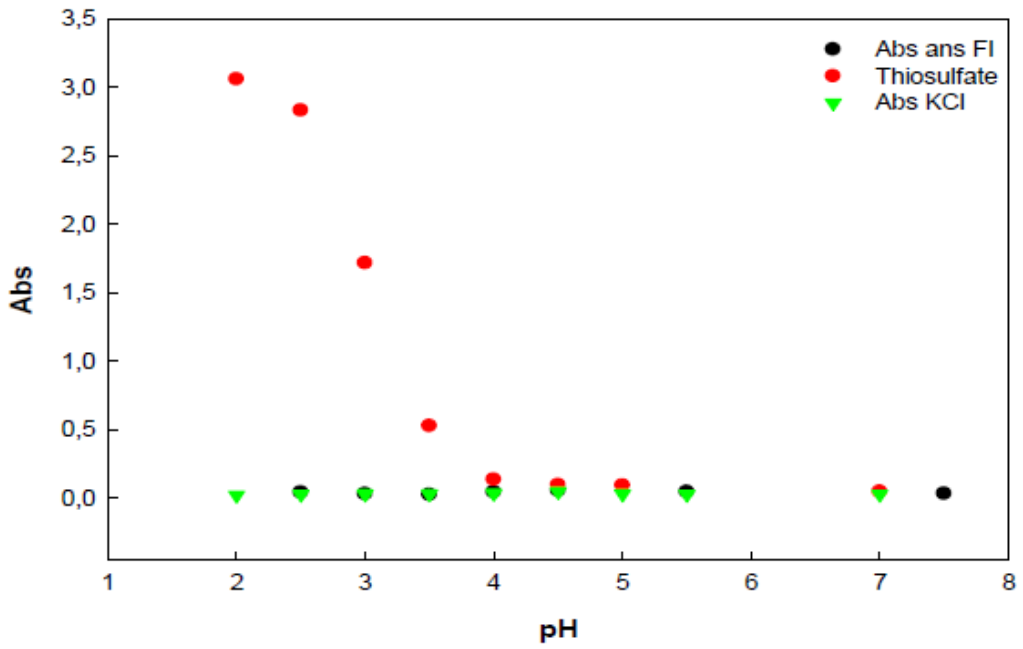

Figure 3: Variation in absorbance as function of pH for the ratio Alg-Na/HPMC 0/3 (KCl,$Na_2S_2O_3$) 0.1M

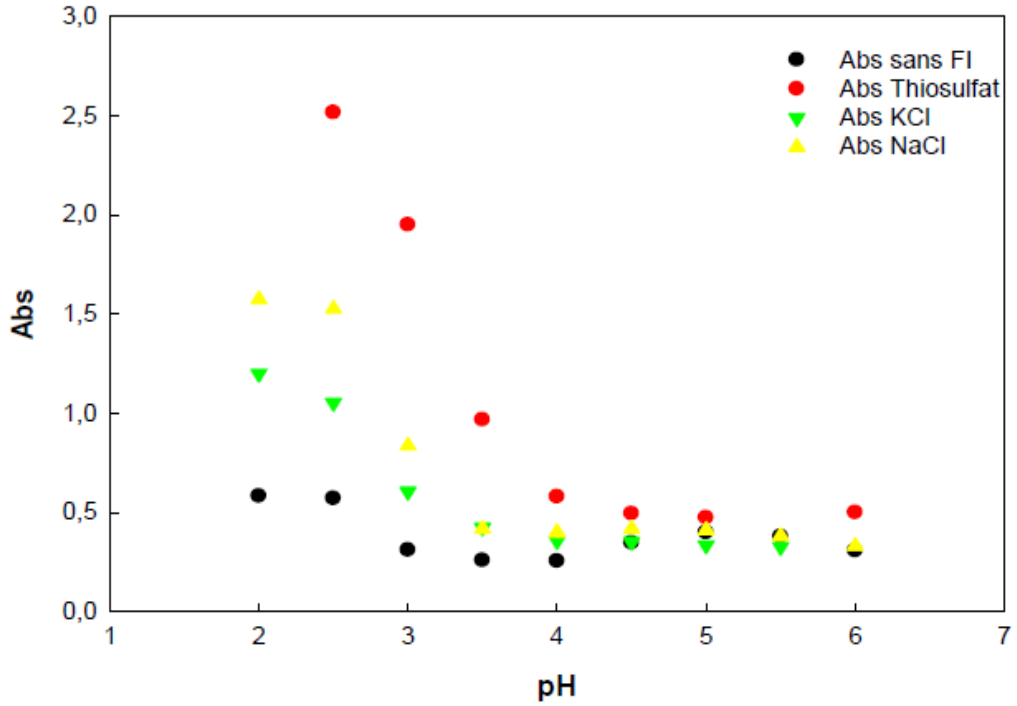

Figure 4: Variation de l`absorbance as function of pH for the ratio Alg-Na/HPMC 1/2 (KCl, $Na_2S_2O_3$, NaCl) 0.1M

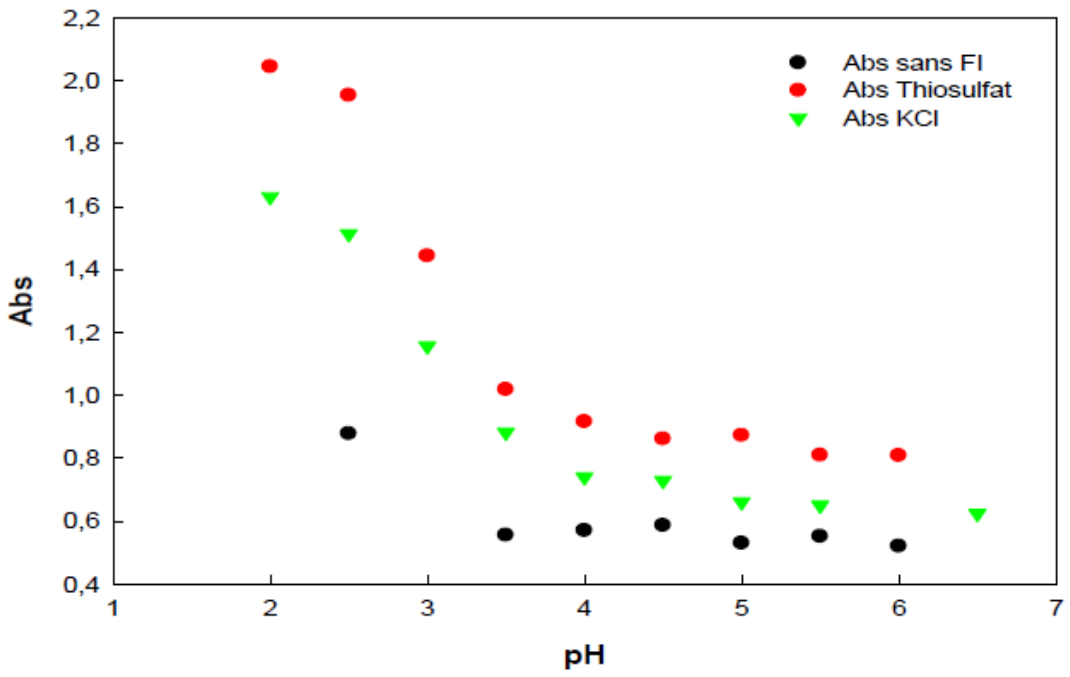

Figure 5: Variation in absorbance as function of pH for the ratio Alg-Na/HPMC 2/1 (KCl, $Na_2S_2O_3$) 0.1M

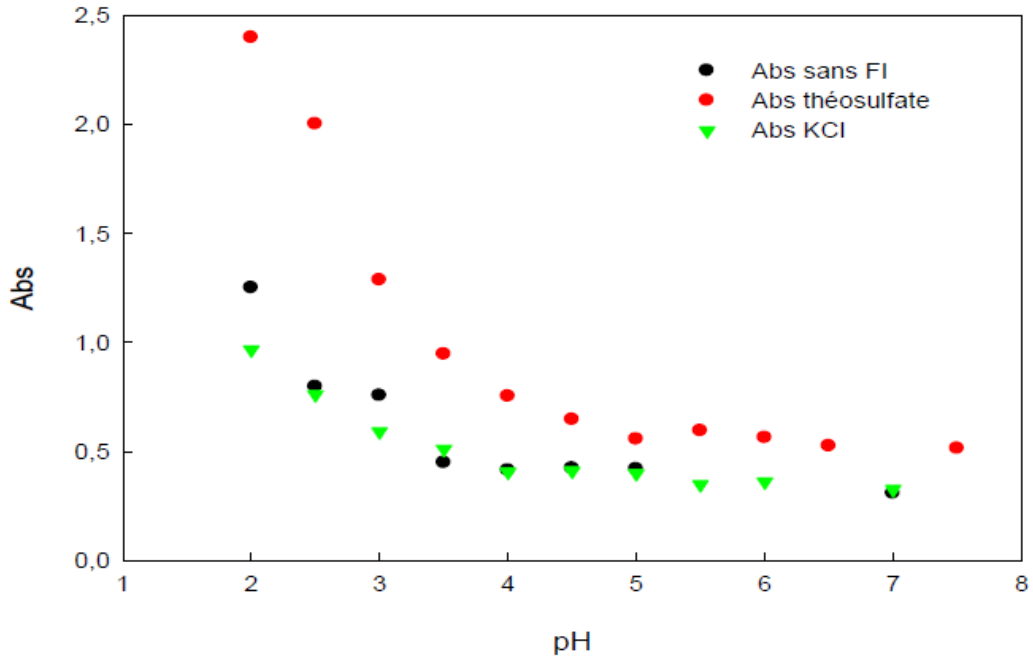

Figure 6: Variation in absorbance as function of pH for the ratio Alg-Na/HPMC 1.5/1.5 (KCl, $Na_2S_20_3$,0.1M)

Figures (1-6) representing the variation in absorbance for ratios (3/0, 0/3, 1/2, 2/1, 1.5/1.5) show maximum absorbance at around pH 2 and 2.5 in the presence of ionic strengths ($Na_2S_2O_3$, KCl , and NaCl) 0.1M. Under these conditions, the electrostatic interactions between Alg-Na and HPMC cause a phase separation known as segregation. These results are consistent with the macroscopic findings. The highest absorbance is observed at the sodium thiosulphate level, which is probably due to its dissolution in water.

Rheological characterization of binary Alg-Na/HPMC mixtures at different ratios without ionic forces

The flow curve of the graph representing the five ratios is shown in (Figure 7).
These solutions exhibit two types of behaviour: the first is Newtonian behaviour at low shear rates and rheofluidising behaviour at intermediate shear rates, reflecting the structural behaviour of the two biopolymers. At low shear rates, the physical bonds resist flow. As the shear rate increases, shear causes the physical bonds between the macromolecules to break, resulting in partial destructuring depending on the intensity of the shear, which manifests itself in their dispersion. When the shear is very strong, the macromolecules are completely dispersed and oriented along the flow, and their resistance becomes constant, as does the viscosity. The same type of behaviour was found by Belhadji et al. [81] in the case of sodium alginate solutions with xanthan.

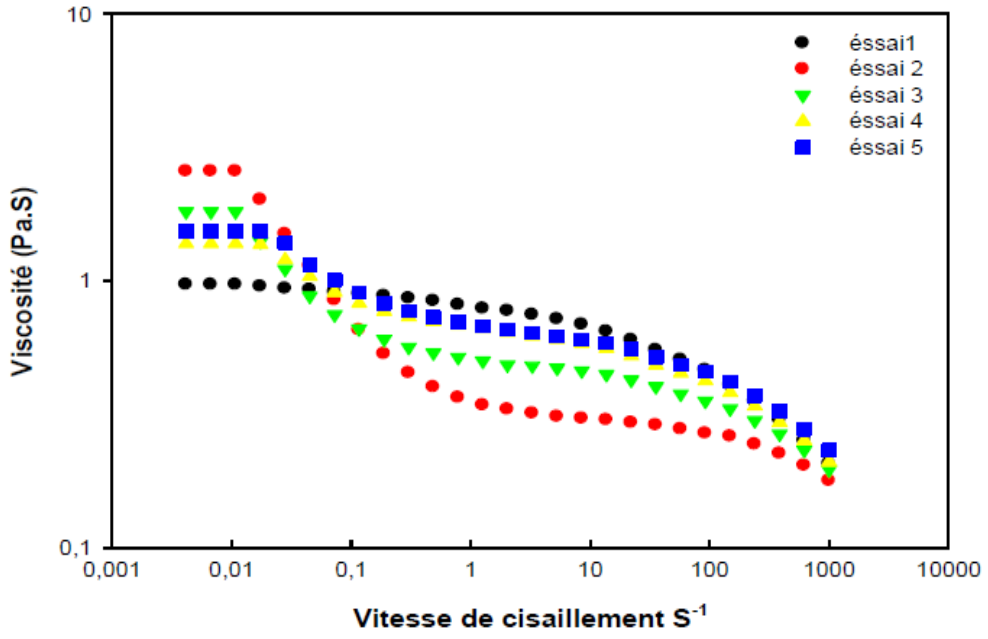

Figure.7 Variation in viscosity as a function of shear rate at different ratios

Viscoelastic behavior

In order to analyze and compare the results obtained, we presented the conservation modulus curves for the different ratios and pH values (Figures 8-10) without ionic forces

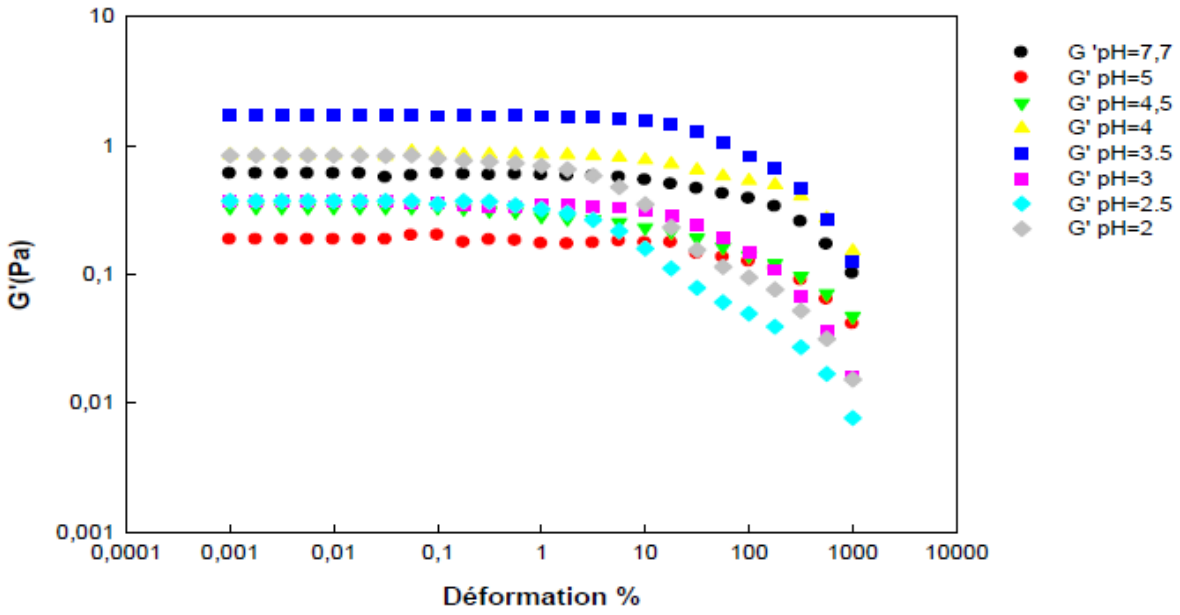

Figure . 8: Variation of the storage modulus G′ as a function of deformation for R1/2 with an ionic strength of $Na_2S_2O_3$ 0.1M

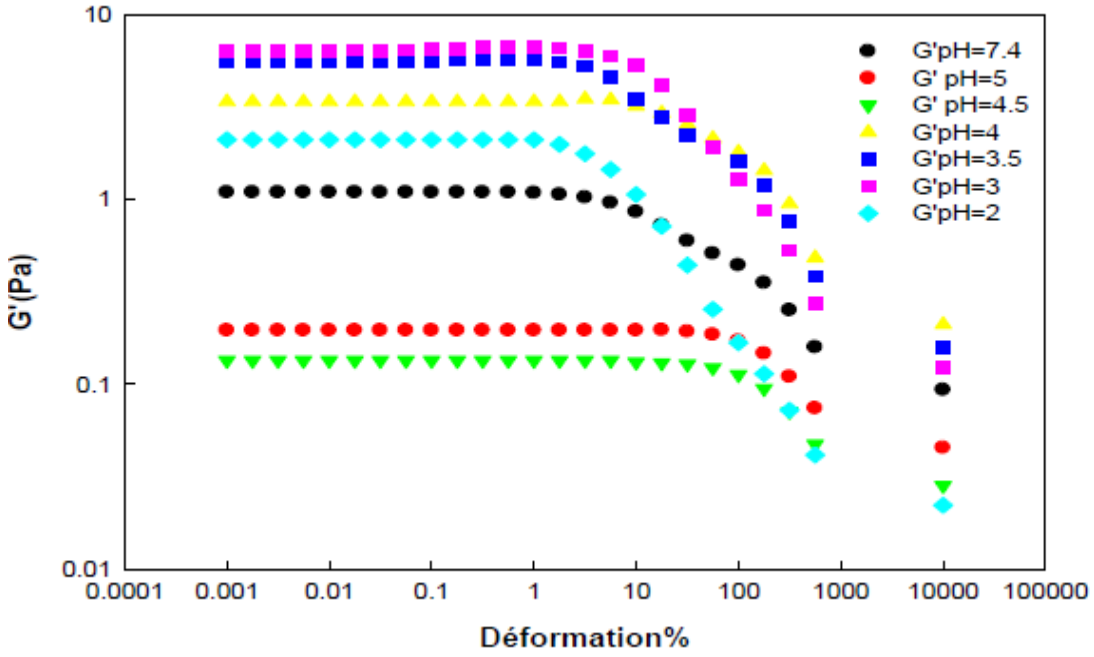

Figure . 9: Variation of the storage modulus G′ as a function of deformation for R1/2 with an ionic strength of 0.1M KCl

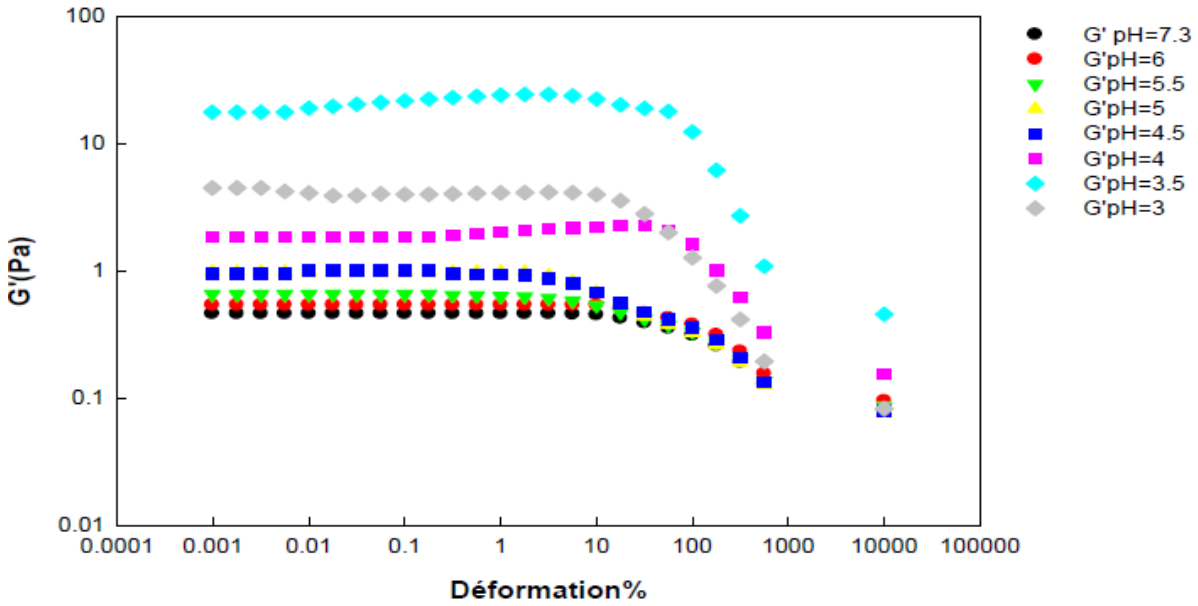

Figure . 10: Variation of the storage modulus G′ as a function of deformation for R1/2 with an ionic force NaCl

For the 1/2 ratio for the three salts ($Na_2S_2O_3$, NaCl, KCl), the maximum G′ values are recorded for pH values of 2 and 2.5, where phase separation is observed. Based on the macroscopic appearance, this is an associative phase separation. [1] These results are consistent with the turbidity results, for which we recorded maximum absorbance values at the same pH values highlighted above.

CONCLUSIONS

The objective of this work is to study the physicochemical and rheological characteristics of a binary polymer blend in order to observe their interactions. Several binary blends were prepared at different ratios, for which we studied the effect of pH and ionic strength variation.
To do this, we combined two polymers: sodium alginate and HPMC.
At the end of this study, we obtained the following results:
- For the alginate solution alone, gel formation was observed at pH=3.5 with no precipitate formation, as demonstrated by microscopic examination, which revealed the formation of transparent aggregates.
- For the other ratios 1/2, 2/1 and 1.5/1.5, visual examination of the test tubes showed cloudiness from pH 3.5 onwards, with an opalescent appearance, confirming the interaction between the polymers, but of a weak nature since no precipitate was observed.
□□The concentration of salt added has an effect on the macroscopic and microscopic appearance of the different polymer ratios.
□□For the other ratios 1/2, 2/1 and 1.5/1.5, we observe that phase separation is even more visible at acidic pH values (pH 3.5, 3, 2.5) with the formation of a white precipitate and a transparent supernatant. Under the microscope, we can see more or less dense aggregates resulting from the interaction of the two polymers in the presence of salt.
KCl and NaCl appear to have the same effect on alginate solutions alone and polymer blends, as precipitation occurs with phase separation at acidic pH (from 3 onwards) with aggregates visible under an optical microscope.

□□The highest absorbance is observed for sodium thiosulphate, probably due to its dissolution in water.
The 1/2 ratio for the three salts (, the maximum G′ values are recorded for pH 2 and 2.5, where phase separation is observed (Tables III.2, III.3 and III.4). Based on the macroscopic appearance, we have associative phase separation.

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THE ROLE OF SOIL CONDITIONERS IN CEREAL PRODUCTION

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ABSTRACT

Soil conditioners are crucial for improving soil quality and sustaining cereal production in the face of mounting environmental and agronomic challenges. This study examines the various types, mechanisms and effects of organic, inorganic and synthetic soil conditioners on soil physical properties, water retention, nutrient availability, microbial activity and soil structure as a whole. Through a comprehensive review of recent research and experimental evidence, we evaluate the influence of conditioners on short- and long-term cereal crop productivity, considering factors such as improved soil fertility, reduced compaction, and mitigation of the effects of salinity and erosion. Organic amendments, such as compost, manure and humic acids, improve aggregation and biological activity, whereas inorganic agents, such as gypsum and lime, enhance ion exchange and pH balance. Synthetic polymers contribute to erosion control and moisture conservation. Furthermore, integrating conditioners with slow-release fertilisers and sustainable management practices has demonstrated synergistic effects on yield improvement and soil health. Our findings emphasise the importance of judicious application of soil conditioners for achieving resilient and sustainable cereal production systems, particularly in degraded or marginal soils.

Keywords: Soil Conditioners, Cereal Crops, Sustainable Agriculture, Soil Health

INTRODUCTION

Cereal crops such as wheat, maize, barley, and rice are the cornerstone of global food security, yet their production is increasingly threatened by soil degradation, nutrient depletion, and climate variability. Sustainable intensification of cereal systems requires strategies that improve soil physical, chemical, and biological functions. Soil conditioners including organic amendments, inorganic inputs, and synthetic products are central to these strategies because they directly influence soil structure, water relations, and nutrient cycling while also contributing to long term soil health (Jarecki & Lal, 2003).

One of the most pressing challenges is the decline of soil organic carbon (SOC) due to intensive cultivation and conventional tillage, which has reduced soil fertility and resilience across many agroecosystems. Restoring SOC stocks through organic amendments, cover crops, and reduced tillage has been shown to improve aggregation, enhance microbial activity, and reduce erosion, all of which are critical for sustaining cereal productivity (Morya et al., 2023). In addition, soil conditioners act as vehicles for carbon sequestration, offering mitigation potential against greenhouse gas emissions while improving nutrient retention in cropping systems (Zhang et al., 2024).

Beyond their role in carbon management, conditioners influence the efficiency of nutrient use. Integrating conditioners with precision nutrient application technologies can optimize fertilizer delivery, minimize losses, and improve nutrient uptake in cereals (Raza et al., 2023). This synergy between conditioners and nutrient management is particularly important under conditions of high variability in soil fertility and water availability, as it promotes yield stability while reducing environmental externalities.

The adoption of conditioners is also aligned with climate smart agriculture principles, as they contribute to soil resilience under stresses such as drought and salinity. For instance, organic inputs such as compost and biochar improve soil water holding capacity, while gypsum and lime alleviate sodicity and acidity constraints (Morya et al., 2023; Jarecki & Lal, 2003). Together, these interventions highlight the dual role of soil conditioners in enhancing immediate crop performance and securing long term ecological sustainability.

This paper explores the classification, mechanisms, and impacts of soil conditioners in cereal production systems. By synthesizing experimental evidence and recent reviews, it provides a framework for understanding how conditioners contribute to improved soil quality, enhanced nutrient use, and resilient crop yields under diverse environmental and management contexts.

SOIL CONDITIONERS CLASSIFICATION

Soil conditioners encompass a wide spectrum of materials deliberately applied to soils to improve their physical, chemical, and biological properties. In the context of cereal production, these conditioners are typically grouped into organic, inorganic, and synthetic amendments. Each category provides unique advantages and limitations, and their comparative effectiveness often depends on soil type, climate, and cropping system.

Organic conditioners such as compost, farmyard manure, green manure, crop residues, and biochar are indispensable for building soil organic matter (SOM). Their value lies not only in supplying nutrients but also in enhancing soil structure, microbial diversity, and water dynamics. Paustian (2000) emphasized that adoption of practices like residue retention, cover cropping, and reduced disturbance is crucial for maximizing organic matter inputs and thereby stabilizing carbon in soil systems.

Ingram and Fernandez (2001) also underlined the ecological dimension of organic conditioners, stressing their role in sustaining carbon inputs over multiple rotations, which supports aggregate stability and minimizes nutrient leaching. Application of biochar and compost has shown promising results in increasing soil cation exchange capacity (CEC), thereby enhancing nutrient retention and reducing fertilizer losses. Over time, these organic materials build a more resilient soil system, capable of buffering cereals against stress conditions such as drought or nutrient fluctuations. However, a limitation of organic conditioners is that their effects may take several years to manifest fully, as stabilization of carbon and improvement in aggregation are gradual processes.

Inorganic amendments such as gypsum, lime, and phosphogypsum are widely recognized for their capacity to correct chemical imbalances. Gypsum application reduces sodium induced dispersion,

improves infiltration, and restores root growth in sodic soils. Lime, conversely, neutralizes soil acidity, reduces aluminum toxicity, and enhances phosphorus availability. West and Post (2002) demonstrated that combining mineral amendments with conservation tillage significantly improved soil carbon sequestration rates, indicating their dual role in both fertility restoration and carbon management.

A major strength of inorganic conditioners is their relatively rapid effect compared to organic amendments. For example, liming acidic soils often results in visible yield responses within the first season. Nevertheless, repeated application may be required to maintain soil chemical balance, and over application can lead to secondary issues such as micronutrient deficiencies. Thus, their use requires careful calibration based on soil testing and site specific conditions.

Synthetic conditioners, including hydrogels, polyacrylamides, and other water retaining polymers, represent a more recent technological innovation. They are particularly valuable in regions where water scarcity and erosion are limiting factors for cereal production. Six et al. (1998) illustrated that aggregate stability plays a central role in determining the persistence of organic matter in soils, and synthetic conditioners can directly enhance this stability by reducing crusting and surface sealing.

Hydrogels, for instance, can store many times their weight in water and release it gradually to plant roots, significantly improving water use efficiency under dry conditions. Polyacrylamides, when applied to the soil surface, have been shown to reduce runoff and sediment loss, contributing to both water conservation and soil protection. Despite these advantages, concerns remain about the cost of application and the long term ecological impacts of synthetic materials, such as their persistence in soil and potential interactions with soil biota.

The effectiveness of soil conditioners increases when they are used in a complementary manner. Doran and Smith (1987) highlighted that conservation systems integrating crop residues with minimal soil disturbance not only enhance microbial biomass but also improve nutrient cycling efficiency. Wairiu and Lal (2003) further demonstrated that the integration of conditioners with crop rotation and reduced tillage can restore carbon pools and maintain soil fertility on sloping lands, where erosion pressure is high.

The integrative use of conditioners highlights a critical point: while organic amendments build long term soil resilience, inorganic materials provide immediate corrections for chemical imbalances, and synthetic conditioners deliver short term technological solutions to address acute stress factors. A holistic approach that combines these inputs ensures sustained cereal productivity while simultaneously contributing to soil carbon sequestration and ecological sustainability

Table 1. Soil conditioners and their primary functions in cereal production

Conditioner Type	Examples	Main Effects	Impact on Cereals
Organic	Compost, manure, biochar	Improves SOM, aggregation, microbial biomass	Enhances yield stability, nutrient uptake
Inorganic	Lime, gypsum, phosphogypsum	Corrects pH, reduces sodicity, improves CEC	Boosts root growth, improves P availability
Synthetic	Hydrogels, polyacrylamides	Increases water retention, reduces erosion	Improves drought tolerance, water-use efficiency

SOIL PROPERTIES EFFECTS

The influence of soil conditioners on soil properties is multifaceted, impacting the physical, chemical, and hydrological characteristics of soils. These changes are central to ensuring that cereal crops thrive under both optimal and stress prone conditions. By modifying soil texture, aggregation,

nutrient pools, and erosion dynamics, conditioners create a more balanced and resilient environment for sustainable cereal production.

One of the primary contributions of soil conditioners is the improvement of soil structure through aggregation and stabilization. Tisdall and Oades (1982) reported that the addition of organic residues enhances the formation of water stable aggregates, which protect soil organic matter and reduce susceptibility to erosion. These stable aggregates improve porosity, allowing better gas exchange and root penetration.The flow of energy, organic matter transformation, and nutrient cycling within the soil ecosystem occur through interconnected processes (Figure 1).

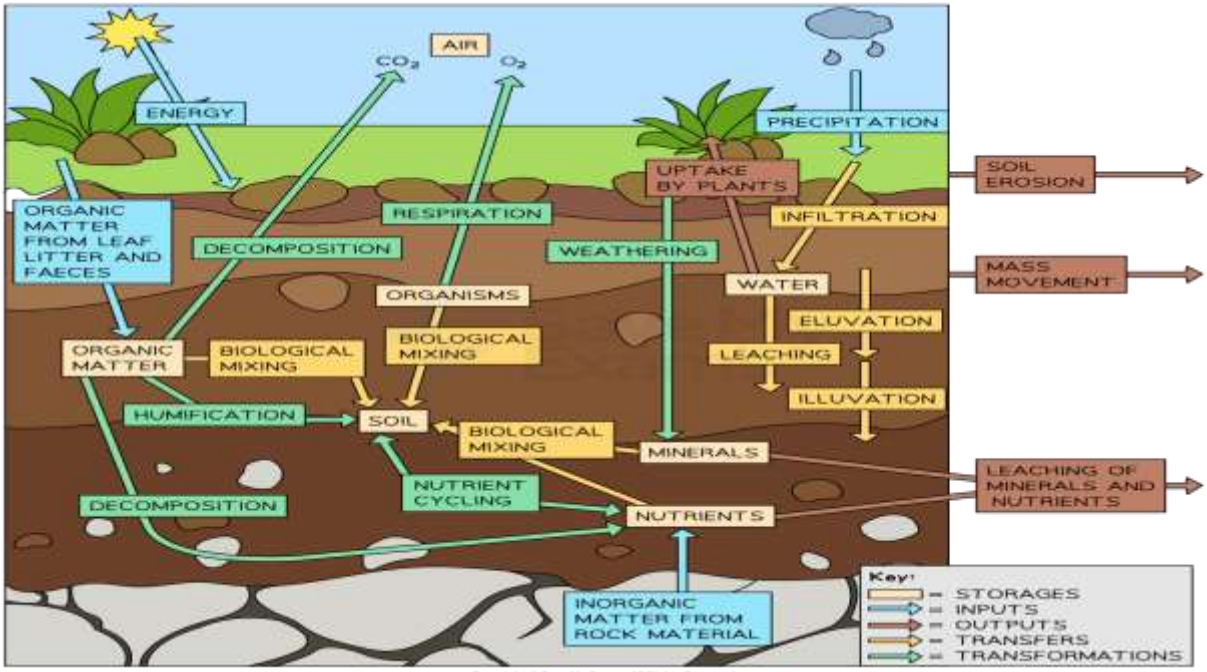

Figure 1. Schematic representation of major soil processes influencing nutrient cycling and organic matter dynamics. The diagram illustrates pathways of decomposition, mineralization, weathering, infiltration, and leaching, which together determine nutrient availability, soil formation, and plant uptake in cereal-based systems.

Bulk density is another parameter directly influenced by conditioners. Cameron et al. (1981) demonstrated that continuous cultivation without organic inputs leads to higher bulk density and reduced pore space, restricting water infiltration and root development. The application of conditioners reduces bulk density and increases micropore volume, which is particularly advantageous for cereals like wheat and maize, where root proliferation in the upper soil layers is critical for water and nutrient uptake. Improved porosity also enhances infiltration rates, reducing surface runoff and making water more available for plant use.

Conditioners also exert a strong influence on soil chemistry, particularly nutrient dynamics and pH balance. Dalal and Mayer (1986) highlighted that intensive cultivation without amendments depletes total and mineralizable nitrogen pools, leading to nutrient imbalances and reduced soil fertility. Incorporating conditioners such as manures, composts, or lime helps restore nitrogen fractions and maintains a steady supply of nutrients for cereals.

pH regulation is another important function. Mann (1986) demonstrated that acid soils lose significant amounts of soil carbon after cultivation, a process that can be mitigated through liming. Lime reduces soil acidity, enhances phosphorus availability, and alleviates aluminum toxicity, all of which are essential for robust cereal growth. Gypsum also plays a role by supplying calcium and

improving cation exchange capacity (CEC), which counteracts sodicity and improves root zone conditions. These chemical corrections ensure that essential nutrients remain available and accessible throughout the growing season.

Soil conditioners also affect the hydrological properties of soils by enhancing water retention and infiltration. Improved aggregation and porosity facilitate deeper water movement into the profile, while organic conditioners such as compost and biochar increase the soil's capacity to store moisture. For cereals grown in semi arid environments, this enhanced water holding capacity is critical for bridging dry spells and ensuring yield stability. Tisdall and Oades (1982) further emphasized that organic matter plays a dual role by not only binding particles into aggregates but also acting as a sponge to retain soil water, improving drought resilience.

The long term application of conditioners contributes to the stability of soil organic carbon (SOC) pools and nutrient cycling. Follett et al. (1987) found that conservation tillage combined with residue retention decreases the loss of SOC associated with eroded sediments by nearly half compared to conventional tillage. This demonstrates that conditioners are essential not only for improving soil fertility but also for mitigating soil erosion and carbon loss. Over time, these effects create a positive soil carbon balance, transforming soils from carbon sources to carbon sinks.

The preservation of SOC is particularly important for cereals, as it directly correlates with yield stability and soil resilience. By minimizing erosion and leaching, conditioners maintain critical topsoil layers rich in nutrients and organic matter. This ensures that cereal cropping systems remain productive while simultaneously supporting global efforts to mitigate greenhouse gas emissions.

SOIL BIOLOGICAL ACTIVITY AND MICROBIAL DYNAMICS

Soil conditioners not only modify the physical and chemical environment of soils but also exert strong control over the biological functioning of the soil ecosystem. Microorganisms—bacteria, fungi, and actinomycetes—respond sensitively to changes in organic matter, pH, nutrient levels, and moisture. Through these responses, soil conditioners shape nutrient availability, soil resilience, and cereal crop productivity.

Organic amendments such as compost, manure, crop residues, and biochar provide energy rich substrates that stimulate microbial growth. Jenkinson and Ladd (1981) demonstrated that the addition of fresh organic matter significantly increases microbial biomass carbon, which acts as a labile reservoir of nutrients. Swift (1987) further explained that the quality of organic residues—for example, residues with low lignin and high nitrogen content—accelerates decomposition and promotes microbial turnover, while resistant materials such as straw with high lignin content decompose more slowly, stabilizing soil carbon. This balance between easily decomposable and recalcitrant fractions underpins both immediate nutrient release and long term carbon sequestration.

Inorganic conditioners, particularly lime and gypsum, indirectly influence microbial communities by altering soil chemical conditions. Haynes and Naidu (1998) reported that liming acidic soils reduces aluminum toxicity and enhances base saturation, creating an environment where microbial diversity can flourish. Gypsum, by improving soil structure and reducing sodicity, provides better aeration and root growth conditions, which in turn stimulate rhizosphere microbial activity. These changes are particularly beneficial in cereal systems where root–microbe interactions strongly affect nutrient uptake.

Soil conditioners influence microbial processes at a biochemical level. Balota et al. (1998) observed that microbial biomass and enzyme activities—such as dehydrogenase, phosphatase, and urease—are consistently higher in soils with organic inputs compared to conventional systems. These enzymes are crucial for breaking down organic compounds and releasing nutrients like nitrogen,

phosphorus, and sulfur in forms readily available to cereals. Increased enzymatic activity not only accelerates nutrient cycling but also enhances the efficiency of fertilizer use, thereby reducing nutrient losses to the environment.

Microbial activity contributes directly to soil aggregation and stabilization of soil organic matter. Powlson et al. (1987) showed that soils receiving straw or compost amendments maintained higher microbial biomass, which was closely linked to improved aggregate stability. Greenland and Szabolcs (1994) highlighted that resilient soils are characterized by active microbial populations that produce polysaccharides and glomalin like substances, binding soil particles together and protecting carbon within aggregates. In this way, microbial activity creates a feedback loop where conditioners stimulate microbes, and microbes in turn stabilize soil structure and protect organic matter.

Enhanced microbial diversity promoted by conditioners also contributes to disease suppression. Competitive exclusion, production of antimicrobial compounds, and stimulation of beneficial organisms such as mycorrhizal fungi help protect cereal roots from soil borne pathogens. While not a direct function of conditioners themselves, the enrichment of microbial communities indirectly fosters a healthier rhizosphere, reducing the need for chemical disease control.

Over longer time scales, conditioners build microbial resilience. Powlson et al. (1987) demonstrated that repeated organic inputs maintain microbial populations even under intensive cereal cropping, preventing declines in soil fertility. Greenland and Szabolcs (1994) emphasized that this resilience is fundamental to sustainable land use, as biologically active soils are more capable of withstanding climatic and management stresses. By maintaining microbial driven carbon and nutrient cycles, conditioners thus provide cereals with a stable supply of essential resources across years and crop rotations.

SOIL FERTILITY, STRESS TOLERANCE AND YIELD RESPONSES IN CEREALS

Soil conditioners directly influence nutrient uptake, stress tolerance, and yield stability in cereal crops. Their role extends beyond short term fertility enhancement, as they improve the soil's ability to supply essential nutrients under both favorable and stress conditions. Soil conditioners increase the efficiency of nutrient use in cereal systems by improving soil structure and nutrient availability. Blevins et al. (1983) reported that no tillage systems combined with organic amendments enhanced nitrogen use efficiency in maize by reducing leaching losses. Similarly, Thomas et al. (1996) showed that the incorporation of crop residues improved phosphorus availability in acidic soils, directly supporting cereal growth. These findings underline the role of conditioners in ensuring sustained nutrient supply throughout critical growth stages.

Conditioners contribute to resilience against abiotic stresses such as drought and salinity. Unger and McCalla (1980) demonstrated that residue management increases soil water storage and reduces evaporation, thus improving drought tolerance in wheat and sorghum systems. In sodic soils, the application of gypsum improves soil structure and root growth, which in turn enhances water and nutrient uptake. Additionally, Salinas Garcia et al. (1997) observed that long term organic amendments increased soil organic carbon and aggregate stability, providing cereals with greater buffering capacity against both drought and salinity stress.

The integration of conditioners into cereal production systems has consistently improved yields. Power et al. (1986) reported that manure application increased maize grain yield by improving nitrogen availability and soil moisture retention. Similarly, long term trials highlighted by Rasmussen and Collins (1991) demonstrated that soils with continuous organic inputs maintained higher yields under intensive cereal cropping compared to conventionally managed soils. These responses reflect both immediate improvements in soil fertility and the cumulative benefits of enhanced soil structure and biological activity.

Conditioners also play a crucial role in sustaining yields across multiple seasons. By improving nutrient use efficiency and stabilizing soil carbon pools, they reduce the dependence on high fertilizer inputs. Over time, these practices enhance soil fertility and resilience, allowing cereals to maintain productivity even under fluctuating climatic conditions. The cumulative evidence suggests that conditioners should not be viewed solely as yield boosting inputs but as integral components of sustainable soil management systems that balance productivity with long term ecological stability.

Table 2. Reported effects of soil conditioners on cereal crop performance

Parameter	Reported Effect	Example Reference
Nitrogen use efficiency	+15–25% with organic residues	Blevins et al. (1983)
Soil organic carbon	Increased by 0.2–0.4 Mg ha^{-1} yr^{-1}	West & Post (2002)
Grain yield	+10–30% with manure + N fertilizer	Power et al. (1986)
Water holding capacity	Increased by 10–20% with biochar	Tisdall & Oades (1982)
Microbial biomass	20–40% higher in conservation systems	Balota et al. (1998)

INTEGRATION WITH FERTILIZER MANAGEMENT

The integration of soil conditioners with fertilizer management has been shown to significantly enhance nutrient use efficiency, soil fertility, and cereal productivity. Conditioners improve the physical and chemical environment of soils, thereby amplifying the effectiveness of both organic and inorganic fertilizers. When combined, these inputs create synergies that improve nutrient retention, minimize losses, and sustain soil health over the long term. The joint application of organic conditioners and inorganic fertilizers enhances nutrient cycling and soil fertility. Stevenson (1994) highlighted that organic matter improves cation exchange capacity (CEC), allowing mineral fertilizers such as ammonium and potassium to be retained in the root zone for longer periods. This interaction reduces leaching losses and provides cereals with a more sustained nutrient supply. Similarly, Doran et al. (1990) found that combining manure with nitrogen fertilizers improved soil microbial activity, which accelerated nutrient mineralization and increased crop yields. Conditioners can also enhance the performance of controlled release and slow-release fertilizers. Cooke and Hislop (1993) demonstrated that the use of soil conditioners alongside coated nitrogen fertilizers reduced volatilization losses and improved nitrogen uptake efficiency. This is particularly relevant in cereal systems where high nitrogen demand coincides with stages of rapid growth. By regulating nutrient release, conditioners ensure that fertilizers align more closely with plant requirements.Phosphate-solubilizing microorganisms (PSMs) enhance phosphorus availability to plants by converting organic and inorganic phosphorus compounds into bioavailable forms through enzymatic activities (Figure 2).

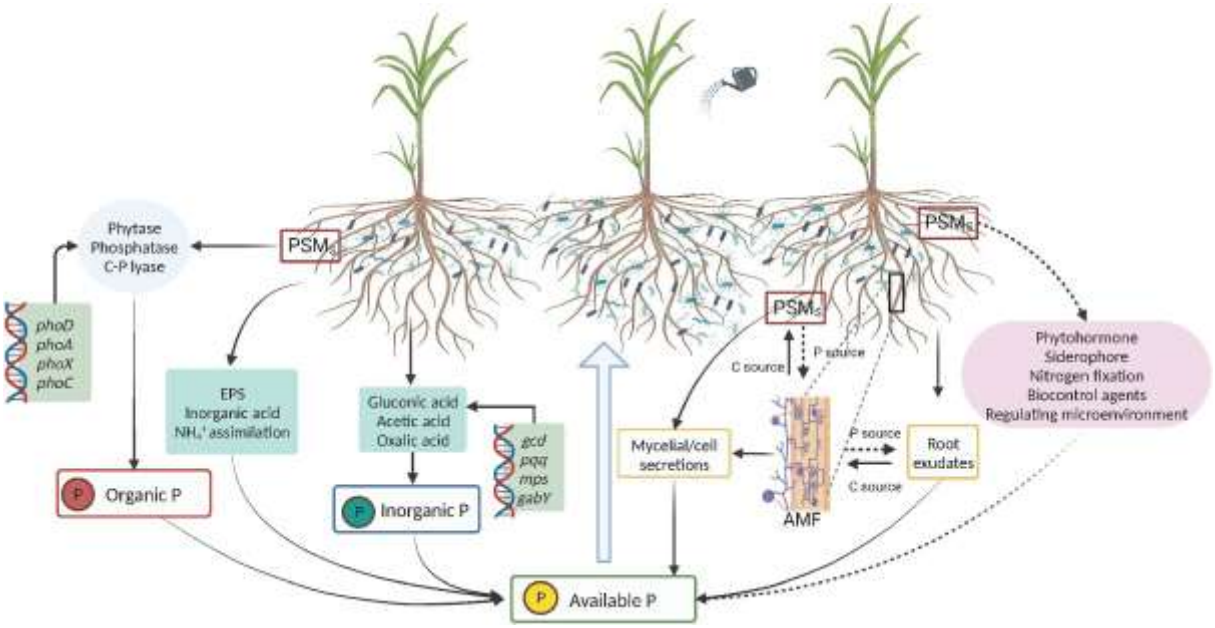

Figure 2. Role of phosphate-solubilizing microorganisms (PSMs) and root–microbe interactions in enhancing phosphorus availability and nutrient cycling in cereal-based systems.

The integration of conditioners with biofertilizers, such as nitrogen fixing bacteria and phosphate solubilizing microbes, creates additional synergies. Giller et al. (1997) reported that organic amendments provide energy substrates that support the establishment of microbial inoculants in soils. As a result, cereals benefit not only from enhanced microbial nitrogen fixation and phosphorus solubilization but also from improved soil structure and water relations.

The role of conditioners is increasingly important in the context of precision nutrient management. By improving soil heterogeneity and buffering capacity, conditioners allow variable rate fertilizer applications to be more effective. Cassman et al. (2002) emphasized that nutrient use efficiency in cereals can be maximized only when soil physical constraints are addressed simultaneously with fertilizer management, underscoring the value of conditioners as complementary inputs.

Long term trials have consistently shown that conditioner–fertilizer combinations lead to more resilient soils. Sanchez (1999) demonstrated that integrated fertility management systems, combining organic and inorganic inputs, improved yields while maintaining soil fertility under continuous cropping. These findings suggest that conditioners should be considered as essential components of integrated nutrient management strategies for sustaining cereal production under both intensive and resource limited farming systems.

CONCLUSION

Soil conditioners are indispensable tools for sustaining and improving cereal production systems worldwide. Their benefits extend well beyond short term soil amendments, as they enhance the physical structure of soils by improving aggregation, porosity, and water retention; correct chemical imbalances by adjusting pH, nutrient availability, and cation exchange capacity; and stimulate biological processes by increasing microbial biomass, enzymatic activity, and nutrient cycling. These combined effects provide cereals such as wheat, maize, barley, and rice with healthier root environments, more efficient nutrient uptake, and greater resilience under stress conditions.

The classification of conditioners into organic, inorganic, and synthetic groups highlights their complementary contributions. Organic amendments (e.g., compost, manure, biochar) support long term soil fertility and carbon sequestration, which directly benefits cereal yield stability. Inorganic inputs (e.g., gypsum, lime) resolve critical soil constraints that often limit cereal root development and nutrient acquisition. Synthetic conditioners provide targeted technological solutions for moisture conservation and erosion control, which are particularly relevant in cereal production areas prone to drought and soil degradation.

Integration of conditioners with fertilizer management further strengthens cereal productivity. When combined with mineral fertilizers, conditioners reduce nutrient losses, improve nitrogen and phosphorus use efficiency, and sustain yields across seasons. Their synergy with biofertilizers supports beneficial microbial populations in the rhizosphere, further enhancing nutrient cycling and stress tolerance. Long term trials confirm that these integrated systems maintain soil fertility while ensuring consistent grain yields under intensive cereal cropping.

From a broader perspective, soil conditioners contribute directly to the goals of climate smart agriculture, linking productivity with environmental stewardship. By sequestering soil carbon, enhancing nutrient use efficiency, and improving tolerance to drought and salinity, they provide cereal systems with the resilience needed to meet future food security challenges.

In conclusion, the judicious use and integration of soil conditioners are not optional but essential for the sustainable intensification of cereal production. Their role in maintaining soil health, supporting high yields, and buffering cereals against environmental stress makes them a cornerstone of future agricultural strategies worldwide.

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A THERMODYNAMIC STUDY OF A SOLAR ADSORPTION AIR-CONDITIONING SYSTEM

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ABSTRACT

This study indicates the simulation analysis of a solar powered solid adsorption machine using activated carbon AC35/methanol pair. For this purpose, a mathematical model based on a transient model of heat and mass transfer equations of the solar reactor (adsorber) has been developed. This model based on the hypothesis of homogeneity of pressure and non-homogeneity of temperature distribution inside the adsorber. The adsorbed methanol mass is given by the equation of DUBININ-ASTAKHOV to describe the phenomenon of adsorption. The discretization equations system is carried out using the method of finite differences with a fully implicit scheme and the resolution of the discretized system is carried out using the iterative method. This study enables us to calculate the profile of temperature, pressure adsorbed mass inside the adsorber according to time and during cloudy day, in order to study the effect of the adsorber dimensioning on the performance of the machine.

Key words: Adsorption, Solar refrigeration, Heat and mass transfer, Activated carbon - methanol.

INTRODUCTION

Ecological problems and energy crisis in a globalized world have motivated scientists to develop energy systems more sustainable, having as one of the possible alternative the use of solar energy as source for cooling systems. In the field of the sorption cooling, there are three kinds of system: liquid absorption, solid absorption (chemical reaction) and adsorption. The great advantage of adsorption systems over absorption ones is that they can operate without moving parts, having then lower costs of maintenance. Other advantages in comparison with the compression systems are: simple construction, environmentally benign and noiseless. A lot of applications for adsorption cooling systems have been viewed in both developed and developing countries such as: storage and conservation of vaccine, medical products, food conservation (vegetable, meat, fish, etc), refrigeration, air conditioning, chillers and ice production. Adsorption cooling systems and the enhancement of heat and mass transfer inside it is the most important factor to improve the performance of such systems. Thus, great efforts have been made to investigate the transfer occurring in the adsorption and desorption process.

The previous theoretical studies [1-5] were focused on the ideal cycles and did not take into account the heat and mass in the adsorbent bed, these models assumed uniform temperature and uniform pressure to optimize the performance of the system.

This paper presents the study of a tubular adsorber of solar adsorption cooling machine. The modelling and the analysis of the adsorber are the key point of such studies, because of the complex coupled heat and mass transfer phenomena that occur during the working cycle. The adsorber is heated by solar energy and contains a porous medium constituted of activated carbon AC-35 reacting

by adsorption with methanol. This is because of the activated carbon is chemically stable during the adsorption operation. The methanol can be evaporated at a
largely temperature below 0 C°, have a high enthalpy of evaporation, its working pressure is always lower than atmospheric pressure.
The simulation technique takes into account the variation of ambient temperature and solar intensity along a simulated day, which is corresponds to a total daily insolation of 26,12 MJ/m^2 and an average ambient temperature of 27.7 °C [6]. The obtained solution of system's equations, allows to know the daily thermal behaviour of the tubular adsorber (temperature and pressure).
The model is used to study the influence of the adsorber dimensioning (number of tubes inside the adsorber, and the total solar collector type surface on the system's behavior (temperature and pressure).

MATHEMATICAL MODEL

The solar refrigeration system presented in this study consists of the following main elements:
a solar collector coupled to the multi-tubular adsorber filled with the porous medium; a condenser, an evaporator
and a receiver which is used to store the fluid refrigerant coming from the condenser [6]. The adsorptive reactor coupled to the solar collector which has 1 m^2 surface area, consists of transparent single glass cover, lateral and rear insulation in order to limit the thermal losses, and 8 connected parallel copper tubes, where the total sum of external diameters tubes is equal to 1 m. The porous medium consists of a fixed cylindrical bed of activated carbon grains reacting by adsorption with methanol. It is packed in the annular space between two coaxial tubes. Consequently, the size of adsorbent bed in each tube is defined by (R_2-R_1) (Figure .1). All details of the different constitutions of the adsorber are well presented in our previous studies [7-8-9].

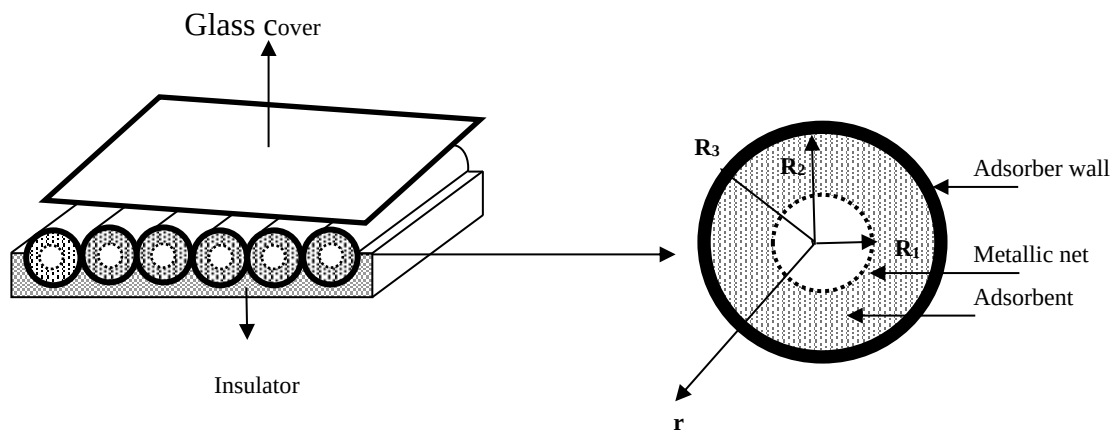

FIGURE 1. Scheme of the adsorptive reactor coupled to the solar collector [6-7]

Adsorption id the important phenomena used in systems' of adsorption refrigeration. It constitutes a solid sorption process by which the binding forces between fluid molecules (adsorbate) and the solid medium (adsorbent) derive from an electrostatic origin or from dispersion-repulsion forces. It is an exothermic and reversible process as a result of the gas-liquid phase change without modification of the solid it self. The adsorbed mass is obtained from the state equation of the bivariant solid-vapour equilibrium using Dubinin- Astakhov model, which is expressed as:

$$m = w_0 \, \rho_l(T) \exp\left[-D\left(T \ln \frac{P_s(T)}{P} \right)^n \right] \tag{1}$$

The energy liberated during adsorption is called isosteric heat of adsorption and its intensity depends on the nature of the adsorbent/adsorbate pair, the amount adsorbed mass and the latent heat L, it is given as:

$$q_{st} = L(T) + RT\ln\left(\frac{P_s(T)}{P}\right) + \left[\frac{\alpha\ RT}{n\ D}\right]\left[T\ln\frac{p_s}{P}\right]^{(1-n)} \tag{2}$$

In order to analyse the evolution of the heat and mass transfer process in the adsorbent bed, this is heart of the system, and to identify the parameters that influence the system performance, a mathematical model has been developed; in order to simplify it. This model is well discussed in our previous work by chekirou et al. [7]. All the basic parameters used in the model are also cited in reference [7].

The energy equation used in this study is given by:

$$\rho_2\left(cp_2 + m\ cp_l + \frac{b\,q_{st}^2}{RT^2}\right)\frac{\partial T}{\partial t} = k\left(\frac{\partial T^2}{\partial r^2} + \frac{1}{r}\frac{\partial T}{\partial r}\right) + \rho_2\ q_{st}\,b\frac{d\ ln\ p}{d\ t} \tag{3}$$

Where: $b = n\,D\,m\,T^n\left(\ln\frac{p_s(T)}{p}\right)^{n-1}$

The heat losses in the adsorber is represented by the top heat losses coefficient U_{top}, which is given by an ampiric relation, for temperatures ranging between 0°and 200° C with an error lower or equal to $\pm\ 0.3W/m^2K$:

$$U_{top} = \left[\frac{N}{\frac{c}{T_w}\left(\frac{T_w - T_{amb}}{N+f}\right)^e} + \frac{1}{h_V}\right]^{-1} + \frac{\sigma\left(T_w + T_{amb}\right)\left(T^2 + T_{amb}^2\right)}{\left(\varepsilon_p + 0.00591\,N\ h_v\right)^{-1} + \frac{2\ N + f - 1 + 0.133\,\varepsilon_p}{\varepsilon_g} - N} \tag{4}$$

Where

$$f = \left(1 + 0.089\ h_v - 0.1166\ h_v\ \varepsilon_p\right)\left(1 + 0.07866\ N\right)$$

$$e = 0.43\left(1 - 100/T_w\right)$$

$$h_v = 2.8 + 3\,V$$

$$c = 520\left(1 - 0.000051\ \beta^2\right)\ \text{ for }\ 0° \prec \beta \prec 70°$$

Where, β is the collector inclination, it is assumed equal to 37.3° the latitude of Constantine, because it permits to receive the maximum annual energy.

The top heat losses coefficient is used in the limit external boundary condition, to find the adsorber wall temperature.

About the boundary and initial conditions, we consider at the beginning of the cycle, the distribution of temperature of the adsorbent bed and adsorber wall can be considered as uniform and equal to the ambient temperature at the sunrise and the pressure equal to the evaporator pressure. As the geometry of the adsorber is cylindrical, therefore, the boundary condition on the cylinder axis is a symmetry condition.

The solar intensity and ambient temperature are modelled in this study as variants throughout the day (hypothetical clear day). These weather conditions are those of the Constantine region, which is situated in north-east Algeria, at 6° ,37' East (longitude) and 37°,17' North (latitude), with an average altitude of about 625 m. This simulated day corresponds to a total daily insolation of 26.12 MJ m^{-2} and an average ambient temperature of 27.7°C.

The given model requires the simultaneous solution of a set of partial differential and algebraic equations. These equations are discretized by using a fully implicit finite differences method in order to obtain four unknowns: The adsorbent bed temperature, the pressure and the adsorbed mass of methanol during a full cycle.

RESULTS AND DISCUSSION

A computer program was done on the basis of the numerical model, which is well discussed in our previous work by Chekirou et al. [7]. Al the basic parameters used in the model are also cited in reference [7].

The estimates of the saturated vapour pressure, the adsorbed phase density and the latent heat of methanol are given by R. C Weast [10], A. Bejan and A. D. Kraus [11].

The average temperature inside the adsorber during one day is presented in figure 2.

The temperature is considered equal to the ambient temperature in the morning before sunrise and under a low pressure of methanol (equal to the evaporation pressure $P_e = 28.72\ hPa$). When the adsorber is heated by solar radiation, the adsorbent bed temperature increases rapidly with time. The heating continues until the condensation pressure is reached (equals to $P_c = 216.76\ hPa$). The reached temperature is known as limit temperature of desorption. The heating continues also as much as the solar radiation is sufficient to increase the temperature of the adsorbent bed and until the maximum regenerating temperature is reached.

Solar flux decreases and the cooling starts down after it reaches the maximum regenerating temperature. The temperature decreases until the pressure becomes equal to that which reign in the evaporator ($P_e = 28.72\ hPa$). The reached temperature is known as limit temperature of adsorption. Cooling continues until the low values of temperatures. The mean of these values corresponds to the initial temperature of a new cycle.

For the effect of the total surface area of the adsorber on the distribution of temperature, from figure 2 we notice that, with the increase in total area of the adsorber, the temperature of the adsorber decreases during the heating phases, and increases during the cooling phases. This can be justified by the fact that, the increase of total surface area induces a reduction in regeneration temperature as it is shown in figure 3. A reduction in regenerating temperature induced a reduction in the performance of the machine as it is found in our previous work [7].

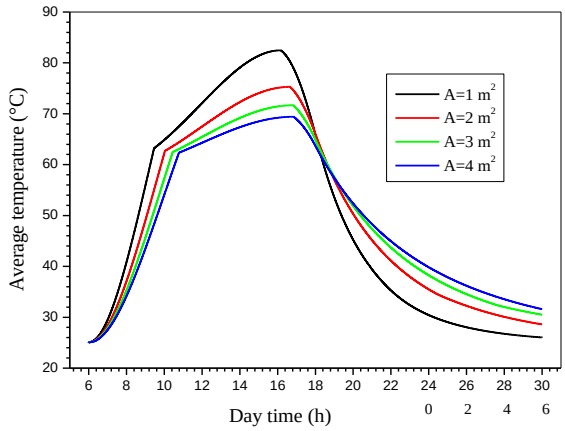

FIGURE 2. Effect of total surface of the reactor on the average temperature inside the adsorber during one cycle

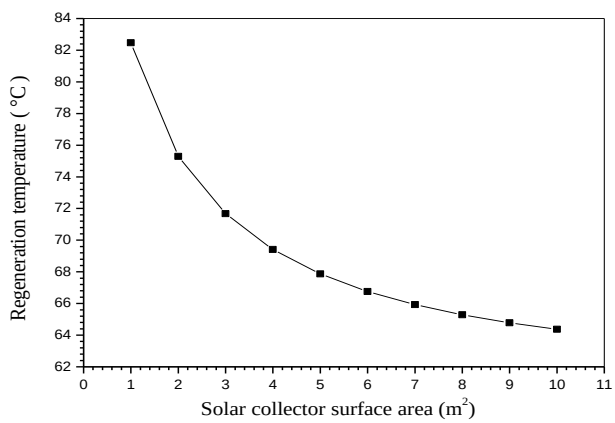

FIGURE 3. Effect of solar collector surface area on regeneration temperature

The pressure behaviour inside the adsorbent bed during one cycle is presented in figure 4 The pressure increases from the initial evaporation pressure $P_e = 28.72$ mbar (equal to the saturated vapour pressure at the evaporation temperature) up to the maximum value corresponding to the condensation pressure $P_c = 216.76$ mbar (equal to

the saturated vapour pressure at the condensation temperature). During the isobaric heating phase, the pressure remains constant and equal to the condensation pressure until the adsorbent bed reaches the maximum regeneration temperature. Then, the pressure starts to diminish down to the initial evaporation pressure and remains constant during the isobaric cooling phase.

About the effect total surface of the reactor on the pressure inside the adsorber during one cycle, we notice the increase in surfce induced a decrese of pressure during the heating phase, and an increase during the heating phase. No effect of total surface of the reactor on pressure during the isobaric phases.

Effect of number of tubes on the average temperature inside the adsorber during one cycle, is presented in figure5. It is clear, that the increase of number of tubes induced an increase in temperature inside the adsorber during the two heating phases. However, during the cooling phases, the increase of number of tubes induced a decrease in temperature inside the adsorber.

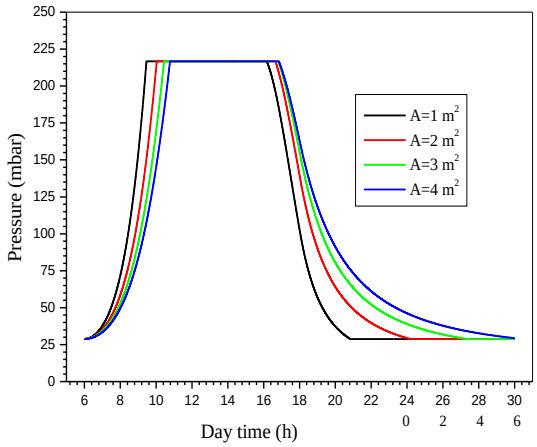

FIGURE 4. Effect of total surface of the reactor on the pressure inside the adsorber during one cycle

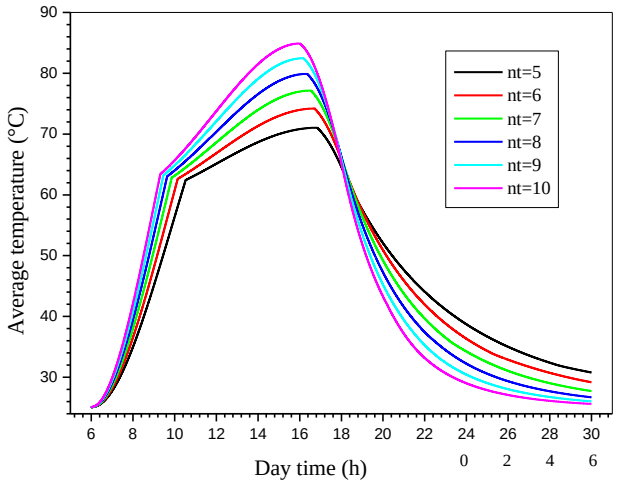

FIGURE 5. Effect of number of tubes on the average temperature inside the adsorber during one cycle

The increase of tubes on the pressure inside the adsorber during the cycle induced an increase of pressure during the isoteric heating phase. However, during the isosteric cooling phase, the increase of tubes induced a decrease of pressure. No effect of number of tubes on pressure during the isobaric phases.

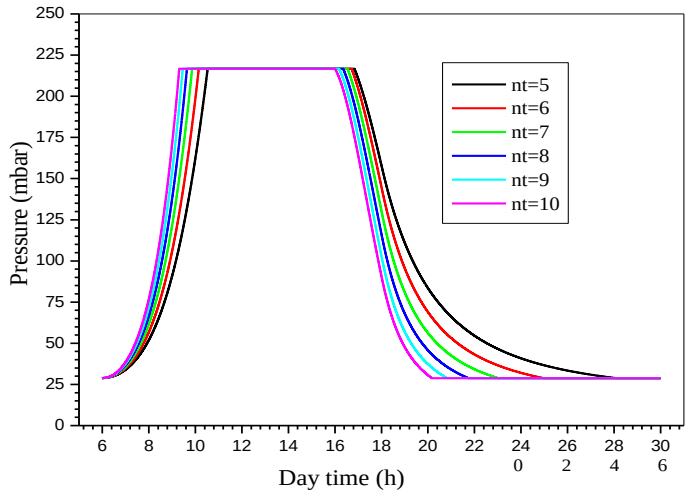

FIGURE 6. Effect of number of tubes on the pressure inside the adsorber during one cycle

CONCLUSIONS

A numerical model has been developed in this study in order to show the effect of dimension parameter (number of tubes, total surface area of the solar reactor) on the distribution of temperature and pressure inside the adsorber. The activated carbon AC-35/methanol is used as a working pair. The ambient and solar radiation time variations are taken into account. A uniform pressure and non uniform temperature model is proposed, in order to describe the behavior of heat and mass transfer in the adsorbent bed, using a meteorological data of Constantine for a hypothetical clear day.

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DETERMINATION OF ELEMENTS IN HONEY, HONEYBEE, SUNFLOWER AND BEE DRINKING WATER SAMPLES COLLECTED FROM THE ENEZ DISTRICT, EDIRNE (TURKEY)

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ABSTRACT

Honey is a natural carbohydrate produced by honeybees. Honeybees are foragers that produce honey from pollen and nectar. Like all living organisms, honeybees require nutrients, including minerals and water, in order to reproduce, grow and develop. The aim of this study was to determine the elemental content of honeybee, sunflower honey, sunflower and bee drinking water samples, and to identify any potential correlations between element concentrations in the samples. This study was conducted in Enez, Edirne, Turkey. Samples of bee drinking water (n = 10), sunflower (n = 10), sunflower honey (n = 10) and dead honeybee (n = 10) were collected from apiaries in the beekeeping area in 2023. In this study, the highest element in honey samples was determined as potassium and the lowest element was determined as cobalt. In the study, the highest element in honeybee samples was determined as potassium, and the lowest element was determined as selenium. In the study, the highest element in sunflower samples was determined as potassium, and the lowest element was determined as molybdenum. In the study, the highest element in bee drinking water samples was sodium, while cobalt content was not detected. In this study, a significant positive correlation was determined between water and honey for molybdenum ($p<0.01$), and a significant negative correlation between water and honeybees for zinc ($p<0.01$), as well as a significant positive correlation for molybdenum ($p<0.01$). No significant correlation was identified between sunflower-honeybee and sunflower-honey. This study demonstrated the elemental content of honeybee, honey, sunflower and bee drinking water, as well as the correlations between the samples in terms of elemental content.

Keywords: Bee drinking water, Honey, Honeybee, Minerals, Trace elements.

INTRODUCTION

Honey is a natural carbohydrate substance produced by honeybees from the honey essence and nectar of various plants and contains many macro elements and trace elements that are carried from the plant nectar (Bilandžić et al. 2011; Silici et al. 2016). Botanical factors have a great influence on the trace element content of honey (Bogdanov et al. 2007). Honeybees are long-range foragers that produce honey from the pollen and nectar they collect from plants and, they are managed in colonies consisting of 10,000 to 80,000 individuals (Cunningham et al. 2022). Like other living organisms, honeybees require carbohydrates, protein, fat, vitamins, minerals and water in their diets for reproduction, growth and development. Their nutrition is completely dependent on the flora (Özbakır and Alişiroğlu 2019). Honeybees collect nectar, pollen and water to meet these requirements. Honeybee try to meet their water needs by searching for it in almost every source close to their colonies. Water can provide the necessary minerals (Ellis et al. 2010). It is reported that the

deficiencies of some elements in pollen are the nutrients that limit the growth and development of honeybees. In addition, it is stated that the lack of phosphorus (P) in sunflower pollen can negatively affect the growth and development of bees (Filipiak et al. 2017). Several studies have been reported on the element content of honey and honeybees (Silici et al. 2016; Bayram et al. 2020; Hungerford et al. 2020; Murashova et al. 2020).

The aim of this study was to determine the elemental composition of honeybee, honey, sunflower and bee drinking water samples and possible correlations between elemental concentrations in the samples in Enez, Edirne, Turkey.

MATERIALS AND METHODS

All samples were collected from the apiaries located area of intensive beekeeping in 2023, Enez, Edirne, Turkey. These apiaries were located around the agricultural area. Bee drinking water (n=10), sunflower (n=10), sunflower honey (n=10) and dead honeybee samples (n=10) were collected from the area. Recently deceased honeybees (for each sample, 50 to 80 forager bees) were collected from at a maximum distance of 1 m from the hive, respecting the colony, without opening or disturbing the hive. All honey samples (100 g) were placed in sterile plastic bottles and transported to the laboratory. Honey samples were stored at 4-8 °C until analysis. Honeybee and sunflower samples were dried and stored at -20 °C until it was analysis. Water samples were collected from water sources where bees consume water in the area. Water samples were acidified and stored at 4°C until chemical analysis.

Sample Preparation and Elemental Analysis

The concentrations of elements in all samples were analyzed using an Agilent Technologies 7700 Inductively Coupled Plasma-Mass Spectrometer (ICP-MS). These measurements were conducted by the Technology Research and Development Application and Research Center (TUTAGEM) at Trakya University. TUTAGEM used the EPA 200.8 method (EPA 1994) for the analysis of water samples and the method adapted from Lee et al. (2022) for the analysis of honeybee, honey and sunflower samples. The methods used by TUTAGEM for sample analysis are detailed below. The calibration of the device was done by the Research Center using certified reference material.

Blank sample preparation: 2 mL of 30% H_2O_2 (MERCK) and 8 mL of 65% HNO_3 (MERCK suprapur) were taken into the microwave vial and processed in the microwave combustion system (Mars 6, CEM, USA) at 200°C for 45 minutes. Then, 9 mL of ultrapure water was added to the tube to be analyzed and 1 mL of the acid solution obtained from the digestion process was taken.

Preparation of Honeybee, Honey and Sunflower Samples: The following method was used to prepare honey, honeybee and flower samples, with each sample being prepared and analysed separately. 0.5 g samples were weighed into microwave vials. 2 mL of 30% H_2O_2 and 8 mL of 65% HNO_3 (suprapur) were added to them and processed in a microwave combustion system (Mars 6, CEM, USA) at 200°C for 45 minutes. Thus, organic compounds in the sample were removed and inorganic compounds were allowed to pass into the acid solution. Then, 9 mL of ultrapure water was added to the tube to be analyzed and 1 mL of the acid solution obtained from the combustion process was taken.

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Preparation of Water Samples: Water samples were filtered using 0.45 micron filters. Then 10 ml of filtered water samples were taken into 15 ml tubes. 200 µL of 1+1 HNO_3/ultrapure water mixture was added to acidify and made ready for analysis.

Data analysis

Statistical calculations were performed using SPSS (ver. 15.0, IBM, USA). Data were presented as mean and standard deviation. Pearson's correlation analysis was conducted to determine the association between the variables.

RESULTS

The concentrations of elements determined in the samples were shown in Table 1 and Table 2. Potassium (K) is the most abundant macroelement (mean concentration 0.96 g/kg) in honey samples. In this study, the lowest level of macroelement in honey was magnesium (Mg) (mean concentration 0.02 g/kg). In the present study, K was the most abundant mineral (mean concentration 7.63 g/kg) in honeybee samples. The most abundant element in the sunflower was K (mean concentration 23.17 g/kg), while the least abundant element was molybdenum (Mo) (mean concentration 0.07 mg/kg). The highest macroelement in the water was sodium (Na) (mean concentration 67.22 mg/L), the lowest macroelement was P (mean concentration 0.05 mg/L). No cobalt (Co) was detected in the water. The correlation results of the study are shown in Table 3. In this study, a significant positive correlation was determined between water and honey for Mo ($p<0.01$), a significant negative correlation between water and honeybees for zinc (Zn) ($p<0.01$), and a significant positive correlation for Mo ($p<0.01$). No significant correlation was found between sunflower-honeybees and sunflower-honey.

Table 1. Element Content of Honey, Honeybee, Sunflower (Mean±Standard Deviation)

Elements	Honeybee (Dry weight)	Honey	Sunflower (Dry weight)
Ca	0.77±0.08	0.08±0.01	2.47±0.07
Mg	0.94±0.09	0.02±0.00	1.09±0.04
K	7.63±0.68	0.96±0.05	23.17±0.77
Na	0.77±0.06	0.09±0.02	0.29±0.03
P	6.66±0.70	0.18±0.04	1.85±0.07
Cr	4.99±1.29	0.07±0.05	1.45±0.12
Co	0.26±0.03	0.02±0.00	0.16±0.01
Cu	24.92±2.68	0.86±0.06	17.96±1.55
Zn	101.53±11.26	8.70±15.70	22.37±0.97
Se	0.24±0.10	0.07±0.05	0.13±0.05
Mn	106.75±18.87	0.29±0.03	10.65±0.54
Mo	0.61±0.23	0.04±0.01	0.07±0.01

Ca, Mg, K, Na, P are expressed as g/kg, others as mg/kg.

Table 2. Element Content of Bee Drinking Water (Mean±Standard Deviation)

Elements	Water
Ca	54.38±16.31
Mg	8.51±0.50
K	1.88±0.38
Na	67.22±2.93
P	0.05±0.03[a]
Cr	8.16±0.40
Co	ND
Cu	4.70±2.26[b]
Zn	1.24±0.87
Se	5.62±2.96
Mn	0.80±0.42
Mo	7.13±5.97

Ca, Mg, K, Na, P are expressed as mg/L, others as µg/L.
[a]P was not detected in a 1 sample.
[b]Cu was not detected in a total of 7 samples.
ND Not detected

Table 3. Correlation coefficients for element concentrations in honey, honeybee, water and sunflower samples.

Elements	Water/Honey	Sunflower/Honey	Water/Honeybee	Sunflower/Honeybee
Ca	0,019	-0,254	0,039	0,228
Mg	-0,115	-0,046	-0,101	-0,008
K	0,238	0,486	0,071	0,387
Na	-0,002	-0,378	0,107	0,151
P	-0,538	-0,403	0,324	0,039
Cr	-0,458	-0,509	0,134	0,279
Co	-	0,473	-	0,483
Cu	0,942	0,323	0,448	0,568
Zn	0,376	-0,311	-,794**	0,271
Se	-0,254	0,277	-0,565	0,482
Mn	-0,605	0,173	-0,546	0,059
Mo	,805**	0,048	,945**	0,166

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed).

DISCUSSION

Consistent with the results of this study, K was observed to be the most abundant element in honey in studies conducted in various countries such as Turkey (Tutun et al. 2019), Bulgaria (Atanassova et al. 2016) and Serbia (Ćirić et al. 2021). The concentration of calcium (Ca) found in this study was lower than the values obtained for honey samples collected from Iran (Arabameri et al. 2024) but higher than the levels obtained in Turkey (Kılıç Altun et al. 2017; Tutun et al. 2019). The mean Na of honey determined in this study was lower than the value (99.7 mg/kg) reported by Hungerford et al. (2020), while the determined result was higher than the results reported in Iran (Arabameri et al. 2024), Bulgaria (Atanassova et al. 2016) and Serbia (Ćirić et al. 2021). The P value obtained in our study was higher than that determined in honey by Atanassova et al. (2016) and Hungerford et al. (2020). The Mg level in our study was found to be lower than the results reported by Altunatmaz et al. (2019) and Tutun et al. (2019), while it was consistent with the result reported by Perna et al. (2021). The mean copper (Cu) concentration determined in the present study was higher than 243 µg/kg determined by Aghamirlou et al. (2015) in Iran, 0.35 mg/kg found by Murashova et al. (2020) in Ryazan region from Russia, 68.5 ppb reported by Kılıç Altun et al. (2017) and 0.38 mg/kg determined by Tutun et al. (2019) in Turkey. But Cu concentrations in this study were lower than those reported by Altunatmaz et al. (2019) and Scivicco et al. (2022) in honey samples from Turkey and Italy (11.03 µg/g and 1049 µg/kg, respectively). Aghamirlou et al. (2015) reported that the differences in the Cu content of honey were due to the use of different fertilizers or diversity of crop management practices in the region studied. This may be the reason why the honey Cu content determined in this study differs from the results of other studies. The mean concentration of chromium (Cr) was lower than those determined in Iran (Aghamirlou et al. 2015) and Turkey (Altunatmaz et al. 2019). The mean Cr level determined in the present study was higher than 17.57 µg/kg reported by Perna et al. (2021) in Italy. The mean Zn concentration in this study was higher than that found in Turkey, Australia, Russia and Italy (Tutun et al. 2019; Hungerford et al. 2020; Murashova et al. 2020; Perna et al. 2021), but lower than that reported by Altunatmaz et al. (2019). The mean selenium (Se) concentration in this study was lower than that reported by Altunatmaz et al. (2019) (2.183 µg/g), but higher than that reported by Hungerford et al. (2020) (0.0052 mg/kg). Similar result were also reported in previous study in Turkey (Kılıç Altun et al. 2017). Atanassova et al. (2016) determined the Co content in honey collected from two different areas to be 0.01 and 0.02 mg/kg, respectively. This result is consistent with the result of the present study. The mean Co level was higher than that determined in Australia, Italy and Kosovo (Hungerford et al. 2020; Perna et al. 2021; Kastrati et al. 2021). Kanbur et al. (2021) determined that the mean manganese (Mn) level in chestnut honey was 19.49 mg/kg. Perna et al. (2021) found the Mn content of honey to be 1603.06 µg/kg and Scivicco et al. (2022) found the Mn level of honey to be 881 µg/kg. Altunatmaz et al. (2019) reported an average Mn level of 1.985 µg/g in honey samples collected from different regions of Turkey. While the honey Mn concentrations reported in the above mentioned studies were higher than those found in the present study, the 45.6 ppb reported by Kılıç Altun et al. (2017) was lower, and the 0.25 mg/kg reported by Tutun et al. (2019) was comparable to the levels observed in this study. In this study, the mean Mo content of honey was higher than those from Australia (0.0115 mg/kg) (Hungerford et al. 2020) and Italy (4.71 µg/kg) (Perna et al. 2021), but lower than the 0.078 ppm reported by Perna et al. (2012).

The Cr content of honeybees determined in this study was higher than the results of the study conducted in Nigeria by Adeoye et al. (2021). Murashova et al. (2020) determined Zn and Cu levels in honeybee samples collected in Russia. The levels of Zn and Cu in this study were higher than those reported in bee samples from Russia. In another study, Goretti et al. (2020) reported mean concentrations in honeybee samples from Umbria (central Italy) as follows: Zn 132.47 mg/kg, Cr 0.27 mg/kg, Cu 14.39 mg/kg and Mn 98.98 mg/kg. In comparison with the above mentioned study, the Zn value obtained in the present study was seen to be lower, while the Mn, Cr and Cu levels were higher.

Zarić et al. (2016) analyzed metals in honeybee samples. In a rural area with agricultural and forested areas, similar to the sampling site in this study, the levels of Cr, Co, Cu, Mn and Na were found to be lower than those observed in this study, while the Zn concentration was comparable to presented results.

Tokatlı (2019) collected drinking water samples from 30 stations in the Ergene River Basin during the summer season. In that study, the mean contents of Mg (12735 ppb), Na (73417.85 ppb), Co (0.12 ppb), Zn (18.73 ppb), Mn (5.13 ppb), Se (10.07 ppb), Mo (19.37 ppb) were higher than those observed in the present study, whereas the mean contents of Ca (24479 ppb), K (1335 ppb), Cr (3.47 ppb) and Cu (1.11 ppb) were lower. The levels of Zn (18.75 mg/kg) and Cu (4.33 mg/kg) in plant samples reported by Murashova et al. (2020) were lower than the results of this study.

CONCLUSION

In this study, honeybee, honey, bee drinking water and sunflower samples were analyzed by ICP-MS. In the study, the highest element concentration was determined to be potassium in honeybee, honey, and sunflower samples, and sodium in water samples. The lowest element concentrations were found to be selenium in honeybee samples, cobalt in honey and molybdenum in sunflower samples. Cobalt was not detected in water samples. In this study, a significant positive correlation was found between water and honey for molybdenum, a significant negative correlation between water and honeybee for zinc, and a significant positive correlation for molybdenum. No significant correlation was found between sunflower-honeybee and sunflower-honey.

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GENERATIVE ADVERSARIAL NETWORKS AND THEIR APPLICATION AREAS IN SMART AGRICULTURE

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ABSTRACT

The rapid advancement of digital transformation in agricultural technologies in recent years has led to a growing emphasis on artificial intelligence (AI)-based approaches. This study systematically examines the applications of Generative Adversarial Network (GAN) models in smart agriculture applications. The potential offered by GAN-based AI solutions in fundamental agricultural activities such as plant breeding, animal husbandry, and soil characterization is highlighted. GAN applications are detailed through current studies in the literature on topics such as plant health detection, weed control, phenotyping, stress tolerance assessment, harvest timing, and product quality analysis, underscoring their significance in enhancing agricultural productivity and promoting sustainable practices. In animal husbandry, GANs are particularly notable for their success in synthetic data generation and classification in areas such as animal health monitoring, behavioral analysis, and image-based growth monitoring. In soil studies, GAN-based models have been shown to produce highly accurate synthesized images for the development of maps of soil structure, moisture content, and nutrient distribution, making significant contributions to precision agriculture applications. Furthermore, strengthening data infrastructure, integrating technological solutions into agricultural systems, increasing interdisciplinary collaborations, creating open-access, high-quality datasets, and developing application-oriented standards will enable more effective use of GAN technologies in agricultural research and applications. Consequently, GANs are positioned to play a pivotal role in the digital transformation of agriculture, and leveraging their capabilities in alignment with sustainable agriculture goals is imperative.

Keywords: Machine learning, Generative adversarial networks, Smart agriculture

INTRODUCTION

Due to adverse developments in global factors such as population growth, climate change, and environmental pollution, there is growing concern both about the preservation of biodiversity and the need to ensure productivity and sustainability in agricultural production. In this context, the demand for sustainable and intelligent solutions is steadily increasing. It is anticipated that the effective use of advanced technologies such as artificial intelligence (AI) and machine learning (ML) in the agricultural sector will accelerate optimization processes aimed at addressing these challenges (Bao and Xie, 2021).

In recent years, artificial intelligence-supported computer vision systems, Internet of Things (IoT-edge) technologies, robotics, smart automation solutions, and microclimate controllers, systems

based on machine learning, have gained increasing importance. These systems are effectively utilized not only in research areas such as early detection of plant and animal diseases, yield prediction, and phenotyping, but also in the development of smart greenhouses, barns, and irrigation systems (Sengupta et al., 2021; Olasehinde et al., 2023; Mohammed et al., 2024). However, agricultural data can be affected by seasonal, geographical, and biological factors, which poses various challenges in data prediction processes. Additionally, the lack of sufficient labeled data in visual-based applications such as disease detection, land analysis, and crop classification can limit the performance of machine learning models (Yang et al., 2021). In this context, Generative Adversarial Networks (GANs), an unsupervised learning-based subfield of machine learning have recently come to the forefront, as they enable the transformation of datasets collected under varying conditions into balanced datasets. GANs have attracted significant attention in areas such as image synthesis, data augmentation, and predictive modeling, and have increasingly been adopted in smart agriculture applications (Akkem et al., 2024; Awate & Nagne, 2025).

The aim of this study is to examine the conceptual foundations of Generative Adversarial Network (GAN) models and to highlight their role and significance within smart agriculture applications. Accordingly, this review presents examples and explanations of GAN implementations across various domains, including plant and animal production, soil morphology and structure, as well as land use prediction. Furthermore, the review provides a comprehensive evaluation of the potential challenges associated with the use of these technologies and outlines future directions for research and development.

GENERATIVE ADVERSARIAL NETWORKS (GANs): BASIC CONCEPTS

GANs, as a popular machine learning model among artificial neural networks, consist of two complementary neural networks: (i) the generator and (ii) the discriminator. The generator is responsible for producing synthetic data that closely resemble real data, while the discriminator evaluates whether the generated data are real or artificial. Through the adversarial interaction between these two networks, GANs establish a self-supervising model architecture (Shah, 2022).

Owing to their ability to generate synthetic data, GANs offer a significant advantage in agricultural studies where datasets are often limited or imbalanced. In the agricultural sector, various GAN applications have been developed to address specific problems or focus on particular data types. These applications are briefly described below:

a) *Deep Convolutional Generative Adversarial Networks (DCGAN)*: This is an enhanced version of the traditional GAN architecture, incorporating deep convolutional neural networks (CNNs). DCGANs are effectively used for generating more stable and realistic outputs from visual data (Guo et al., 2021; Espejo-Garcia et al., 2021).
b) *Semi-Supervised GAN*: This model is applied in situations where only a portion of the training data is labeled. It leverages the limited labeled data to extract meaningful representations from larger unlabeled datasets (Kerdegari et al., 2019).
c) *Cycle-Consistent GAN (CycleGAN)*: This architecture enables transformation between two different datasets (e.g., diseased vs. healthy) without requiring one-to-one pairing of samples for training (Barth et al., 2020).
d) *Boundary Equilibrium Generative Adversarial Network (BEGAN)*: BEGAN is a balance-oriented GAN variant that evaluates the quality of generated samples based on reconstruction errors (Luo et al., 2020).
e) *Conditional Generative Adversarial Network (CGAN)*: In this model, data generation is conditioned on specific inputs such as labels, temperature, or soil type. CGANs allow for customized data generation based on crop type, region, or climatic conditions, and are used in

applications such as plant growth simulation and visual annotation (Olatunji et al., 2020; Singh et al., 2021).

f) *Self-Attention Generative Adversarial Network (SAGAN)*: Thanks to its attention mechanism (self-attention), it is used to model visual details and relationships between distant regions. It enables high-quality and detailed image synthesis during the generation process (Li and Tang, 2020).

g) *Conditional Tabular GAN (CTGAN)*: This GAN model was developed to generate artificial tabular data and is particularly used in structural data synthesis (Ahmed et al., 2021).

h) *Wasserstein GAN (WacGAN)*: Using Wasserstein distance as the loss function, this model aims to mitigate the "mode collapse" problem. Thanks to these features, it is effectively used in the prediction of complex datasets such as weather patterns and soil samples (Madsen et al., 2019).

i) *Customized GAN*: These GAN models are designed to develop functionality for specific agricultural problems by combining or adapting existing GAN types (Chou et al., 2019).

j) *Transformer-based GAN (TransGAN)*: GAN architecture uses transformer blocks instead of convolutional neural networks (CNNs). It is particularly successful in producing high-resolution and detailed images thanks to its attention mechanism (Wang & Xiao, 2021).

USE OF GANs IN SMART AGRICULTURE APPLICATIONS

GANs are primarily used in smart agriculture applications in crop and animal husbandry, but also in various smart agriculture areas such as soil morphology, structure, and land use estimation. Therefore, examples of smart agriculture applications are presented under three subheadings: (i) GANs in plant breeding, (ii) GANs in animal husbandry, where livestock and aquaculture are considered together, and (iii) GANs in soil properties and agricultural area change estimation.

GANs IN PLANT CULTIVATION

To date, applications of GANs in plant cultivation have encompassed research areas such as plant phenotyping, detection of plant health in terms of pathogens and pests, weed identification, determination of product quality traits, post-harvest quality assessment, as well as monitoring and detection in biotechnological studies.

The investigation of the effects of various pests and pathogens on plant traits constitutes a significant area of research in plant cultivation. These agents, particularly those adversely affecting photosynthesis in leaves, pose a serious threat to plant development and productivity. Such impacts can slow down the natural growth processes of plants, reduce crop yields, and consequently lead to economic losses. In this context, early detection of pests and pathogens is of paramount importance (Hu et al., 2019; Maqsood et al., 2021). In a study employing GANs, synthetic images were generated using a Conditional Generative Adversarial Network (C-GAN) for the detection of diseases on tomato leaves. These synthetic data were then used to train a DenseNet121 model via transfer learning. The model was tested on the PlantVillage dataset and achieved accuracy rates of 99.51%, 98.65%, and 97.11% for 5, 7, and 10 disease classes, respectively. This work demonstrated superiority over existing methods by improving classification accuracy through GAN-based synthetic data generation (Abbas et al., 2021). Similarly, weeds compete with crops for nutrients, negatively affecting yield. Research utilizing GANs for weed control has been reported to offer effective solutions in this field (Khan et al., 2021; Espejo-Garcia et al., 2021). For instance, in a study by Li et al. (2024), a GAN model enhanced with a channel attention mechanism (CA-GAN) was employed to generate realistic synthetic weed images. These images were used to train deep learning-based weed detection models. The model

achieved an accuracy rate of 93.46% on the ISAS dataset, demonstrating the potential of GAN-based data augmentation to improve model performance under limited data conditions.

Abiotic stress factors such as temperature, drought, and salinity also exert direct and adverse effects on plant yield. Developing stress-resilient varieties requires appropriate species and phenotype selection alongside effective breeding strategies. In this context, a study by Kaushik (2025), GANs were used as a part of multilayered artificial intelligence approaches. GANs were utilized to propose novel gene combinations for high-altitude extremophile plants thriving under extreme environmental conditions. This AI-based platform integrated multi-omics data and environmental simulations to successfully predict plant growth and stress tolerance. It was reported that the GAN-suggested gene combinations improved stress tolerance by up to 15%. This study demonstrates the innovative and promising potential of GANs as tools for developing abiotic stress-resistant plant varieties.

Similarly, successful phenotyping applications have been found in smart greenhouse systems integrated with GANs (Giuffrida et al. 2017; Zhu et al. 2018; 2020; Zahid 2025). In this context, in a study conducted by Liu et al. (2023), ripeness stages and damage levels of apple fruits were classified using data augmentation with GAN. In this study, a lightweight CNN-integrated GAN architecture was developed, generating realistic synthetic apple images in datasets with no class imbalance, thereby improving the recognition accuracy of the model. The results showed 95.6% accuracy, 93.8% recall, and 95.0% overall success rate in apple fruit ripeness and damage classification. This study demonstrates that GAN-based approaches can be effectively applied even with limited data for fruit ripeness estimation and phenotyping, especially in greenhouse conditions. The ARIGAN study conducted by Valerio Giuffrida et al. (2017) developed a GAN model that generates synthetic images dependent on the leaf count of *Arabidopsis thaliana* plants. The model produced realistic rosette images for different leaf counts, thus creating new image datasets that can be used for data augmentation in plant phenotyping. The model developed using these synthetic images resulted in a significant improvement in leaf count accuracy. This study demonstrates that GANs offer an effective solution for plant phenotyping in greenhouses or controlled environments, increasing data diversity, addressing the lack of labeled data, and enhancing data-based model training processes using synthetic images. Furthermore, GANs are reported to be effective in solving object detection problems associated with vision systems in intensively cultivated agricultural areas (e.g., orchards) (Fei et al., 2021). In this context, a study by Fei et al. (2021) generated synthetic fruit images from a 3D model using a semi-semantic constrained GAN model. This method preserved fruit positions and yielded images compatible with real-world datasets. The resulting synthetic images strengthened the training dataset, particularly in dense orchard environments where challenges such as overlap and hidden regions existed, and significantly increased the accuracy of object detection models by reducing the need for data labeling.

Through control systems integrated with GANs, quality standards and fruit deformities can be reliably detected. The identification of deformities and decay in commercially valuable fruits such as lemons, coffee beans, and kiwis holds significant importance. In such quality inspections, GAN-based data augmentation approaches provide an effective solution to improve overall model performance (Lu et al., 2022). For instance, in a study by Bird et al. (2022), Conditional GAN (cGAN)-based data augmentation was applied for lemon surface quality and defect classification. To expand the limited number of existing lemon images, synthetic images of defective fruits were generated using cGAN, and a fine-tuned VGG16-based classifier was trained with this enhanced dataset. As a result, the model's classification accuracy improved from 83.77% to 88.75%, substantially reducing generalization issues caused by data scarcity.

Table 1 presents some examples of studies conducted to date using GAN-based methods in plant breeding. The table summarizes the plant material used in each study, the specific applications to

which GAN models have been applied (e.g., disease detection, phenotyping, quality assessment, etc.), and metrics related to model performance, such as accuracy and F1 score.

Table 1. Applications of GAN models in plant health monitoring and crop production

Usage Area	**Review Material**	**Performance Measure / Ratio**	**References**
Plant health determination	Determining tea leaf diseases (red scab, red leaf spot and leaf blight) Detection of wheat yellow rust Detection of grape leaf diseases Detection of tomato leaf diseases Detection of cucumber leaf diseases	ACC gains 28%; ACC gains 83% 98.7% accuracy rate 98-99.8% accuracy rate 96,11-90,67 accuracy rate	*Hu et al. (2019)* *Maqsood et al. (2021)* *Liu et al. (2020)* *Deng et al. (2021)* Zhang et al (2021a)
Weed control	Weed control in pea and strawberry fields Weed control in sugar beet Weed control in tomato and nightshade	% 90 accuracy rate 85% F1 Accuracy 99.1% F1 Accuracy and 0.85% gain	*Khan et al. (2021)* *Kerdegari et al. (2019)* *Espejo-Garcia et al. (2021)*
Plant phenotyping	Orchid Viability Phenotyping in *Arabidopsis* plants and error reduction in GAN model Phenotyping via corn tassels	21% F1 gain 0.95 R^2 gain and 16% error reduction Structural similarity index measurement 0.84-0.89	*Zhu et al. (2020)* Valerio Giuffrida *et al (2017)* *Zhu et al. (2018)* *Shete et al. (2020)*
Fruit detection	Kiwi fruit detection Fruit detection on grape vines	5%-7% error Worked with 0.75–0.98 model correlation.	*Olatunji et al. (2020)* *Kierdorf et al (2021)*
Postharvest quality assessment	Quality determination in coffee beans Detection of fruit shape deformities in lemon Quality classification in dates	% 80 accuracy rate 88.8% ACC gain %98 accuracy rate	*Chou et al. (2019)* *Bird et al. (2022)* *Guo et al. (2021)*

GANs IN ANIMAL HUSBANDRY (FARM ANIMALS AND AQUACULTURE)

There are various GAN applications in farm animals and aquaculture for research areas such as animal phenotyping, determination of health status, and determination of yield and quality traits in animal products.

Animal production, which constitutes a significant portion of the food industry, continues to present numerous challenges, including animal welfare, ensuring quality standards, and establishing disease-free facilities. The inadequacy of traditional methods in these areas allows GAN models to offer remarkable solutions, particularly for needs such as image generation, data augmentation, and modeling phenotypic variation. For example, GAN-based systems integrated with IoT technologies have been reported to yield effective results in improving product quality and monitoring animal health

in poultry farming (Elmessery et al., 2023). Similarly, in the field of chick breeding, GAN models trained with data obtained from time sensors have demonstrated high accuracy in distinguishing healthy from diseased individuals (Abdoli et al., 2018).

GANs are also widely used in areas such as animal behavior analysis and phenotyping. They offer an effective alternative, particularly in situations where collecting and labeling animal data is difficult (Li et al., 2023). For example, in a study conducted by Shang et al. (2023), a GAN model was trained based on differences in hair shape between Angora goats and sheep and was found to be successful in phenotypic identification of Angora goats. On the other hand, in a study conducted to assess animal meat quality, fatty acid profiling was performed on sheep, beef, and pork; the resulting data was analyzed using a GAN model for quality classification (Cui et al., 2024).

In aquaculture, digital underwater monitoring methods are preferred to identify cultivated species, particularly those at risk from adverse underwater conditions, and to accurately identify underwater species (Li et al., 2021). Among these digital approaches, the success of GAN-based applications in species identification using visual data is noteworthy. Indeed, a GAN model trained on shrimp egg datasets has been reported to yield effective results in this field (Zhu et al., 2017).

GAN-based studies used in animal production systems in the context of smart agriculture applications are presented in Table 2. This table summarizes the animal materials used, the purpose for which GAN models were chosen, and the basic evaluation criteria for the performance of these models.

Table 2. Applications of GAN models in livestock and aquaculture systems

Usage Area	**Review Material**	**Performance Measure / Ratio**	**References**
Phenotyping in animals	Cattle identification and classification Identification of Angora goats Species identification using cattle mouth and face images Phenotyping of sheep from CT scans	98.9% accuracy rate 93.75% accuracy rate 97.27% accuracy rate 98% accuracy rate	*Singh et al. (2021)* *Shang et al. (2023)* *Gunda et al. (2022)* *Robson et al. (2021)*
Animal health determination	Disease detection in poultry Body condition scoring in cattle	% 97 accuracy rate % 80 accuracy rate	*Ahmed et al. (2021)* *Li et al. (2024)*
Yield determination & quality assessments in animal products	Estimation of polyunsaturated fatty acids in red meat	R^2 accuracy rate 0.2998	*Cui et al (2024)*
Aquaculture	Fish species detection Shrimp egg detection Aquarium density detection	%80.5%-83.1% ACC gain 99.2% model accuracy 97.57% model accuracy	*Zhao et al. (2018)* *Zhang et al. (2021b)* *Hu et al. (2024)*

GANs IN PREDICTING SOIL PROPERTIES AND AREA CHANGE

GAN models are used within the scope of smart agriculture applications in many soil-focused studies such as determining soil temperature, estimating the properties of soil nutrients and detecting changes in agricultural land use.

Soil physical properties such as texture, porosity, and aggregate structure; chemical factors such as pH, cation exchange capacity, and ion activity; and biological elements such as microbiota population and activity directly affect plant growth and development (Delgado and Gomez, 2024). Furthermore, the uptake of macronutrients (N, P, K, etc.) and micronutrients (Fe, Zn, Mn, etc.) by plants is also largely dependent on climatic variables such as temperature and precipitation (Voltr et al., 2021). Accurately modeling and analyzing these complex interactions is of strategic importance for the design of sustainable agricultural systems and adaptation to climate change (Jansson & Hofmockel, 2020). Modeling approaches provide effective results in predicting the effects on root development, plant nutrition, and yield resulting from the interaction of environmental factors such as temperature and precipitation, as well as properties related to soil structure and morphology. One of the studies conducted in this direction was conducted by Li et al. (2020). In this study, a GAN model was trained to estimate subsoil temperature using meteorological data such as wind speed and humidity. Following training, the model successfully predicted soil temperature at a depth of 5 cm. Similarly, in another study by Jiang et al. (2023), alpine meadow soil samples from the Qinghai-Tibet Plateau were evaluated and a GAN model was trained. This model was found to be effective in estimating the ratios of nitrogen, soil organic matter, potassium oxide, and phosphorus pentoxide to varying levels of accuracy at the regional level.

Table 3 presents some GAN-based sample studies on soil property determination and land use change prediction, along with GAN performance metrics for these studies.

Table 3. Applications of GAN models for soil property analysis and land use change estimation

Usage Area	**Review Material**	**Performance Measure / Ratio**	**References**
Soil temperature (Ts)	Hourly meteorological data, including air temperature (Ta), wind speed (W), relative humidity (RH), solar radiation (SR), and vapor pressure deficit (VPD)) from two stations in China (Changbai Mountain and Haibei) were used for model training.	Model performance for estimating 5 cm soil depth temperature; Model efficiency coefficient: 0,915 Willmott index of agreement: 0,978 Legates and McCabe index: 0,686	*Li et al. (2020)*
Soil nutrients	It was used to estimate soil nutrients (nitrogen (TN), soil organic matter (SOM), total potassium oxide (TK_2O), and phosphorus pentoxide (TP_2O_5)) in degraded Alpine Grasslands in the Qinghai-Tibetan Plateau.	Accuracy rate; 50-60 for TN 25-35 for SOM 30-40 for TK_2O 30-35 for TP_2O_5	*Jiang et al. (2023)*
Detection of changes in land use	UKCEH Land Cover Map data was used.	The model covers a transition between arable and grazing land cover classes by 71%.	*Harrison (2025)*

		It predicted spatial patterns among forested, arable, grassland, freshwater, and urban areas 89% of the time.	

Soil-based area modification refers to the optimization of land use and agricultural activities in a region based on the physical, chemical, and biological properties of the soil. This approach, particularly in smart agriculture applications, enables land management strategies to be addressed with different soil management strategies by taking soil variations into account (Taghizadeh-Mehrjardi et al., 2020; Pandey and Pandey, 2023). In a study by Harrison (2025), a GAN model was trained using data from 1990 to validate the original UKCEH Land Cover Map data from 2019. This model was found to be effective in accurately predicting arable land, pasture, freshwater, and urban areas.

FUTURE PERSPECTIVE OF GANs and CONCLUSION

As a machine learning model, GANs are used in many smart agriculture applications such as phenotyping in plant and animal production, disease and pest detection, determination of product yield and quality characteristics, soil physical and chemical analysis and land use monitoring. However, factors such as data incompleteness, environmental variability, and uncertainties related to application factors can lead to data inconsistencies in AI-based applications. However, GANs' ability to model through data augmentation and synthetic data generation suggests that they can offer more flexible and effective solutions to overcome these challenges compared to other methods.

With technological advances in image generation, data augmentation, and deep learning-based model training, GANs hold high potential for solving agricultural problems. Indeed, researchers such as Hong et al. (2020), Saxena and Cao (2021), Abbas and Bouzid (2024), Islam et al. (2024), Zhou et al. (2024), and Murad et al. (2023) emphasize that there are many areas for future development of GANs in the agricultural sector. These areas include:

- *Increasing data quality and diversity*: High-quality and diverse datasets are crucial for GAN-based models to perform more effectively in agriculture. Future advancements in sensor technologies, remote sensing systems, and imaging devices will make data access easier, significantly improving the accuracy and generalization capacity of GANs.
- *Improving training techniques*: Problems such as mode collapse, unbalanced learning, and slow convergence that arise during training of GAN models limit their stability and performance. Therefore, the development of new optimization algorithms, the application of regularization techniques, and the support of GANs with hybrid architectures will enable the creation of more robust and efficient models.
- *Real-time applications*: While current GAN applications are largely limited to experimental applications, in the future, it will be possible to integrate these models into real-time agricultural decision support systems. For example, agricultural robots or unmanned aerial vehicles (UAVs) will be able to autonomously perform tasks such as disease detection by generating and analyzing snapshots using GANs.
- *Advanced multi-data (multimodal) models*: The need for multimodal GAN models that integrate soil, climate, plant health, imaging, and sensor data is increasing. Such holistic approaches will enable much more comprehensive and accurate assessments of crop yield predictions, environmental stress analyses, and the impacts of climate change on agriculture.
- *Integration of policy development and sustainability*: The simulation and modeling capabilities offered by GANs can enable agricultural strategies to be tested under climate

change scenarios. This will contribute to the development of sustainable agricultural policies based on scientific data and facilitate more balanced environmental, social, and economic decisions.

In conclusion, GAN-based approaches hold great promise for enhancing data diversity, improving model accuracy, and increasing the reliability of decision support systems in precision agriculture applications. However, for these technologies to be more effectively utilized within agricultural systems, there is a need for interdisciplinary collaborations, high-quality open-access datasets, and the development of application-oriented standards.

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GENETIC AND SPECIES DIVERSITY OF FISH IN ALBANIA'S MAJOR LAKES

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ABSTRACT

Lake Shkodra, shared between Albania and Montenegro; Lake Ohrid, located between Albania and North Macedonia; and the two Prespa Lakes, which span the borders of Albania, North Macedonia, and Greece, together form the most remarkable lake complex in Europe in terms of habitat, species, and genetic diversity. This publication presents the unique characteristics that sustain the stable and resilient condition of each lake, as well as one of their most important features - the genetic and species diversity of their fish populations. The main characteristics of Albania's large lakes should be at the center of management activities. Particularly important is the fish community, especially endemic species threatened by pollution and unregulated fishing.

Keywords: Genetic diversity, Lake Shkodra, Lake Ohrid, Prespa Lakes, species diversity.

INTRODUCTION

The hydrological complex formed by the Drin and Buna rivers, along with the natural lakes (Shkodra, Ohrid, Great Prespa, and Small Prespa), is situated on the Balkan Peninsula, within one of the regions characterised by intense tectonic, hydrographic, geomorphological, biogeographical, and evolutionary processes. This complex is part of the Adriatic Sea Basin—one of the most ecologically and scientifically intriguing areas of the Mediterranean—and belongs to the South Adriatic–Ionian zoogeographic water region, noted for its distinct features (Dhora, 2017). Although the four lakes are hydrologically interconnected and in communication with one another, each possesses unique characteristics that endow it with ecological individuality and significance.

Among the most important features of these lakes is the genetic and species diversity of their fish populations. Genetic diversity refers to the variability of genes within a species' population, while species diversity denotes the variety of species present and their relative abundance (Biba et al., 2013).

This article outlines, for each lake, the key characteristics that sustain the ecological stability and resilience of the ecosystem, as well as the genetic and species diversity of their ichthyofauna.

MATERIAL AND METHOD

The characteristics of the lakes have been primarily sourced from Dhora (2016, 2017), Dhora et al. (2016) and Group authors (2015). These characteristics have been revised and adapted according to two main aspects: the stable condition of the lakes and the genetic and species diversity of their fish. The updated fish lists for Albania's major lakes have been derived from Dhora (2020), Marić (2018), Talevski et al. (2009), Barbieri et al. (2015), and Crivelli & Catsadorakis (2012).

The discussion on species diversity was conducted by comparing data for each lake regarding the number of fish species, the number of species within the Cyprinidae family, the number of species within the Salmonidae family, the number of species migrating to the sea, the number of introduced species, and the number of endemic species.

The genetic diversity of the fish is examined based on data presented in a previous publication (Dhora, 2024), also providing several insights on *Salmo* species from the Moraça River, three *Salmo* species from Lake Ohrid, *Alosa agone* Bonaparte, 1846 from Lake Shkodra, two *Rutilus* species from Lakes Shkodra and Ohrid, and on the autochthonous common carp of Lake Shkodra, which maintains genetic equilibrium (Biba et al., 2013).

RESULTS AND DISCUSSION

Below are presented the characteristics of Albania's major lakes that support the stable and resilient condition of these ecosystems, as well as the genetic and species diversity of their fish populations.

Lake Shkodra

Lake Shkodra is classified among the major lakes of the Earth (100–10,000 km²) and is the largest lake on the Balkan Peninsula in terms of surface area.

The subsiding movement of the block on which Lake Shkodra lies began at the end of the Paleogene and the beginning of the Neogene period. It is believed that this downward movement has been offset by uplift due to sediment deposition from inflowing rivers, a key geological factor contributing to the lake's long-term stability.

Karst modeling is evident in the karstic landscape, hydrographic features, lake morphology, and its high catchment potential.

Approximately 44.3% of the lake's minimum surface area lies below sea level. This feature classifies the lake as a cryptodepression—a rare phenomenon in Europe and globally. This aspect contributes to its classification as a stereotypical lake, endowing it with unique natural values, regenerative capacity, and longevity.

Lake Shkodra is a lowland lake, yet it is supplied with water from the high-altitude, high-precipitation areas of its extensive catchment basin.

The lake functions as a hydrological stabiliser. When the Drin River brings low water flow, Lake Shkodra freely discharges its waters into the Buna River. Conversely, when the Drin carries a large volume of water, it impedes the normal outflow of the Buna, thus blocking the discharge from the lake. As a result, the lake expands by increasing its water level, surface area, and volume.

Historically, Lake Shkodra has been described both as a riverine system (lotic system), where the lake was considered the potamic segment of the Morača–Buna River, and as a marsh system (lentic, palustrine) along the lowland stretch of the river. Today, Lake Shkodra represents a true lake system (lentic, lacustrine), characterised by a large, permanent water surface, distinguishing it from marshlands.

The lake's water temperature very rarely drops below 0°C, which classifies it within the subtropical thermal regime. It holds more water during the wet seasons and less in the summer months.

Lake Shkodra is marked by significant seasonal fluctuations in water level, which can reach up to 5 metres. These fluctuations are ecologically essential. During the warmer seasons and the lowering of the water level, aquatic macrophytes flourish, and biological processes such as reproduction and migration are activated.

As autumn approaches, macrophytes undergo decomposition. The decayed biomass is distributed across the lakebed sediments, while a portion is carried via the Buna River to the sea, contributing to the natural cleansing of the lake's waters.

In the colder seasons, when water levels reach their maximum, the lake undergoes a reversal in ecological dynamics. The number of habitats decreases and fauna seek shelter for overwintering.

Lake Shkodra is considered a littoral-type environment. Its shallowness and water level oscillations prevent the development of well-defined vertical zonation within the lake.

The lake is characterized by clear, oligotrophic waters with low phytoplankton biomass. The key feedback mechanisms that maintain water quality within normal parameters include: nutrient concentrations in the water versus humic production in wetlands; nutrient levels versus the productivity of riparian forest habitats; and food web structures that transfer phosphorus from littoral to pelagic zones versus biogeochemical processes that inhibit phosphorus recycling from sediments.

Lake Shkodra comprises three habitat systems: The lacustrine system, which includes limnetic and littoral habitats; The palustrine system, consisting of habitats that vary in vegetation type and the presence or absence of standing water; The riverine system, which encompasses both permanently and seasonally flooded habitats.

Aquatic macrophytes are the most characteristic feature of the littoral zone and, to a certain extent, of the entire Lake Shkodra ecosystem.

The lake is notable for its wide-ranging species diversity. It also holds significant ornithological value at a regional scale. In and around Lake Shkodra and its catchment area, a total of 283 bird species have been recorded, 168 of which are associated with aquatic habitats.

Lake Ohrid

Lake Ohrid is situated at an altitude of 693 metres above sea level, nestled between the Mokra Mountains to the west and the Galicica Mountains to the east.This lake is listed among the nine most ancient lakes selected from a group of 21 of the oldest lakes worldwide and is considered the oldest in Europe.

Lake Ohrid belongs to the group of large lakes of the globe. It has an average depth of 167 metres and a maximum depth of 288.7 metres, ranking as one of the deepest lakes in Europe—second in term-s of average depth—and the largest in the Balkans by water volume. Lake Ohrid and Lake Prespa are part of the Dessaret basin group, formed by a geotectonic depression during the Pliocene epoch.

Uniquely among global lakes, Lake Ohrid is primarily spring-fed. Approximately 50% of its inflow originates from underground sources coming from Lake Prespa, which lies about 10 km to the southeast and roughly 150 metres higher in elevation. The lake has three thermal water zones. The epilimnion, the uppermost water layer, extends to about 20 metres and can reach temperatures of 24–27°C. The metalimnion lies beneath, where temperature decreases by approximately 0.5°C per metre of depth. The hypolimnion is the deepest, coldest layer, which does not receive heat from sunlight or oxygen from the atmosphere. At 150 metres depth, the temperature is about 6°C.

The open waters of Lake Ohrid are oligotrophic, whereas the nearshore zones are considered mesotrophic.

Lake Ohrid is characterised by outstanding biodiversity, including: Habitat diversity, often physically and/or ecologically isolated; Species diversity, particularly of endemic taxa; Genetic diversity in several biological groups. The lake's biota exhibits strong resistance to biological invasion, not only due to its physical isolation from surrounding waters but also because its biocenosis is homogeneous, stable, and a product of long-term evolutionary processes.

Lake Ohrid holds the highest density of endemic species per surface area of any lake in the world. The presence of cold, oxygen-rich underwater springs, combined with both horizontal and vertical isolation, are the main drivers of the lake's unique evolutionary pathways, its high level of endemism, and its ecological stability.

Lake Ohrid is often described as a "museum of relict species" or a "repository of living fossils," as many species that were once widespread millions of years ago and are now extinct elsewhere have survived only in this lake. The most notable relict groups include Diatoms, Gastropods, and Salmonids, with the benthic community being among the most interesting.

Zooplankton plays a vital role in the lake's food web, serving as the primary food source for smaller fish, which in turn sustain predatory fish, including trout.A total of 270 bird species have been recorded in Lake Ohrid and its surrounding catchment.

The Prespa Lakes

The Prespa Lakes—Great Prespa Lake and Small Prespa Lake — form part of the Dessaret basin group, which originated from a geotectonic depression during the Pliocene epoch. Great Prespa Lake is included in the list of the 21 oldest lakes in the world. Located at altitudes ranging from 844 to 852 metres above sea level, they are considered the highest tectonic lakes in the Balkans**.**

Great Prespa Lake

Great Prespa Lake belongs to the group of large global lakes (100 – 10,000 km²). It receives water primarily through **surface inflows**, while its outflows are subterranean, passing through the karstic systems beneath **Mount Galicica** and **Mount Mali i Thate**, and emerging as springs in **Tushemisht** and **Saint Naum**, which subsequently feed **Lake Ohrid**.

The lake has a **maximum depth of 52 metres**. Over the past few decades, the water level has dropped by approximately **8 metres**, likely due to changes in the underground karstic water flow, or possibly for other **natural or anthropogenic reasons**. Unlike Lake Ohrid, **thermal stratification** in Great Prespa Lake is **less distinct**, although a general layering does exist. For the majority of the year, the **open waters** of Great Prespa exhibit **oligotrophic** characteristics, shifting towards **mesotrophic conditions** during the summer months.

Ecological stability in Great Prespa Lake is attributed to its **clean waters** and a **dual scheme of primary production**, supported by two key components of the ecosystem: **phytoplankton**, which thrives in the surface thermal layer, and **rooted macrophytes** in the littoral zone.

Small Prespa Lake

Small Prespa Lake is **shallow**, with a **maximum depth of only 7.7 metres**, and exhibits **eutrophic conditions** due to higher levels of **phosphorus concentration**. It is rich in emergent vegetation and wetland habitats, making it ecologically significant, especially for birdlife.

Landscape and biodiversity

The lakes are surrounded by **diverse habitats**, scenic **bays**, and **remarkable islands** such as **Golem Grad**, **Maligrad**, **Agios Achillios**, and **Vidronisi**, which, together with the **pristine nature** and **clear waters**, contribute to the lakes' **aesthetic and ecological value**. The **habitat diversity**,

species richness, and **presence of endemic taxa**—particularly among **gastropods** and **fish**—highlight the lakes' **conservation priority** status. In total, **over 200 bird species** have been recorded in the Prespa Lakes. In recent decades, the **wintering waterbird populations** have reached up to **50,000 individuals**.

In terms of **biogeographical classification**, Great Prespa Lake is generally grouped within the **Adriatic–Ionian Lake system**, which also includes **Lake Ohrid**. However, it can also be considered **ecologically distinct** from Lake Ohrid and the broader **Eastern Lake Group**, which comprises two Balkan and three Anatolian lakes. To date, **28 species of aquatic macrophytes** have been recorded in Great Prespa Lake. **Over 200 bird species** have been recorded in the two Prespa Lakes. In the past two decades, the **maximum number of waterbird individuals** counted during the winter period has approached **50,000**.

On the genetic diversity of fish

The species *Salmo obtusirostris* Heckel, 1851 is considered a complex species. This group includes *Salmo zetensis* Karaman, 1938 from the Zeta River, which some authors regard as a subspecies of *S. obtusirostris*. Two other species, *Salmo montenigrinus* Karaman, 1933 from the Morača River, considered by some authors as a synonym of *S. obtusirostris*, and *Salmo taleri* Karaman, 1933 from the upper course of the Zeta River, morphologically resembling *S. cf. farioides* Karaman, 1938 have so far been recorded only at a single locality. Therefore, the *Salmo* species of the Morača River basin, which includes the Zeta and Cem tributaries, are still not well known, although six *Salmo* species are reported. It is necessary to deepen research to accurately determine these species or subspecies, including the use of genetic analyses.

A thorough study is required on the non-migratory *Alosa agone* population of Lake Shkodra. This study should also investigate whether migration to the sea occurs, and gradually reveal further relevant data. For years, a portion of individuals older than three years may not have participated in migration to the sea. Over time, a non-migratory population may have formed, adapted to life along the northeastern shore of the Albanian part of the lake, and possibly reproducing there as well. Individuals of this population are smaller than migratory ones, have shorter heads, and fewer spots on both sides of the body (Kottelat & Freyhof 2007). This long-established population is *Alosa agone*, living in sympatry with *Alosa fallax* Lacépède, 1803 (Rakaj & Crivelli, 2001). These findings were also discussed in a previously published article (Dhora, 2024).

In Albania, the common carp (*Cyprinus carpio* Linnaeus, 1758) was introduced by the Romans, who cultivated it. Considering its adaptive capacity, the carp population in Lake Shkoder can be regarded as an autochthonous species of the lake. Microsatellites have been used as molecular markers to assess genetic variability at the DNA level in carp populations of Lake Shkodra and Lake Ohrid. This study found that only the carp population of Lake Shkodra, at the MFW6 locus, is in genetic equilibrium (Biba et al., 2013). The conservation of the native common carp in Lake Ohrid, and possibly also in the Prespa Lakes, should be ensured through careful stock enhancement based on selected individuals, using controlled technology.

Rutilus karamani Fowler, 1977 and *Rutilus ohridanus* Karaman, 1924, previously considered distinct species in Lake Shkodra, are regarded by Bianco & Ketmaier (2014) as synonyms of a single species, *Leucos basak* Heckel, 1843. They were once classified as subspecies: *karamani* as the yellow roach and *ohridanus* as the white roach (Dhora et al., 2008). In the publication of Dhora (2024), it is suggested that this variation may represent intraspecific diversity, possibly as colour morphs related to the characteristics of their aquatic habitats and their adaptive abilities.

Equally important is the study of trout in general, and particularly the species of Lake Ohrid: *Salmo aphelios* Kottelat, 1997; *Salmo balcanicus* Karaman, 1927; *Salmo lumi* Poljakov, Filipi &

Basho, 1958, and *Salmo ohridanus* Steindachner, 1892. In many cases, trout populations are on the brink of collapse due to pollution, declining water levels, and unregulated fishing.

On the species diversity of fish

The list of freshwater fish species in Albania, according to Dhora (2020), comprises 100 species. The two most significant families in terms of species number are Cyprinidae with 40 species and Salmonidae with 15 species.

The Table 1 below provides, for each lake, the following data: the number of fish species, the number of species from the Cyprinidae family, the number of species from the Salmonidae family, the number of migratory species to the sea, the number of introduced species, and the number of endemic species.

Table 1

Lakes	Species number	Cyprinidae	Salmonidae	Migratory to the sea	Introduced	Endemic
Lake Shkodra	45	26	7	8	16	6
Lake Ohrid	28	15	6	2	4	5
Great Prespa lake	30	19	3	1	14	7
Small Prespa lake	18	14	?	1	8	7

As shown in the table, Lake Shkodra has the highest number of fish species with 45, followed by Great Prespa Lake with 30 species, then Lake Ohrid with 28 species, and finally Small Prespa Lake with 18 species.

The number of species in the family Cyprinidae is as follows: Lake Shkodra has 26 species, Great Prespa Lake 19 species, Lake Ohrid 15 species, and Small Prespa Lake 14 species. The total number of species and those of the Cyprinidae family is greatest in Lake Shkodra due to its subtropical characteristics, large surface area, and shallow depth over most of its extent. After Lake Shkodra, Great Prespa Lake follows with a considerable difference in both total species and Cyprinidae species. This is because it is shallower and more eutrophic, especially along the gently sloping shores with abundant vegetation. The difference becomes more pronounced in the other lakes.

Lake Shkodra also has the greatest number of species from the family Salmonidae, as this count includes the trout of the Morača River, which flows into the lake and contributes over 60% of the lake's total water inflow. Lake Ohrid has deep waters sourced from both surface and subterranean springs, which are well suited to salmonids.

Due to its close proximity and connection to the Adriatic Sea via the Buna River, Lake Shkodra also hosts numerous migratory species that move to the sea.

Both Lake Shkodra and Large Prespa Lake have many introduced species, numbering 16 and 14 respectively. Many of these species were introduced to increase fish production for consumption. By contrast, Lake Ohrid has 4 introduced species, while Small Prespa Lake has 8. The latter is a shallow and eutrophic lake.

Each lake hosts endemic species. The endemic species of the two Prespa lakes are almost identical due to their close hydrological connection. Lake Shkodra shares some endemic species with the Morača River. Meanwhile, Lake Ohrid's endemic species have developed within the lake itself, primarily due to geographical and ecological isolation.

CONCLUSIONS

Many of the most prominent values of the lakes are considered unique. Lake Shkodra, for instance, is a rare cryptodepression in Europe and worldwide. Lake Ohrid is unique globally because it is primarily fed by springs and ranks as the richest lake in endemic species relative to its surface area on the entire planet. The Prespa Lakes, situated at an altitude of 852 metres, are the highest lakes in the Balkans. Water entering these lakes through surface flow disappears beneath Mount Galicica and Mount Thate, re-emerging as springs at Tushemisht and Saint Naum, which feed Lake Ohrid.

The main characteristics of Albania's large lakes should be at the centre of management activities. Particularly important is the fish community, especially endemic species threatened by pollution and unregulated fishing.

Care must be taken to recognise the high genetic and species diversity of the fish populations in these lakes. The three endemic trout species of Lake Ohrid need to be confirmed as distinct species through genetic analyses. For the six trout species in the Morača River, genetic studies are necessary to differentiate species and subspecies. A comprehensive study of the non-migratory *Alosa* population in Lake Shkodra is required, including morphological, genetic, embryological, ecological, and evolutionary analyses. *Rutilus karamani* and *Rutilus ohridanus* have been unified into one species; however, further wider population and genetic studies are needed in both Lake Shkodra and Lake Ohrid to clarify whether these represent subspecies or distinct forms.

Lake Shkodra, Lake Ohrid, and the Prespa Lakes have been declared protected areas by their respective countries. In the future, it is envisaged that the entire hydrological complex and its catchment basin should be declared a protected area, which would involve five countries in an integrated and much more successful management effort.

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DETERMINATION OF CHLOROPHYLL CONTENT IN MAIZE AND BEANS (2M+1B) SOWN AT DIFFERENT RATES IN THE SAME ROW

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ABSTRACT

The aim of this study was to determine the effect of intercropping maize and beans on changes in chlorophyll content and yield. Plots were set up with two maize seeds followed by one bean seed in a row. Four irrigation levels were applied to the plants. The control treatment (S100) was defined as the treatment where the plants' water requirements were supplied in entirety. The treatments with 20% (S80), 40% (S60), and 60% (S40) water deficits were defined as the deficit irrigation treatments. The chlorophyll content values of the plants were as follows: in maize, S100: 50.85, S80: 49.85, S60: 48.10, S40: 46.35; while in beans, it was measured as S100: 39.95, S80: 40.80, S60: 38.70, S40: 38.30. The highest chlorophyll content was determined in maize for S100 and in beans for S80. The average total green forage yield was determined as S100: 9951, S80: 9102, S60: 8325, S40: 7927 kg da^{-1}.

Keywords: intercropping, deficit irrigation, forage yield, evapotranspiration

INTRODUCTION

The effects of climate change around the world have made the proper management of natural resources, especially water, a priority. Irregular rainfall, the depletion of drinking and irrigation water sources, and environmental problems caused by industrialization make careful use of water essential. With the growing population, the need for water is increasing at the same rate; this demand is becoming more pronounced in both daily life and production. Under these conditions, the amount of water used in agriculture is decreasing, and the conscious use of water is becoming increasingly important. Water conservation is not only an environmental responsibility but has also become a fundamental part of economic development and social welfare. Therefore, the conservation and efficient use of water resources is now an inevitable necessity.

The continuity of agricultural production must be ensured in order to meet the increasing demand for food. The balanced and efficient use of water resources is vital for the sustainability of this production. Success in water management increases agricultural yield on the one hand and reduces the environmental burden on the other. In particular, the adoption of modern irrigation methods and the application of techniques for the economical use of water will increase efficiency in agricultural production while also contributing to the sustainability of water resources.

In recent years, the decline in water resources and the corresponding increase in water demand have brought to the forefront the concept of producing more with less water in agricultural production. The methods developed in this context aim to adapt production to these new conditions by focusing on saving water. Deficit irrigation systems and intercropping techniques, which aim to maintain yields with limited water, are notable effective applications in this regard. Thanks to these methods, continuity in production can be ensured even with scarce water resources.

Intercropping also offers various advantages against harmful insects and diseases. Having different plants in the same area makes it difficult for pests to target a single species, thereby limiting the spread of disease and protecting production (Altieri, 2018). This reduces the need for chemical pesticides and creates an environmentally friendly production model.

Beans preserve soil fertility with their nitrogen-fixing properties, while maize contributes to moisture balance by utilizing groundwater thanks to its deep roots (Frankenberger et al., 2002). This synergy enables more efficient use of both water and nutrients, thereby improving product quality and preserving the balance of the agricultural ecosystem. Li et al. (2023) conducted a study to investigate the effects of soil interactions in maize-pea intercropping systems on crop yield and nutrient uptake. This research, conducted between 2017 and 2020, utilized field and pot experiments. The findings showed that intercropping increased the yield of both species. For example, in 2018, fava bean yield increased by 4.19%, while maize yield increased by 6.01%. In terms of nutrient accumulation, it was determined that nitrogen and phosphorus uptake in fava beans increased significantly in intercropping systems.

In conclusion, intercropping complementary crops such as silage maize and beans increases agricultural productivity, offers an environmentally friendly production model, and contributes to the sustainable use of natural resources. Therefore, the widespread adoption of intercropping methods is of strategic importance for the success of future agricultural policies.

The aim of this study is to determine the yield, leaf area index (YAI), and chlorophyll content obtained from the intercropping system by applying different levels of irrigation water to maize and beans intercropped together.

MATERIAL AND METHOD

Material

This study was conducted on land belonging to the Eastern Mediterranean Transition Zone Agricultural Research Institute (DAGTEM) located in the province of Kahramanmaraş. The study area is 465 meters above sea level and is located between 37°55′08″ north latitude and 36°55′09″ east longitude. In September and October of both years, a significant decrease in minimum temperatures was observed. Temperatures of 8.6 °C were recorded in October 2022 and 9.5 °C in October 2023. These temperatures are at levels that could cause cold stress in some plants during their late stages of development.

When evaluating precipitation data, 2022 was a notable period in terms of drought. Precipitation amounts were quite low in July and September. The year 2023 stood out with higher rainfall values; 16.4 mm of rainfall was recorded in June and 17.7 mm in July. These values are above the long-term average for the period 1960–2021. This indicates that soil moisture was naturally supported, especially during the 2023 season.

When examining the physical properties of the soil in the study area, it is observed that the bulk density values range between 1.54–1.64 g cm^{-3}. These values indicate a medium texture and a structure favorable for plant root development. The field capacity determined on a weight basis varies between 21.4% and 23.5%, while the wilting point varies between 12.2% and 14.3%. Based on these parameters, calculations show that for the 0–90 cm soil profile, the field capacity (FC) is 327.8 mm, the wilting point (WP) is 189.3 mm, and the available water holding capacity (AWHC) is 138.6 mm (Keten, 2020).

In this study, two field crop varieties adapted to the region's climatic and soil characteristics were selected. The varieties named “Colonia” for silage maize and “Bulduk” for beans were preferred. Both types stand out for their fundamental agricultural characteristics, such as high yield potential and

tolerance to environmental stresses. The Colonia maize and Bulduk dry bean varieties used in the study offer significant advantages in agricultural production due to their high yields, environmental tolerance, and nutritional value. Thanks to these characteristics, they provide both economic returns and contribute to sustainable agricultural systems.

Method

The intercropping method used in the study has been evaluated as an important strategy for increasing agricultural productivity and more effective use of resources. In this system, maize and beans were grown together in the same field plot and in the same row. The plants were arranged in a "2M:1F" pattern; that is, one bean plant was added after every two maize plants. Maize plants were planted 15 cm apart in the row, while bean plants were planted approximately 30 cm apart.

This structure provided an environment that preserved the maize's dominant vertical growth advantage while also providing shade and support for the beans to climb. Based on the planting pattern obtained, plant densities were calculated to be 9,524 plants/da for maize and 4,762 plants/da for beans. These values reflect the population level necessary for both species to thrive in the same environment. When maize and beans are planted together, they exhibit complementary structural and functional characteristics. The sturdy and upright stem structure of maize supports beans, facilitating their vertical growth; while the bean plant fixes atmospheric nitrogen into the soil, contributing to the natural fulfillment of maize's nitrogen requirements (Kumar et al., 2019).

In this study, different irrigation irrigation topics were determined to identify the most appropriate water restriction percentage to maintain silage yield. Four different irrigation topics were planned in this experiment, and these topics were planned and implemented as described below:

S100 (Full irrigation level): The entire amount of water required to reach field capacity (100%) was applied. This is the control treatment. S80 (Deficit irrigation): Water was reduced by 20% from the control treatment level, and 80% of the control treatment level was applied.

S60 (Deficit irrigation): Water was reduced by 40% from the control treatment level, and 60% of the control treatment level was applied.

S40 (Deficit irrigation): Water was reduced by 60% from the control level, providing 40% of the control level.

The trial studies were conducted on plots, each 7 m long and 3.5 m wide, with a total area of 35 m². A distance of 2 m was left between plots and 3 m between blocks. This layout was chosen to prevent the different applications from affecting each other.

Within the scope of the research, two different plant species, silage maize (M) and beans (F), were planted together using the intercropping method. Four different irrigation treatments (100%, 80%, 60%, 40%) were applied to these plants, allowing for a systematic analysis of the effects of water deficiency on plant growth, physiological indicators, and yield. Each treatment was applied in a randomized block design with three replications. This established an experimental design that was both scientifically valid and field-applicable (Figure 1).

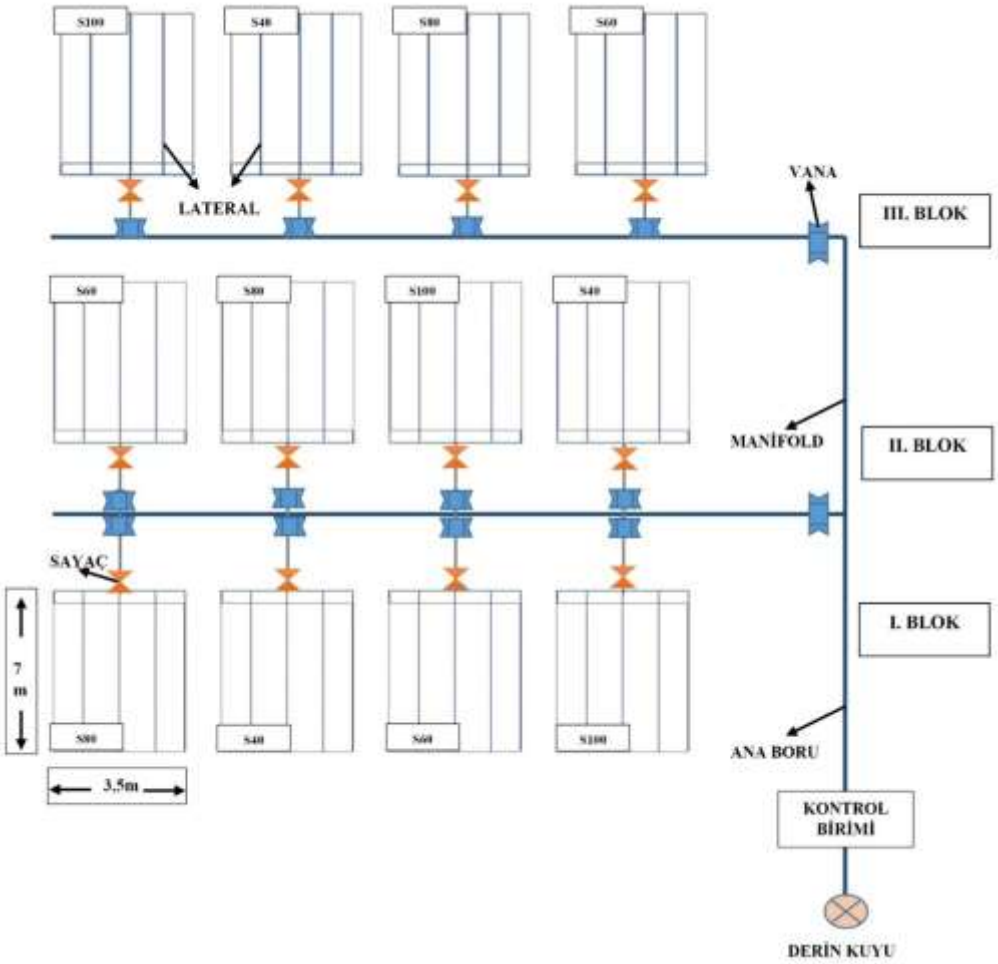

Figure 1. Schematic diagram of the trial design

Chlorophyll content, one of the important indicators of plant physiology, is a fundamental parameter reflecting photosynthetic capacity and overall metabolic status. In this study, chlorophyll density was measured using the SPAD device, a non-destructive and rapid method.

The SPAD-502 Plus (Konica Minolta, Japan) model device measures how much light the leaf absorbs by emitting light at red and infrared wavelengths onto the leaf surface, reflecting chlorophyll content as an index value (Uddling et al., 2007). The advantage of this device is that it is fast, portable, and can take measurements without damaging the leaf.

Measurements were taken before and after irrigation throughout the plant growth period. In each plot, measurements were taken at five separate points selected from middle-aged leaves, the device was placed perpendicular to the leaf, and care was taken to avoid shadows. The average of the obtained values was taken and used in the analyses.

Due to the high correlation between the SPAD value and the nitrogen content in the leaf, this method is also considered an indirect tool for monitoring plant nutrition (Chang et al., 2003). A decrease in the SPAD value under stress conditions, such as irrigation restriction, reveals the plant's physiological response to water.

The LAI is a dimensionless measure that indicates the total leaf area per unit of soil area. It is considered one of the fundamental indicators for assessing a plant's photosynthetic capacity, how

effectively it utilizes sunlight, and for monitoring processes such as water loss through transpiration (Watson, 1974; Jonckheere et al., 2004). LAI is also widely used to track changes in plant development over time, analyze responses to stress conditions such as drought, and evaluate the effects of applied agricultural methods.

In this study, LAI measurements were performed using the AccuPAR LP-80 model device, which operates based on light transmittance. This device indirectly calculates leaf density by simultaneously measuring sunlight intensity from the upper and lower parts of the leaf canopy (Norman et al., 1989). These measurements provide information about the light interception rate and leaf distribution of the plant canopy.

In practice, measurements were taken in two separate periods, before and after irrigation, in each plot to evaluate the effect of irrigation. In each measurement, lower and upper readings were taken from five different points within the plot as recommended by the device, and the leaf area index for that plot was determined using the average of these measurements.

Leaf area index measurements have been an important assessment tool for revealing the density of plant cover and the effect of agricultural practices on leaf development.

RESULTS AND DISCUSSION

Determination of chlorophyll content

In the study, the leaf chlorophyll content of silage maize and bean plants was determined using the SPAD-502 portable measurement device. Measurements were taken at regular intervals before and after each irrigation throughout the production period. Measurements for the first year (2022) began on August 10, and measurements for the second year (2023) began on August 20. Graphs of these values are provided in Figures 2, 3, 4, and 5.

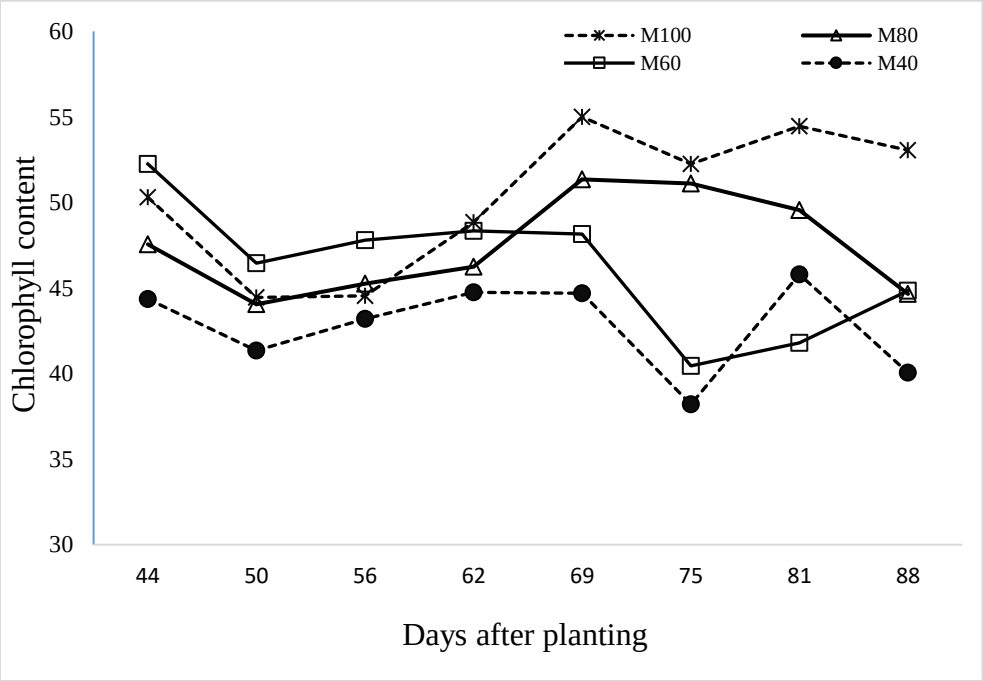

Figure 2. Chlorophyll meter values for maize in 2022

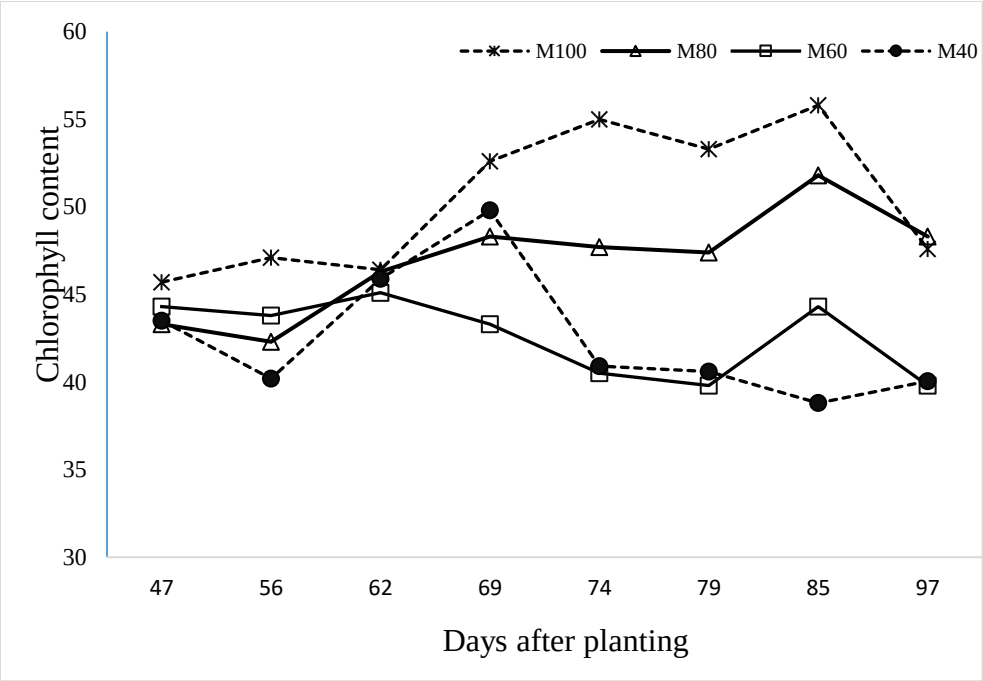

Figure 3. Chlorophyll meter values for maize in 2023

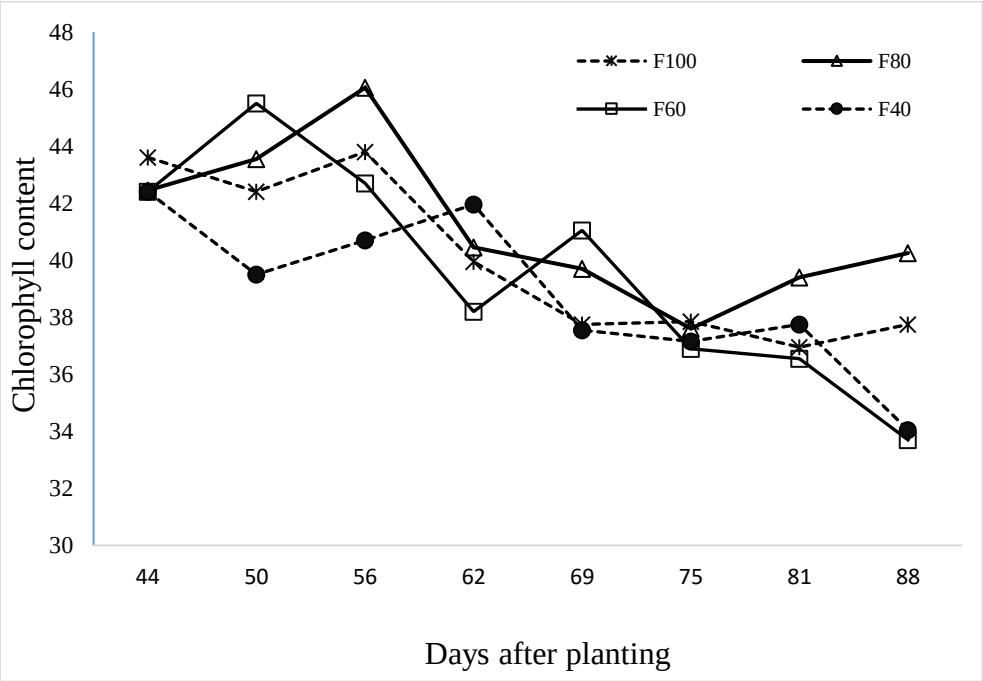

Figure 4. Chlorophyll meter values for bean in 2022

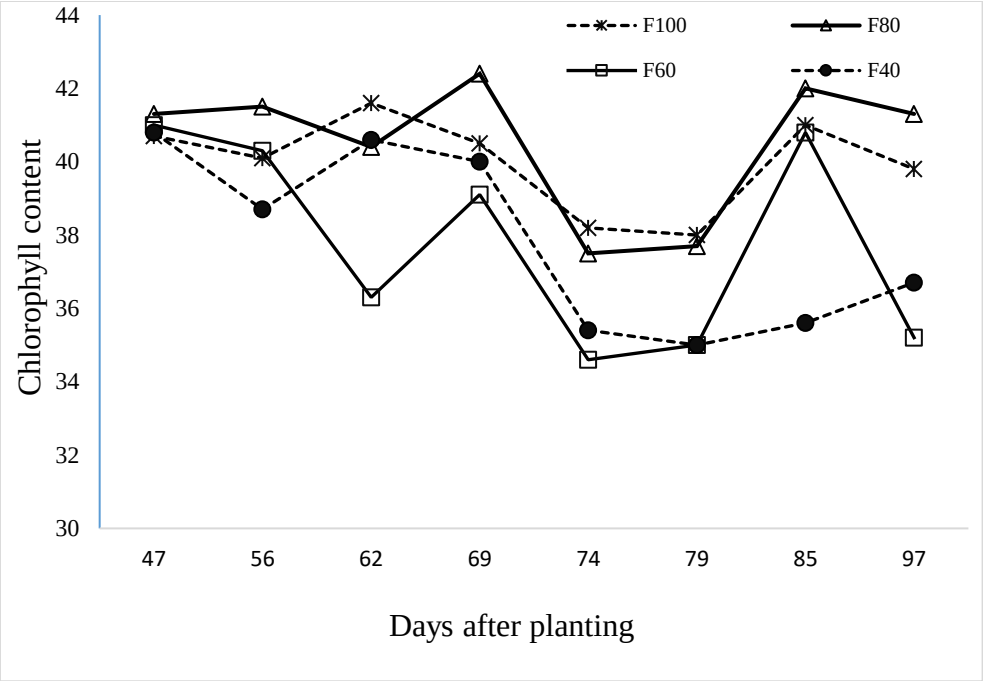

Figure 5. Chlorophyll meter values for beans in 2023

The chlorophyll content values obtained in this study were determined as an average of 50.3 for the maize plant in the S100 application in 2022 and 50.4 in 2023; it was observed that these values gradually decreased at reduced irrigation levels. The lowest average value was recorded at 42.4 in the S40 application in 2023. In the bean plant, the S100 average was 40.0 in 2022 and 39.9 in 2023; the lowest values were again determined in the S60 and S40 applications, where water restriction was intense. These data reveal that chlorophyll content is higher in full irrigation applications and that chlorophyll synthesis is suppressed due to water stress associated with restricted irrigation.

Leaf Area Index (LAI) measurements were taken at regular intervals over two growing seasons. Measurements began one day before irrigation started and were repeated before and after irrigation throughout the irrigation period. Graphs of the measured leaf area index values are shown in Figure 6 and Figure 7.

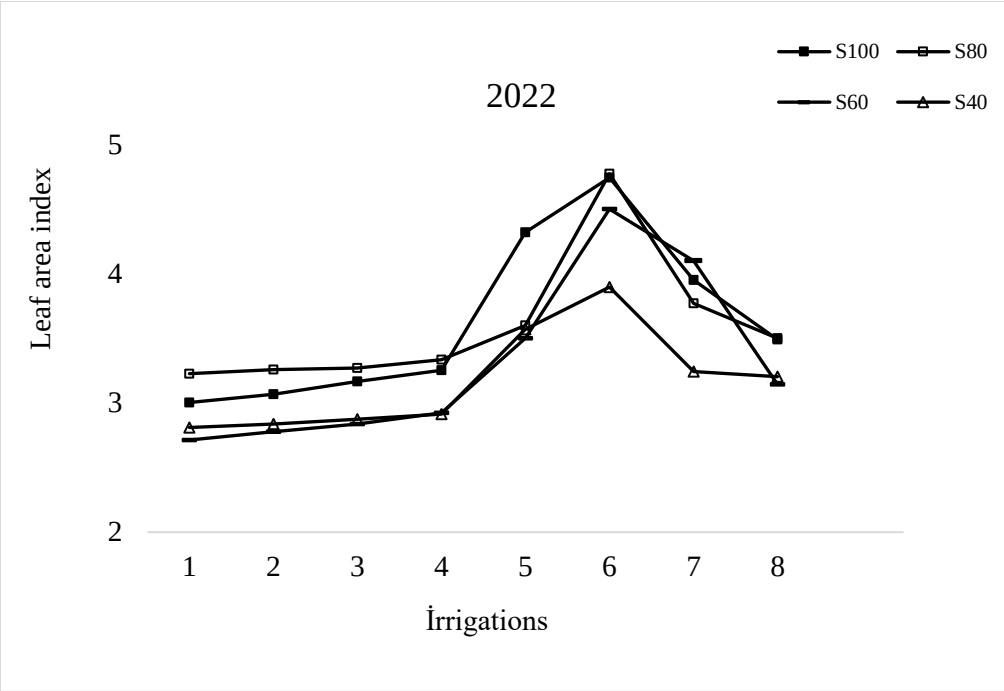

Figure 6. Leaf area index (LAI) values for 2022

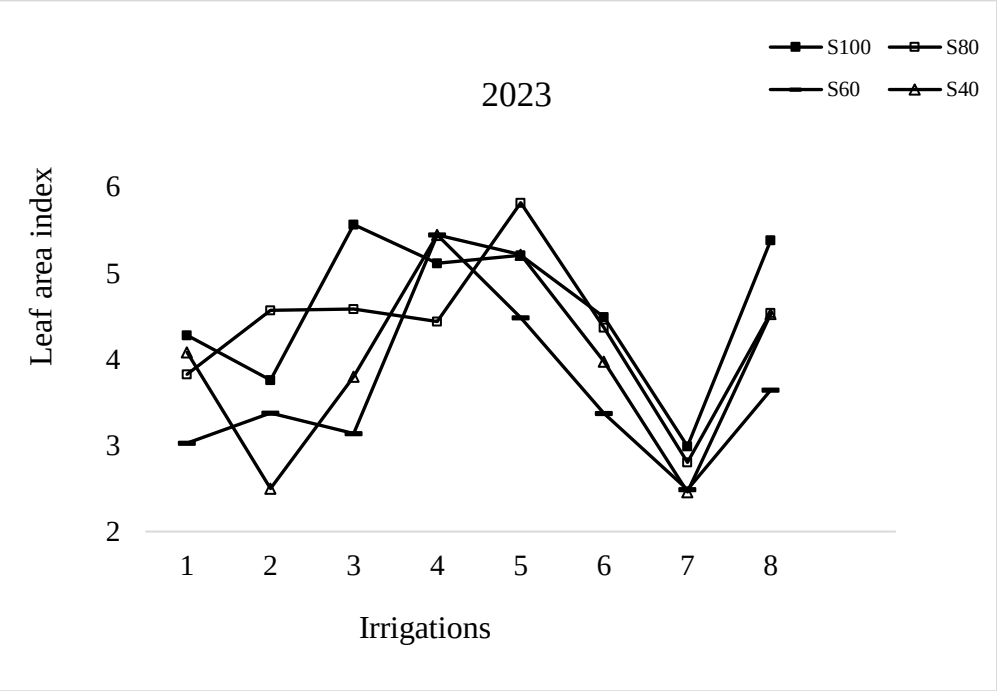

Figure 7. Leaf area index (LAI) values for 2023

-Measurements taken throughout the 2022 production season determined that the average YAI value was 3.06 $m^2\ m^{-2}$ for the S100 application, 3.26 $m^2\ m^{-2}$ for S80, 2.91 $m^2\ m^{-2}$ for S60, and 2.67 $m^2\ m^{-2}$ for S40. The value of 4.69 $m^2\ m^{-2}$ obtained in the sixth irrigation period on the S60 plot in the same year is the highest LAI value among all measurements. The lowest value was recorded in the first irrigation period on the S60 plot, at 2.21 $m^2\ m^{-2}$. The average leaf area index (LAI) values for

2022 ranged between 2.67 and 3.26 m² m⁻². YAI data for the 2023 production year was higher than in 2022. Average LAI values were measured as 4.10 m² m⁻² in S100, 3.87 m² m⁻² in S80, 3.12 m² m⁻² in S60, and 3.50 m² m⁻² in S40. The highest value was 5.32 m² m⁻², recorded in the fifth irrigation period on the S100 plot. This suggests that the second year was more favorable in terms of plant development. The average leaf area index (LAI) values for 2023 ranged from 3.12 to 4.10 m² m⁻². The LAI values obtained show that successful results can be achieved with the intercropping system under the environmental conditions specific to Turkey's semi-arid transition zone. In particular, the value of 4.10 m² m⁻² achieved in 2023 indicates a leaf density close to maximum light interception capacity. This result reveals that the applied 2M:1F row pattern provides an optimal growth environment for both maize and beans by balancing light and space competition between species. Plant canopy density increased due to the effect of the planting pattern.
The YAI levels determined in the study showed a high degree of consistency with data reported in the literature, such as Çiftçi et al. (2006), Arıkan and Yılmaz (2008), and Cammarano et al. (2023). However, it is thought that the much higher YAI values reported in studies such as Iqbal et al. (2006) and Shah et al. (2022) were obtained due to the different physiological structure of the species used (e.g., cowpea, soybean) and higher plant density.

In 2022, the average green forage yield was determined to be 9240 kg ha⁻¹. This value was found to be 9088 kg ha⁻¹ in the S80 plot, 8905 kg ha⁻¹ in S60, and 8455 kg ha⁻¹ in S40. The highest green forage yield was obtained from the S100 application, at 10,621 kg da⁻¹.

In the 2023 growing season, the total green forage yield was generally lower. The average yield was calculated as 8365 kg da⁻¹, with the highest yield value of 9282 kg da⁻¹ obtained from the S100 plot. Yields of 9116 and 7552 kg da⁻¹ were obtained in the S80 and S60 applications, respectively, while the lowest value of 7509 kg da⁻¹ was observed in the S40 treatment. Table 1 shows the total green forage yield (kg ha⁻¹) values and Duncan groups for different irrigation levels by year.

Table 1. The total green forage yield of varieties

Year	Total green forage yield kg da⁻¹				
	S100	S80	S60	S40	**Average**
2022	10621a	9088b	8905b	8345b	**9240a**
2023	9282b	9116b	7552b	7509b	**8365b**
2022-2023	**9951a**	**9102ab**	**8228b**	**7927b**	**8802**

When evaluated based on the two-year averages, the total green forage yield ranged between 7927 and 9951 kg ha⁻¹. The highest average yield of 9951 kg ha⁻¹ was obtained with the full irrigation (S100) treatment. This treatment was followed by S80 (9102 kg da⁻¹), S60 (8325 kg da⁻¹), and S40 (7927 kg da⁻¹), respectively. Based on the two-year averages, S100 was in the a letter group and provided statistically significantly higher yields than all other irrigation levels. The S80 treatment was in the abc letter groups and was not statistically distinct from the S100 treatment. The S60 treatment was in the c–f letter group and showed medium yield. The S40 treatment was in the d–g group and was statistically close to the lowest yield group.

These findings show that full irrigation conditions (S100) provide maximum yield in green forage production, while water restrictions statistically limit yield, especially at levels of 60% and below. It is understood that as the irrigation level increases, plant growth is encouraged and, consequently, green forage production increases.

In studies conducted on intercropping systems, the total green forage yield values for silage maize and legumes were as follows: Şimşek et al. (2005), under Turkey-Harran Plain conditions, in a 2M:1S (maize + soybean) strip intercropping system, 4800–5700 kg ha^{-1}; Kızıl Aydemir (2018), under Turkey-Iğdır conditions, in an M+70%S mixture, 9410–10280.1 kg ha^{-1} was found.

CONCLUSION

This study presents important findings demonstrating the effectiveness of intercropping systems, particularly in semi-arid regions. Intercropping silage maize and beans in a 2M:1F row pattern yields extremely efficient results in terms of both biomass production and water use efficiency. In particular, the fact that the S80 irrigation level provides significant water savings while maintaining yield levels with an acceptable loss of 8–10% makes it necessary to recommend it as an effective strategy for the sustainable use of water resources.

One of the most noteworthy findings of the study, chlorophyll content, is recommended to be used as a direct indicator in the evaluation of irrigation levels, in line with its close relationship with the plant's access to water. The high chlorophyll values remaining in the S100 and S80 applications indicate that the plants did not enter physiological water stress, proving that the S80 level, in particular, can provide maximum physiological performance with minimum water use compared to the S100 irrigation level.

In conclusion, based on the data presented in this study, the integration of dual cropping in a 2M:1F pattern, restricted irrigation at the S80 level, and chlorophyll content measurements into agricultural decision-making processes is among the main strategies recommended for achieving high yields and efficient resource management in semi-arid regions.

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RESPONSE OF FENNEL (Foeniculum vulgare MILL.) TO SUB-LETHAL RATES OF GLYPHOSATE AND FLAZASULFURON

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ABSTRACT

Weeds are among the most significant biological agents in many agricultural systems, and have an adverse impact not only on crop yield but also on crop quality. Although chemical weed control is recommended only as a complementary component of integrated weed management, it remains widely used by growers for its rapid efficacy, despite off-target risks such as spray drift. Non-selective herbicides applied in orchards or non-agricultural fields to control weeds may move from the applied fields to adjacent agricultural fields, sometimes the fields that were grown with medicinal and aromatic crops such as fennel (*Foeniculum vulgare* Mill.). This study was conducted to determine the adverse impacts of sub-lethal rates (12.5%, 6.25%, 3.125%, and 1% of the recommended rates) of glyphosate isopropylamine salt (1440 g ai ha^{-1}) and flazasulfuron (50 g ai ha^{-1}) under climate room conditions. Herbicides were applied to fennel seedlings when they reached 2-4 true leaves, and they were placed in a climate room adjusted to 16h/8h photoperiod, 24/20˚C (day/night) temperature. The impact of the herbicides on fennel seedlings was evaluated 21 days after treatment. Glyphosate and flazasulfuron reduced fennel dry biomass by up to 53% and 62.7% in a rate-dependent manner, respectively.

Keywords: Glyphosate, flazasulfuron, fennel, *Foeniculum vulgare*, drift

INTRODUCTION

Fennel (*Foeniculum vulgare* Mill.) represents a commercially significant perennial herb within the Apiaceae family. The species is distinguished by its characteristic production of anise-flavored volatile compounds and its versatile applications spanning the culinary, pharmaceutical, and agrochemical industries (Rather et al., 2016). Native to the Mediterranean region, fennel has been cultivated for centuries and has spread globally due to its economic importance. The plant's aromatic properties are attributed to its essential oils, primarily composed of compounds like trans-anethole, fenchone, α-phellandrene, and estragole, which collectively dictate its characteristic flavor and contribute to its recognized medicinal and aromatic properties (Díaz-Maroto et al., 2006). These volatile compounds not only contribute to fennel's distinctive flavor and fragrance but also possess various biological activities, making it a valuable resource for natural product research and development. These properties have led to increased interest in fennel as a potential source of natural preservatives and therapeutic agents in the food and pharmaceutical industries (Jadid et al., 2023). Furthermore, the plant's adaptability to various climates and its relatively low maintenance requirements have contributed to its widespread cultivation, making it an economically important crop in many regions worldwide.

While fennel holds considerable economic value, its cultivation is vulnerable to certain abiotic stressors, such as herbicide drift, which can substantially reduce yields and compromise the quality of the harvested product. Therefore, it is essential to investigate the non-target effects of sub-lethal herbicide concentrations on fennel's early growth patterns, particularly considering the widespread use of glyphosate and flazasulfuron. Glyphosate, a widely used non-selective herbicide, exerts its phytotoxic effects by disrupting the shikimate pathway, a crucial metabolic route in plants responsible for the synthesis of essential aromatic amino acids like phenylalanine, tyrosine, and tryptophan. Flazasulfuron, in contrast, inhibits plant growth by targeting acetolactate synthase, an enzyme pivotal in the synthesis of branched-chain amino acids such as valine, leucine, and isoleucine, consequently disrupting protein production and overall plant metabolism.

Impact of sub-lethal glyphosate rates on non-target crops varies depending on the crop species, environmental conditions, and application timing; these factors may initiate a complex cascade of physiological and morphological alterations. For instance, plants exposed to sub-lethal rates of glyphosate may exhibit reduced photosynthetic efficiency, impaired nutrient uptake, and altered carbon allocation patterns, ultimately leading to stunted growth and reduced yields. Brown et al. (2009) showed glyphosate drift rates significantly reduced corn height and shoot dry biomass up to 45% and 46%, respectively, depending on the rate. Moreover, if this drift occurs before in-crop herbicide application, the adverse impact of glyphosate drift is more destructive than alone. Similarly, sub-lethal rates of ALS/AHAS inhibitor herbicides can trigger a range of responses in non-target plants, including stunted growth, chlorosis, and altered reproductive development, potentially leading to yield reductions and diminished product quality. Bailey and Kapusta (1993) indicated that soybean plants subjected to sub-lethal exposures of nicosulfuron and primisulfuron exhibited reduced shoot growth and developed leaf cupping, leaf chlorosis, and leaf necrosis. Although numerous studies have been conducted on the impact of glyphosate and ALS/AHAS inhibitors on major crops, there is currently limited information available about the physiological responses of fennel to sub-lethal concentrations of glyphosate and flazasulfuron. Because of these gaps in knowledge, we measured how fennel responded to sub-lethal levels of glyphosate and flazasulfuron in a controlled setting, looking at visual injury, height, and biomass as outcomes.

The study was conducted to determine the physiological impacts of sub-lethal rates of glyphosate and flazasulfuron on fennel seedlings in climate room conditions.

MATERIAL AND METHOD

A dose-response study was conducted in a climate room adjusted to 24 ± 1 °C/20 ± 1 °C with a 16/8 h day/night photoperiod to determine the response of fennel to sub-lethal rates of glyphosate isopropylamin salt (Forfosat 48 SL; Koruma Klor Corporation, Kocaeli, Turkey) and flazasulfuron (Coswel 250 WG; Adama Corporation, Izmir, Turkey). The climate room was set light to LED lamps (10 W). Five fennel seeds were sown in plastic pots (7 × 7 × 8.5 cm) filled with white peat bedding substrate + white perlite (4/1 w/w). After emergence seedlings were removed so that only two plants remained per pot. The seedlings were irrigated using tap water as needed.

Sub-lethal rates (12.5%, 6.25%, 3.125%, 1% of the recommended rates) of Glyphosate (1440 g ai ha^{-1}) and flazasulfuron (50 g ai ha^{-1}) were dissolved in deionized water without any surfactant to simulate drift conditions. These rates were applied using a sprayer equipped with an even flat-fan nozzle mounted (Teejet EVS11001) at the 2-4 true leaf stage. Sprayer was calibrated to deliver 195 L ha^{-1}. Untreated control was included. The impact of the rates of the herbicides on fennel seedlings was evaluated 21 days after treatment (DAT). Herbicide efficacy on the fennel seedlings was visually

evaluated using a 0-100 scale, 0 indicated control plants, whereas 100 indicated dead plants. The height of the seedlings was measured from the soil surface to the top of them at 21 DAT, and they were cut from ground level. The seedlings were placed in a drying oven at 60 °C for 48 h to determine the above ground dry weight.

Treatments were arranged in a completely randomized design with four replications per treatment, and the experiment was repeated twice over time. The data were subjected to variance analysis using the Agricolae package (Mendiburu & Yaseen, 2020) in R statistical software (R Studio Team, 2024). The means were compared using the Least Significant Difference test at the 5% probability level.

RESULTS AND DISCUSSION

Glyphosate rates resulted in a statistically significant, dose-dependent increase in the severity of visual injury observed on fennel seedlings, where high glyphosate rates (above 45.13 g ai ha^{-1}) resulted in a statistically significant difference compared to the untreated control, whereas the lowest rate (14.44 g ai ha^{-1}) did not (Figure 1). Similar to the observations with glyphosate, flazasulfuron application also elicited a statistically significant and dose-responsive increase in the visual injury scores of fennel seedlings, except for the lowest rate (0.50 g ai ha^{-1}) (Figure 2). However, observed visual injury in fennel seedlings applied at the rates of 6.25% and 3.125% of the recommended rates of flazasulfuron was higher than that observed in fennel seedlings applied at the rates of 12.5% and 6.25% of the recommended rates of glyphosate. These results confirmed flazasulfuron's phytotoxic effect on fennel even when applied at low rates.

Figure 1. Response of fennel to glyphosate isopropylamine salt rates at the climate-room pot experiment.

Figure 2. Response of fennel to flazasulfuron rates at the climate-room pot experiment.

Plant height exhibited a significant reduction in response to increasing rates of both glyphosate and flazasulfuron, which is consistent with visual injury data (Table 1). Specifically, the application of flazasulfuron resulted in a more evident reduction in plant height compared to the application of glyphosate due to greater impact on the early vegetative development of fennel seedlings. Unlike dry biomass results, the lowest rates of both herbicides could not result in a significant height reduction compared to the control treatment. Consistent with the observed reductions in plant height, the above-ground dry weight of fennel seedlings exhibited a similar trend for both herbicides (Table 1). Increasing rates of them caused a decline in biomass accumulation, suggesting a direct impact of herbicide exposure.

Table 1. Response of fennel seedlings to sub-lethal rates of glyphosate isopropylamine salt and flazasulfuron in the climate room experiment

Herbicide	Rate (g ai ha^{-1})	Dry biomass (mg)	Height (mm)	Visual Injury (%)
Glyphosate isopropylamine salt	0	44.88 (5.87) a*	178.88 (7.20) a	-
	14.44	40.75 (2.79) a	175.75 (7.56) a	3.75
	45.13	35.25 (3.57) ab	148.13 (11.95) ab	16.25
	90.25	22.25 (1.81) bc	119.75 (6.32) bc	28.75
	180.50	21.00 (1.77) c	104.25 (10.05) c	45
Flazasulfuron	0	55.25 (7.73) a	135.13 (3.15) a	-
	0.50	49.50 (5.08) ab	139.38 (11.58) a	5
	1.56	32.50 (1.73) bc	134.75 (5.88) a	17.5
	3.13	22.75 (2.50) c	76.00 (7.51) b	31.25

	6.25	20.63 (1.92) c	70.88 (4.39) b	50

*Within each herbicide, means followed by different letters are significantly different at $P \leq 0.05$ (LSD).

Sub-lethal rates of glyphosate negatively affected fennel growth, similar to other susceptible crops, such as sunflower, soybean, wheat, and tomato, despite these plants exhibiting varying sensitivities to it. Sunflowers exposed to a rate of 86.4 g a.e ha^{-1} of glyphosate resulted in a 55% height reduction and a 50.47% decline in the number of leaves per plant (Vital et al., 2017), whereas soybeans exposed to a higher glyphosate rate, such as 140 g ai ha^{-1}, showed less sensitivity to glyphosate (Ellis and Griffin, 2002). Roider et al., (2007) indicated that glyphosate applied at a 140 g ai ha^{-1} reduced wheat height up to 36% 14 DAT while causing an injury up to 61% 28 DAT. Tomato is among the most sensitive plants, and a rate of 138.75 g a.e. ha^{-1} of glyphosate resulted in nearly 90% dry biomass reduction (de Paula Medeiros et al., 2023). Our results showed that fennel was highly sensitive to glyphosate, similar to other susceptible crops. However, fennel exhibited lower sensitivity than tomato but greater sensitivity than soybean.

Sulphonylurea herbicides are widely used, yet their unintended off-target movements, such as drift and runoff, have not been comprehensively studied (Askew et al., 2022; Jester et al., 2022). Askew et al. (2022) demonstrated that application of flazasulfuron at 5.2 g ai ha^{-1} or trifloxysulfuron sodium at 3.0 g ai ha^{-1} to corn seedlings in a corn bioassay resulted in approximately a 40 % reduction in dry biomass compared to non-treated controls. Our biomass reductions at 3.13 g ai ha^{-1} flazasulfuron (~59%) exceed corn bioassay declines reported for drift-equivalent SU doses. This result suggests greater fennel sensitivity under similar sub-lethal exposure.

CONCLUSIONS

This study proved that fennel is highly sensitive to sub-lethal rates of both glyphosate and flazasulfuron under controlled climate room conditions. Both herbicides induced significant reductions in plant height, visual injury scores, and above-ground dry biomass, even if they were applied at relatively low application rates. However, flazasulfuron exhibited greater crop injury than glyphosate. These results emphasized the potential risk of herbicide drift from adjacent fields or orchards to fennel crops. Therefore, strict drift management strategies and careful herbicide application practices should be implemented to protect fennel fields, especially in regions where these herbicides are commonly used in nearby cropping systems. Future research should focus on field-scale evaluations and recovery potential following sub-lethal herbicide exposure to better inform integrated weed management strategies.

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RAPID ASSESSMENT OF THREAT STATUSES OF FISH SPECIES IN DRIN RIVER

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ABSTRACT

Drin River is the longest river in Albania with about 285 km length. Its two branches start respectively from Lake Ohrid and from its waterfall in Western Kosovo. The fish fauna of the Drin River consists of 52 species. Of these, 9 species have a significant threat status according to the IUCN, while only 13 of them are included in the list of threatened species for Albania. In order to assess the global and national threat levels of the fish species of Drin River, after the preparation of the species list, IUCN global and national threat statuses have been established, as well as comparisons and discussions have been made for species or groups of species in different cases. From the list of threatened species for Albania, in 9 cases the national level of threat is higher than the global one, for 3 species it is lower and for one it's the same. More than half of the fish species of the Drin River belong to the group of market fish species that are used for consumption. The river Drin should be considered as an important habitat with many values in terms of fish fauna. Therefore, it is necessary to carry out in-depth studies and take measures to preserve the populations of these species.

Key words: fish, threatened species, Drin River, biodiversity, ecosystem.

INTRODUCTION

There is no doubt that lists of threatened species fulfil important political, social, and scientific needs. For example, endangered species lists are very useful in explaining to people the importance of species recovery or loss. In most circumstances, they are the only tools available that have a clear social mandate and that rely on substantial ecologically sound knowledge. (Possingham et al., 2002). However, these lists have many limitations in effectively addressing the problems they are used to solve, and ill-advised application carries its own risks. (Mace & Lande, 1991)

Biodiversity research and data are very important in several ways, not only for researchers but also for state institutions that design policies and act to protect biodiversity. Too often biodiversity data are not available to institutions, researchers and the general public in many countries or, if available, they are scattered in several different repositories that differ in platform, structure and data semantics. (Edwards et al., 2000, Schuurman & Leszczynski, 2008, Heidorn, 2011, Martellos & Atorre, 2012)

Although with a small area, Albania is internationally known for its high level of biodiversity, habitats and ecosystems. One of these important natural resources is the river Drin.

The Drin river basin covers a geographical area of about 20,000 km2 in the western Balkans; the basin extends through: Albania, Greece, North Macedonia, Kosovo and Montenegro. The Drin river basin is a hydrological interconnecting system consisting of the transboundary basins of Lakes Large Prespa and Little Prespa; Lake Ohrid; Lake Shkodra; The Drin River including its tributaries, the White Drin, the Black Drin and the Buna River. (Pano, 2008)

The Drin River flows through the mountain area of the Western Balkans towards the Adriatic Sea, thus providing the third and largest river discharge in the European Mediterranean. (Dhora, 2017)
Drin River is formed by the confluence of two rivers: Black Drin and White Drin. Black Drin covers an area of 9209 km2 (including the Prespa and Ohrid watersheds); 58% of this area lies in Albania (5,369 km2) and 42% in North Macedonia (3,840 km2) and flows north from North Macedonia to Albania (Kukes), where it joins the White Drin which originates on Mount Zljeb in Kosovo, and encompasses an area of 4,964 km2, 88% in Kosovo, and 12% in Albania. It then flows west for about 335 km, of which 285 km flows within Albania and the rest in Kosovo, North Macedonia and Montenegro. Its hydrological network is dense, consisting of numerous rivers, streams, springs and natural lakes. One of the branches of the Drin joins the Buna/Bojana River near the city of Shkodra and the other branch flows directly into the Adriatic Sea south of Shkodra, near the city of Lezha (Keci et al., 2022). The connection of the Drin river with the Buna River, right next to Shkodra Lake, makes this river affect the fish fauna not only of the Buna river, but also of the Shkodra lake. (Dhora et al., 2016)
The list of fishes of the river Drin contains 52 species which give a considerable value to this ecosystem. (Dhora, 2020). This fact shows the regional fauna-forming importance of the Drin River, as a very valuable resource in the entire hydrological network of the Balkan Peninsula. Considering the great value of this class of vertebrates, we have carried out this study in order to highlight their current situation and the need to preserve these species. The comparison between the global status according to the IUCN (http 1) and the national one, as well as the assessment of their condition in terms of their presence in the ecosystem, is seen as a valuable study by us, presenting the international importance of this group of species.
An important aspect is the treatment we have given to market and common species. Along the banks of the river Drin lives a significant population of inhabitants who have fishing as one of the economic alternatives of living. Many of the Drin fishes are poorly studied and further research will most likely lead to taxonomic revisions of the species (Holcik & Soric, 2004). Consequently, the recognition, monitoring and protection of fish species takes on even greater importance at the national and regional level.

MATERIAL AND METHOD

The list of fishes of the river Drin is taken from the publication of Dhora (2020) where the updated list of fish species of Albania according to ecosystems is given.
After ranking the list, for each species in particular, the verification of the actual global status of the IUCN in 2025 was made. Also, from this same source, the population trend for each species in particular, whether it is increasing, decreasing or stable, was obtained. In the column next to it, the year when the information was obtained and the assessment made by the IUCN for each species is placed (IUCN, 2025). For the populations of the species that this source did
not have information on, the note "?" has been placed.
In the following, based on the publication of Dhora (2018), a column has been placed for the inclusion or not of each species in the list of common species of the fauna of Albania.
Then the threat status in Albania was determined for each species separately. For this purpose, we have mainly relied on Order no. 1280 dated 20.11.2013 of the Minister of Environment "For the approval of the red list of wild flora and fauna".
Also, based mainly on the publication of Dhora (2022) but also on the local tradition, we have identified the market fish species in our country, for which the corresponding note has been placed.
All these data have been put in a table on which we have based to make the relevant comparisons and discussions.

The 9 categories of the Red List (IUCN) are listed below:
Threat categories:
EW - Extinct in the wild
EX - Extinct
VU - Vulnerable
EN - Endangered
CR - Critically endangered

Other categories:
NT - Near threatened
LC - Least concern
DD - Data deficient
NE - Not Evaluated

RESULTS AND DISCUSSION

Below are lists of fish species of the River Drin and relevant discussion of the species in particular. The list of fishes of the Drin River includes 52 species, which are grouped into 11 orders and 18 families. The largest family is Cyprinidae with 19 species.

Table 1. Information about fish species of Drini River

No	**Species name**	**Threatened status IUCN 2025**		**Year of evaluation IUCN**	**Common species Dhora, 2018**	**Albania Red List 2013**	**Market fish species**
	AGNATHA						
	PETROMYZONTIFORMES						
	Petromyzontidae						
1	*Eudontomyzon stankokaramani*	NT	?	2023			
2	*Petromyzon marinus*	LC	Decreasing	2022		VU	
	OSTEICHTHYES						
	ACIPENSERIFORMES						
	Acipenseridae						
3	*Acipenser naccarii*	CR	Increasing	2019		EN	+
4	*Acipenser sturio*	CR	Decreasing	2020		EN	+
5	*Huso huso*	CR	Decreasing	2019			
	ANGUILLIFORMES						

	Anguillidae						
6	*Anguilla anguilla*	CR	Decreasing	2018	+		+
	ATHERINIFORMES						
	Atherinidae						
7	*Atherina boyeri*	LC	Decreasing	2022			
	CLUPEIFORMES						
	Clupeidae						
8	*Alosa agone*	LC	Stable	2022			+
9	*Alosa fallax*	LC	?	2022	+	VU	+
	CYPRINIFORMES						
	Balitoridae						
10	*Barbatula sturanyi*	NT	?	2023			
	Cobitidae						
11	*Cobitis ohridana*	NT	Decreasing	2024	+	LC	
	Cyprinidae						
12	*Alburnoides ohridanus*	NT	?	2023			+
13	*Alburnus scoranza*	NT	Decreasing	2023	+		+
14	*Barbus rebeli*	NT	?	2023	+	LC	
15	*Carassius gibelio*	LC	Decreasing	2023	+		+
16	*Chondrostoma nasus*	NT	Decreasing	2023	+	LC	+
17	*Ctenopharyngodon idella*	LC	?	2020			+
18	*Cyprinus carpio*	LC	Decreasing	2022	+		+
19	*Gobio ohridanus*	EN	?	2023		LC	
20	*Gobio skadarensis*	LC	?	2023	+		
21	*Hypophthalmichthys molitrix*	NT	Decreasing	2011			+
22	*Hypophthalmichthys nobilis*	DD	Decreasing	2010			+
23	*Leucos basak*	LC	?	2023			+
24	*Pachychilon pictum*	NT	Decreasing	2023	+		+
25	*Pelasgus minutus*	VU	?	2023	+		
26	*Phoxinus lumaireul*	LC	?	2024	+		
27	*Pseudorasbora parva*	LC	?	2020	+		
28	*Rhodeus amarus*	LC	Increasing	2022	+		
29	*Scardinius knezevici*	VU	?	2023	+		+
30	*Squalius platyceps*	LC	?	2023			+

	CYPRINODONTIFORMES						
	Cyprinodontidae						
31	*Aphanius fasciatus*	LC	Decreasing	2022		EN	
	Poecilidae						
32	*Gambusia holbrooki*	LC	Stable	2012	+		
33	*Poecilia reticulata*	LC	Stable	2020		EN	
	PERCIFORMES						
	Blennidae						
34	*Salaria fluviatilis*	LC	Decreasing	2022		LC	
35	*Salaria pavo*	LC	Stable	2007			
	Gobiidae						
36	*Gobius paganellus*	LC	Stable	2014			
	Moronidae						
37	*Dicentrarchus labrax*	NT	Decreasing	2022	+		+
38	*Dicentrarchus punctatus*	LC	?	2014			+
	Mugilidae						
39	*Chelon labrosus*	NT	Decreasing	2022			+
40	*Liza aurata*	NT	Decreasing	2022			+
41	*Liza ramado*	NT	Decreasing	2022	+		+
42	*Liza saliens*	NT	Decreasing	2022			+
43	*Mugil cephalus*	LC	Decreasing	2022	+		+
	Percidae						
44	*Perca fluviatilis*	LC	Stable	2022			+
45	*Sander lucioperca*	LC	Stable	2022			
	PLEURONECTIFORMES						
	Pleuronectidae						
46	*Platichthys flesus*	LC	?	2022			+
	SALMONIFORMES						
	Salmonidae						
47	*Oncorhynchus mykiss*	LC	Stable	2020	+		+
48	*Salmo balcanicus*	CR	?	2024			+
49	*Salmo dentex*	DD	?	2024	+		+
50	*Salmo farioides*	NT	Decreasing	2024	+	VU	+
51	*Salmo marmoratus*	VU	Decreasing	2024		EN	+
	SYNGNATHIFORMES						
	Syngnathidae						

52	*Syngnathus abaster*	LC	?	2022			

As can be seen from the list of fish species in Drin River, 15 species have NT status, 26 species with LC status, 3 VU species, 1 EN species, 5 species with CR status and for 2 species there is insufficient DD data. The most endangered family is the Acipenseridae family with all three species in the critical stage of existence. However, if we look at the population trend, for *Acipenser naccarii* it is increasing, which gives hope for the improvement of the situation at the global level.

While for the other two species, the population is decreasing. Regarding the situation in Albania, the species of the genus Acipenser have the EN status of threat, which shows that the situation in our country is not good. While the species *Huso huso* is not evaluated at all in the Red List for Albania. The first two species (*Acipenser sp*.) are also market species in Albania and have had a considerable use until a few decades ago. While in recent years they have become very rare and have disappeared from the market, which is also consistent with the status given to them on the red list of Albania.

The family Petromyzontidae consists of two species in the Drin River, which have a relatively low threat status (NT and LC). However, of interest is the species *Petromyzon marinus* whose population trend is decreasing and such continuity may increase the level of threat to it. At the national level, the situation for this species is even more serious, as it has been assessed with the VU threat status. While for the species *Eudontomyzon stankokaramani* we have a lack of data on the population trend from the IUCN. This species is not even included in the red list of Albania.

The family Anguillidae is represented only by the species *Anguilla anguilla* in the river Drin. Despite the fact that this species has a CR status of global threat and the population is declining, in Albania it is a market species, it is part of the list of common species (Dhora, 2018) and there is no threat level in the red list for our country. The situation of this species should be studied in more detail for Albania, since by comparing the global threat level according to the IUCN, the population trend and the occupations made in recent decades in Shkodra Lake (Dhora, 2022) as one of the largest water ecosystems in Albania, it turns out that this species is not in a very good situation in our country and may have a tendency to decrease.

The family Atherinidae includes only one species in the river Drin, *Atherina boyeri.* This species has the LC level of threat and according to the IUCN its population is declining. As for Albania, apart from the fact of its physical existence, there are no other studies to show its current state.

The Clupeidae family in the Drin River is represented by the two species *Alosa agone* and *Alosa fallax*. Although for the second there is no data on the global population trend, the situation does not seem worrying for both species. They have the threat level LC and for *A. agone* the population is stable. As for Albania, *Alosa fallax* is not in a satisfactory situation. It has a nationally threatened VU status, but is called a common species and continues to be one of the market species for consumption. The situation of this species in Albania should be looked at more carefully, as the national threat status indicates that it is declining and if it continues in this way it may lead to its further threat.

The family Balitoridae is represented by the species *Barbatula sturanyi*, which globally has the NT status of threat, while there are no data on the population trend. As for her situation in Albania, there is no data at all. Of course, it would be worthwhile to conduct scientific studies to assess the situation of this species in Albania.

The family Cobitidae and the species *Cobitis ohridana* are not presented with a high level of threat, either globally or nationally. The global NT status of the IUCN and the LC status for Albania show the relatively good situation of this species. However, two aspects should be taken into account: its

world population is declining and this species is part of the list of common species of Albania (Dhora, 2018), which allows its fishing in significant quantities.

The largest fish family of the Drin River is the Cyprinidae with 19 species. At the world level, 3 species represent the greatest importance in terms of their status: *Gobio ohridanus* (EN), *Pelasgus minutes* (VU) and *Scardinius knozevici* (VU). Currently the IUCN has no information on the population trend for these species globally. While for Albania the situation of these three species is different. Two of these species, *Pelasgus minutus* and *Scardinius knezevici* are part of the list of common species for Albania (Dhora, 2018). Also, they have no threat status in Albania and *S. knezevici* is part of the list of market species (Dhora, 2022). While the species *G. ohridanus* has LC status for Albania. However, considering the global threat status, these three species should be kept in mind in our country as their situation can change rapidly.

For 7 species from the Cyprinidae family, we have a global population decline. For one species *Rhodeus amarus* we have an increase in the population, while for the rest there are no data at the global level. While at the national level, only three species are included in the red list of Albania and have LC status of threat (*Barbus rebeli, Chondrostoma nasus* and *Gobio ohridanus*), while the rest are not included in the red list of Albania at all. In this respect, it would be necessary to study this family in more detail and to know the current population situation for each species. In addition, 12 species of this family are market species and therefore take on greater importance at the national level.

The family Cyprinodontidae and the only species in this river *Aphanius fasciatus*, globally has LC status of threat and the population is in decline. While in Albania its situation seems more worrying, it has the EN status of threat and is not a common species at the national level.

The two species of the Poecilidae family, *Gambusia holbrooki* and *Poecilia reticulata*, are presented in a relatively non-disturbing situation worldwide. They have LC status according to the IUCN and their populations are in a stable state. However, the latest IUCN estimates are made over a decade ago and therefore the trend of their populations is not certain if it continues to be the same in recent years. While for Albania, only *P. reticulata* is of interest as it has an EN status of threat and must be kept in attention with continuous monitoring for the study of its population.

The Blennidae family with its two species does not pose a concern as it has a relatively acceptable situation at the global and national level for Albania. The threat stage LC at the global level, and LC for only one of them at the national level indicates that their situation currently does not appear to be problematic.

The family Gobiidae and the species *Gobius paganellus* appear relatively stable worldwide according to the IUCN, with LC status of threat. As for Albania, there is no inclusion of it in the red list of threatened species.

The two species of the genus *Dicentrarchus* of the family Moronidae are in a globally non-concerning situation, with threat statuses (NT and LC), but the world population trend is decreasing for *D. labrax* while for *D. punctatum* there is no information. As for Albania, both of these species are market species and are not included in the country's red list.

The Mugilidae family has 5 species, whose populations worldwide are declining. While the threat statuses for 4 of them are NT and only one has LC. Since world populations are declining and all are market species for Albania, it is necessary to conduct studies at the national level to control the population trend and the level of threat of these economically important species.

The Percidae family is represented by two species that globally have LC status, but their populations are in a stable state. While in Albania only *P. fluviatilis* is included in the market fish list and none of these two species is included in the Albanian red list.

The species *Platinchthus flesus* of the family Pleuronectidae globally has LC status, but there are no population trend data. While at the national level for Albania, there is no information on the situation of the population and it is not included in the country's red list. This species is considered a market species for the country.

The Salmonidae family consists of 5 species in the Drin River, all market species in Albania. At the world level, the two species *Salmo balcanicus* with CR status and *Salmo marmoratus* with VU status present more interest. While for Albania, in addition to the species *S. marmoratus*, which at the national level has the status EN, the species *S. farioides*, which has the status VU of threat, is also of interest. This species is even part of the list of common species of the Albanian fauna (Dhora, 2018). For the other two species, *O. mykiss* and *S. dentex*, the situation at the world and national level is not worrying, although more scientific studies are needed for the latter.

The family Syngnathidae in the Drin River is represented by only one species *Syngnathus abaster*, who has LC status of global threat, but there is no information on the trend of this population. While at the national level for Albania, it is not included in the red list of the Albanian fauna.

CONCLUSIONS

The fish fauna of the Drin River consists of 52 species. Of these, 9 species have a significant threat status according to the IUCN (VU, EN, CR), while only 13 of them are included in the Red List of threatened species for Albania.

From the list of threatened species for Albania, in 9 cases the national level of threat is higher than the global one, for 3 species it is lower and for one it is the same. Over 50% of the fish species of the Drin River belong to the group of market species that are used for consumption.

It is worth noting that 41 species (79%) of fish are relatively not threatened or threatened to a low degree.

In 9 cases of species included in the Red List of Albania, the threat status for Albania is higher than that at the global or European level, so these species face a higher level of threat in our country. We believe that this is mainly related to the environmental condition of ecosystems and the negative effects they suffer mostly as a result of human activity. It is also found in species whose populations at the global or European level are stable or on the way to improvement.

Recognizing their regional situation as well as the importance that the group of fish has for the local and regional economy, as well as from its position in the food chain of different birds, it is necessary that their populations should be monitored and given even a minimal protection status, since the perspective in relation to the world population trend is not promising.

The species that at the global and/or European levels are really threatened (CR, EN, VU) and that are not included in the red list of fauna species in Albania should be treated with special attention, monitoring their populations and giving the necessary conclusions for their management.

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INFLUENCE OF TEMPERATURE ON FERMENTATION RATE IN PRODUCTION OF DIFFERENT WINE TYPES

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ABSTRACT

Wine production is a complex process that integrates traditional methods with modern scientific approaches. The quality, variety, and processing methods of grapes are among the key factors determining wine characteristics. Grapes, with their natural content of sugars, organic acids, and phenolic compounds, are considered an ideal raw material for producing various wine types. The quality attributes of wine are directly influenced by grape cultivation conditions, the maceration process, harvest maturity, and production techniques. Additionally, control parameters such as temperature during the fermentation process significantly affect both production efficiency and the sensory and chemical properties of the final product. This study investigates the effects of temperature control and maceration on the fermentation process in the production of different wine varieties. Specifically, the impact of temperature on fermentation rate was statistically analyzed using red, rosé, and white wines produced from the Papaskarası grape. The study was conducted based on experimental design principles, and statistical analysis revealed that both wine type and temperature significantly influenced fermentation rate ($\alpha = 0.05$), with wine type having a greater effect than temperature. Red wine completed fermentation in the shortest time, particularly at 25°C, due to enhanced yeast activity from phenolic and nitrogenous compounds during maceration, while rosé and white wines required longer durations. Higher temperatures (25°C) reduced fermentation time compared to 15°C, and lower temperatures preserved aromatic properties in rosé and white wines. The findings are discussed in the context of different wine types, highlighting the critical role of temperature and maceration in optimizing production efficiency and wine quality.

Keywords: Wine, Fermentation, Maceration, Phenolic compounds, Density

INTRODUCTION

Throughout history, grapes have held significant importance not only as a fruit but also as a raw material for various products due to their nutritional value and sensory properties. Grapes are derived from the vine plant known as Vitis vinifera (Parihar & Sharma, 2021). The nutritional composition of grapes is characterized by a high sugar content (Venkitasamy, Zhao, Zhang & Pan, 2019). Additionally, grapes are rich in minerals such as calcium, potassium, sodium, and iron, and serve as an important source of vitamins, including vitamins A, B1, B2, niacin, and C. Generally, grapes consist of water, sugars, organic acids, phenolic compounds, pectic substances, aroma compounds, nitrogenous compounds, enzymes, vitamins, and minerals (Venkitasamy, Zhao, Zhang & Pan, 2019). Factors such as grape variety, soil and climatic conditions, applied technical and cultural practices, and particularly the degree of ripeness, significantly influence the composition of grapes (Çaşkurlu, 2023).

Grapes are cultivated for table consumption, drying, and winemaking purposes. The intense phenolic compounds present in grapes, particularly those grown for winemaking, determine their color

and flavor (sensory properties) (Hornedo-Ortega et al., 2020). The chemical composition of grape must, influenced by factors such as grape variety, harvest maturity, climatic conditions, and soil structure, has a critical impact on wine quality. During the aging process, the stability of wine is primarily affected by its chemical composition, light exposure, oxygen levels, storage temperature, and storage duration. Consequently, the maceration process is the most significant factor distinguishing different wine varieties (Scepanovic, Vuletic, Christofi & Kallithraka, 2024).

Red Wine

In red wine production, harvested grapes are destemmed and crushed. The crushed grapes are then transferred to fermentation tanks via a peristaltic pump. In the fermentation tank, the broken grapes, including skins and seeds, are held under controlled conditions throughout the fermentation period. Alongside a selected constant temperature and mixing process, yeast addition facilitates simultaneous alcoholic fermentation and maceration (Morata, 2018). Fermentation temperatures typically range between 20–30°C for industrial yeasts. Higher temperatures enhance the extraction of phenolic compounds (tannins and color compounds) from the grape skins (Chidi, Rossouw, Buica & Bauer, 2015), resulting in intense, richly aromatic, and full-bodied wines. After fermentation is complete, the grapes are pressed to separate the must from the skins and seeds. Following clarification in the tank, the resulting wine is either aged or bottled based on production decisions.

Rosé Wine

In rosé wine production, destemmed and crushed grapes undergo a short maceration process with their skins and seeds, lasting between 4–24 hours, depending on the grape variety and desired wine characteristics (Radeka, Eukic & Peršuric, 2012). After maceration, the must is separated from the skins and seeds through pressing and transferred to fermentation tanks for fermentation. Following yeast addition, fermentation is completed under controlled conditions. After clarification, the resulting rosé wine is either aged or bottled based on production decisions. Fermentation temperatures for rosé wine typically range between 12–20°C (Salinas, Garijo, Pardo, Zalacain & Alonso, 2005). Rosé wines aim for fresh and fruity aroma similar to white wines, with slight tannin and color extraction, resulting in balanced, aromatic, and lightly tannic wines.

White Wine

Unlike other wine types, white wine production does not involve maceration (Bestulić et al., 2022). Destemmed grapes are crushed and immediately transferred to a press. The must, separated from the skins and seeds, is then moved to fermentation tanks for fermentation. Fermentation temperatures range between 10–15°C (Samoticha, Wojdyło, Chmielewska & Nofer, 2019). Preserving fresh, aromatic, and fruity characteristics is a priority in white wines. Lower temperatures result in slower fermentation, minimizing the loss of aromatic compounds and yielding a fresh, fruity, and vibrant flavor profile.

At the start of fermentation, grape juice has a high sugar content, resulting in a higher initial density (1.090–1.100 g/cm³). During fermentation, yeasts convert the natural sugars in the grape juice into alcohol and carbon dioxide (Figure 1) (Maicas, 2020). As the sugar content decreases and the alcohol percentage increases, the density decreases (Neto, de Castilhos, Telis & Telis-Romero, 2015). Density is typically measured at regular intervals to help winemakers monitor the progress of the process. When fermentation is complete, residual sugar levels are minimal, and the alcohol percentage has increased, causing the density to drop to a specific level. Winemakers generally consider fermentation complete when the density falls to approximately 0.995–0.997 g/cm³.

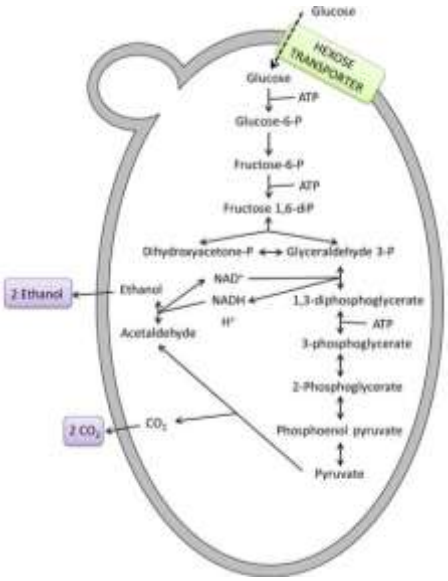

Figure 1. Fermentation metabolism of yeast

MATERIAL AND METHOD

The Papaskarası grape variety was sourced from vineyards in the Kırcasalih region of Edirne (Figure 2). Saccharomyces cerevisiae yeast was used in wine production. Using the same grape variety, red, rosé, and white wine varieties were produced, as shown in Figure 3.

Figure 2. Papaskarası grapes

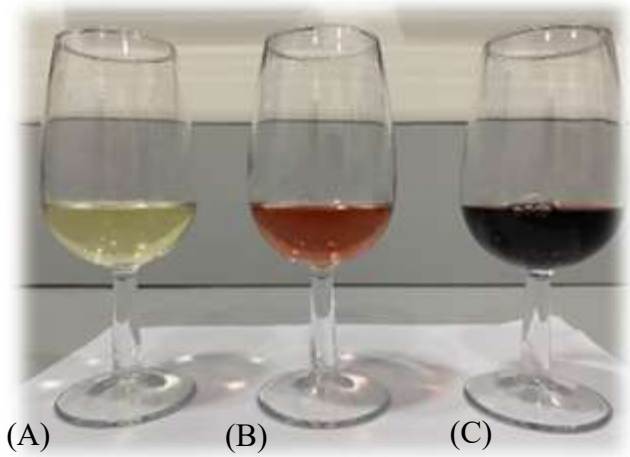

Figure 3. Wine types used in the study: (A) white wine, (B) rosé wine, (C) red wine

All samples were processed in equal quantities under identical production conditions, with the same amount of production chemicals applied to each. Fermentation temperatures were set at 15°C and 25°C. Density measurements of the wines were conducted over approximately 14 days. These measurements were performed using a densimeter, as shown in Figure 4, and fermentation was considered complete when a density of 997 g/cm³ was reached. Statistical analysis was performed using the Minitab 18 program based on the fermentation durations. A general factorial design method was employed, with two factors: wine type and fermentation temperature. The experiments were conducted with two replicates ($\alpha = 0.05$).

Figure 4. Densimeter

RESULTS AND DISCUSSION

Density measurements were conducted over a 14-day period to monitor the fermentation process. Fermentation durations for different wine types at two temperature levels are presented in Table 1, while the data used for statistical analysis are summarized in Table 2. The results obtained from the Minitab program are shown in Table 3.

Table 1. Density Values in the Production of Different Wine Varieties

	Red wine				**Rosé wine**				**White wine**			
	Density (g/cm³)											
ºC	**15**		**25**		**15**		**25**		**15**		**25**	
Days	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**
1	1107	1107	1107	1107	1105	1105	1105	1105	1093	1093	1093	1093
2	1107	1008	1081	1080	1099	1101	1099	1100	1090	1088	1083	1085
3	1070	1079	1040	1037	1092	1099	1087	1085	1084	1080	1072	1074
4	1039	1045	1029	1021	1083	1086	1064	1064	1075	1071	1054	1040
5	1024	1031	1010	1007	1054	1060	1032	1036	1045	1044	1039	1024
6	1012	1018	1002	1001	1031	1029	1020	1022	1027	1021	1003	1009
7	1005	1012	999	997	1013	1009	1002	1003	1012	1013	1001	1001
8	1000	1007	997	997	1003	999	1000	1000	1005	1010	999	1000
9	999	1003	997	996	999	999	997	997	1000	1007	997	997
10	997	997	996	996	999	997	997	997	997	999	997	997
11	997	997	996	996	997	997	997	997	997	997	997	997
12	996	997	996	996	997	997	997	997	997	997	997	997
13	996	996	996	996	997	997	997	997	997	997	997	997
14	996	996	996	996	997	997	997	997	997	997	997	997

Table 2. Effect of Different Temperatures and Wine Varieties on Fermentation Time

Wine types	**Red wine**		**Rosé wine**		**White wine**	
Temperature (ºC)	**Fermentation time (day)**					
15	10	10	10	11	10	11
25	8	7	9	9	9	9

Table 3. ANOVA Table

Variation sources	**Sum of Squares (SS)**	**Degrees of Freedom**	**Mean of Squares (MS)**	**F_0**	**p value**
Temperature	10,0833	1	10,0833	37,23	0,0000
Wine type	2,6667	2	1,3333	4,92	0,040
Interaction	0,6667	2	0,3333	1,33	0,332
Error	1,500	6	0,2500		
Total	14,9167	11			

In this study, the main factors, wine type and temperature, were found to significantly affect fermentation rate ($\alpha = 0.05$). The effect of wine type was observed to be greater than that of the selected temperature values. However, no significant interaction was observed between wine type and

temperature ($p > 0.05$). As shown in the main effects plot, red wine completed fermentation in the shortest time, while rosé and white wines took the longest. Examination of the temperature effect in Figure 5 indicates that fermentation time decreased with increasing temperature.

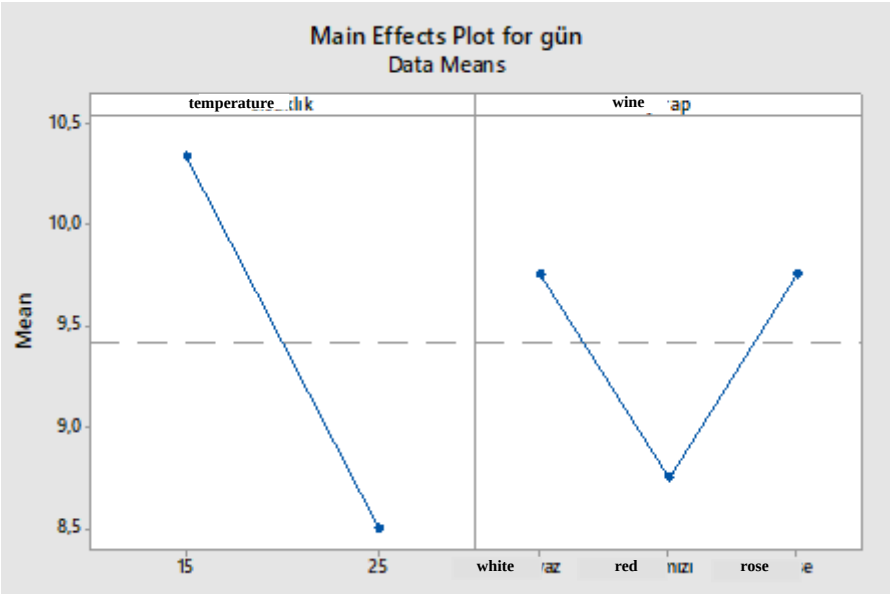

Figure 5. Main Effects Graph

During fermentation, sugars are converted into alcohol and carbon dioxide, resulting in a decrease in wine density. In wine production, a prolonged period of stable low-density values indicates the completion of fermentation (Nelson, Boulton & Knoesen, 2022). Winemakers can monitor this change to determine whether fermentation is complete. In this study, the effects of maceration and temperature were evaluated based on the density values of the wine.

Maceration is a process in winemaking that facilitates the transfer of phenolic compounds, color pigments, and aromatic components from grape skins, seeds, and pulp into the must or wine (Morata, González, Tesfaye, Loira & Suárez-Lepe, 2019). The maceration process influences color and polyphenol extraction, aromatic profile development, tannin extraction, fermentation rate, fermentation temperature, and chemical stability during alcoholic fermentation. Studies on the duration of maceration show that shorter maceration periods yield lighter, fruitier wines, while longer periods produce more tannic, complex wines with higher aging potential (Casassa, Bolcato, Sari & Barda, 2021). In this study, the greater influence of wine type on fermentation rate is attributed to the maceration process. It was concluded that, during red wine production, nitrogen and bioactive compounds present in grape skins enhance yeast activity during alcoholic fermentation, thereby accelerating the process. A study examining the effects of different maceration techniques on red and rosé wines reported that longer maceration periods improved sensory properties, overall wine quality, and aroma intensity. Conversely, short maceration periods resulted in distinct aromas and lower phenolic content, making them suitable for rosé wine production (Radeka, Eukic & Peršuric, 2012).

Temperature control during fermentation is critical for ensuring efficient yeast activity and achieving desired aroma and flavor profiles (Ruiz et al., 2019). Temperature directly affects yeast activity and, consequently, the rate of sugar conversion to alcohol and carbon dioxide. Analysis of the temperature values used for the wine types in this study revealed that higher temperatures positively influenced fermentation rate. Regardless of sensory properties, red wine completed fermentation in

the shortest time at 25°C. A study on the effect of fermentation temperature on rosé wine production used temperatures of 5°C, 10°C, and 15°C. Wines produced at 15°C exhibited the highest color values and the lowest alcohol content. Additionally, sensory analysis tests indicated that the most preferred rosé wine was produced at 15°C (Salinas, Garijo, Pardo, Zalacain & Alonso, 2005).

CONCLUSIONS

This study evaluated the effects of temperature and maceration processes on the fermentation of red, rosé, and white wine varieties produced from the Papaskarası grape. The findings demonstrate that both temperature and wine type significantly influence fermentation rate. Specifically, fermentation processes conducted at 25°C were observed to complete more rapidly compared to those at 15°C. Furthermore, red wine exhibited the shortest fermentation duration, attributed to the contribution of phenolic and nitrogenous compounds from grape skins during maceration, which enhanced fermentation. In rosé and white wines, lower temperatures were found to positively affect the preservation of aromatic properties. The study's findings underscore the critical importance of selecting appropriate temperature and maceration durations in wine production to optimize both production efficiency and product quality. Additionally, this study provides valuable insights into the impact of production techniques on wine quality across different wine types.

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MIGRATION OF FRESHWATER FISHES IN TÜRKİYE AND OBSTACLES ENCOUNTERED BY FISHES DURING MIGRATION

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ABSTRACT

Türkiye's inland waters have significant potential in terms of fish species diversity. Recent studies have reported that 427 fish species, 215 of which are endemic, inhabit Türkiye's inland waters (lakes, rivers, dams, etc.). Some fish species prefer a settled life, while others have to migrate long or short distances. Physical, chemical and biological factors such as light, water depth, currents, pollution, sexual development, spawning, feeding and escape from predators play an important role in fish migration. During migration, various natural or man-made hydraulic obstacles, especially those that are not suitable for the biology and ecological requirements of freshwater fish, put pressure on the development, reproduction and genetic characteristics of many fish species and may cause some species to the point of extinction. In order for fish migrations to take place as they should, the removal or reorganization of some existing obstacles in rivers and even the construction of suitable fish passages on river systems positively affect the continuity of fish populations.

In this study, the barriers that harm the diversity of freshwater fishes in Türkiye and what can be done to prevent the damages caused by these barriers were evaluated.

Keywords: Freshwater fish, Fish migration, Hydraulic barriers, Biodiversity

INTRODUCTION

Freshwater ecosystems are rich and important structures in terms of biodiversity. However, biodiversity in most of these ecosystems is relatively poorly understood (Filiz and Tarkan, 2017). Türkiye is one of the countries in Europe that stands out for its diversity of freshwater ecosystems, with 25 hydrological basins (Ministry of Agriculture and Forestry, 2020). Türkiye's inland waters (lakes, rivers, wetlands, dams, etc.) host a variety of living species and contribute significantly to biodiversity with 427 fish species. Of these species, 215 are endemic (Çiçek et al., 2023). Most fish species generally prefer a sedentary lifestyle. Species that live in a specific environment do not move, or they migrate daily between pelagic and coastal zones. Some species (trout, eels, etc.) migrate over long or short distances between different aquatic ecosystems to continue their generations (Geldiay and Balık, 2009; Tut Deng and Tsegaye Demisse, 2023).

Migration has a significant impact on population structure and can influence species composition in the region. Fish migrate depending on their developmental stages and different functions throughout the year (DSİ, 2016; Avşar, 2005). Migratory fish migrate either individually or in schools and generally return to their place of origin (Tut Deng and Tsegaye Demisse, 2023). But some species undertake non-returnable migrations.

Fish migrate for various reasons. The main reasons are spawning, feeding and sheltering.

Feeding Migration: Both young and mature individuals undertake this migration. It is a type of migration undertaken by adult fish or newly hatched larvae after spawning to waters rich in food sources in order to grow and store energy (Avşar, 2005).

Wintering Migration: At the end of summer, during the winter months, the migration of young and old individuals from their current location to a more suitable area to escape from unfavorable conditions is called sheltering migration (Avşar, 2005).

Reproduction (Spawning Migration): This is a type of migration undertaken by sexually mature fish, consisting entirely of adult individuals, for the purpose of reproduction (Avşar, D. 2005). It is important to find the most suitable, safe and nutrient-rich environment for the juveniles.

Fish may migrate for reasons other than these primary migration causes. Particularly during early developmental stages (eggs, larvae, and immature juvenile fish), they may be drifted along by water currents as they are unable to swim against them. Changes in their habitats may drive them to disperse to different areas (DSİ, 2016).

TYPES OF FISH MIGRATION

Fish migration can be categorised into different types (Şimşek et al., 2022; Geldiay and Balık, 2009).

Diadromous: This is a type of migration that occurs between the sea and freshwater. Fish that undertake this type of migration adapt to the changing salinity of their environment through their osmoregulation abilities, as they move between aquatic ecosystems with different salinity levels.

a-Anadromous: This refers to diadromous migration of species that can reproduce in freshwater but complete their growth phase in the sea. Salmon, sea trout, and sturgeon are examples of this type of fish. *Salmo labrax* (Black Sea salmon) is a species that enters Turkey's inland waters to reach its spawning grounds. These fish, which typically migrate long distances, expend a great deal of energy during their migration and die after spawning.

b-Catadromous: This refers to diadromous migration of species that can reproduce in the sea but complete their growth phase in freshwater. Eels (*Anguilla anguilla*), which live in coastal waters and some lagoons in Turkey and migrate to the Gulf of Mexico to spawn, are an important catadromous fish species.

c-Amphidromous: A special type of diadromous migration undertaken by species that move between saltwater, brackish water and freshwater habitats.

Potamodromous: These are migrations that occur solely in freshwater. Some freshwater fish migrate upstream of lakes and rivers to spawn. A typical example of this group is *Alburnus tarichi* (pearl mullet) found in Lake Van.

Oceanodrom: These are migrations that occur only in the seas. Oceanodromic migrations occur for various reasons and can take place both horizontally and vertically, over both short and long distances.

FACTORS BLOCKING FISH MIGRATION

Fish move not only laterally but also longitudinally (upstream and downstream along the main channel) in their habitats. Fish may encounter various limitations and difficulties while migrating. Changes in habitat conditions affect fish migration behaviour (Tut Deng and Tsegaye Demisse, 2023). Factors hindering fish migration can be classified and examined as physical (structural), chemical, and biological.

Chemical Barriers

When domestic and industrial waste, fertilisers and agricultural pesticides enter aquatic systems, they alter the chemical composition of the water, causing water quality to deteriorate and harming the organisms living there. The wastes cause a decrease in the amount of dissolved oxygen in the water, which can lead to the death of fish. The toxic effects of wastes also cause fish fatalities. Pollutants entering the aquatic environment impair fishes' sense of navigation and smell. (Atamanalp et al., 2013; Küçük, et al., 2018).

Sediment on the bottom of aquatic systems, caused by erosion, deforestation, construction, and improper land use, prevents fish from finding food. Fish eggs and larvae are harmed by the decrease in water and oxygen levels in the environment. Fish whose senses of eyesight and smell and their ability to navigate are impaired find it difficult to migrate.

Biological Barriers

Alien and invasive species compete with native species for food and habitat in the aquatic environments they enter, and they contribute to hybridisation, habitat destruction, and even the transmission of diseases. They can feed on the eggs and larvae of native species living in the habitat they enter. Migrant native species populations, already struggling with other stresses, are further reduced and brought to the brink of extinction under the pressure of these invasive species. *Carassius gibelio* adapts better to the conditions of the habitats it enters than native fish species, is more successful in colonisation, and plays a role in displacing most native species from the environment (Tarkan, 2013; Tarkan et al, 2012).

Migration is the most vulnerable time for fish. Fish schools that gather at specific places for spawning migration (below dams, river narrow passages) are easy targets for poachers. This directly and severely affects the population's capacity to regenerate itself (Küçük et al, 2018).

Structural Barriers

These obstacles are man-made or natural structures that make it extremely difficult or impossible for fish to access their preferred habitats. Dams, hydroelectric power stations, weirs, regulators, embankments, culverts, irrigation channels, bridges on river systems, etc. structures can slow down, stop or change the direction of water flow by blocking rivers. The migratory movements of fish that encounter such changes in the aquatic environments they enter are restricted (İlhan et al, 2024).

Türkiye ranks among the countries with the most dams in Europe, having 861 dams. Nearly all major river systems, such as the Euphrates, Tigris, Çoruh, Kızılırmak, Yeşilırmak, and Sakarya, have been blocked by a series of dams (dsi.gov.tr). It has been reported that the migration of fish has been impeded in the Van Lake basin, one of Türkiye's important basins, due to the failure to construct fish passages and fish ladders to facilitate fish passage during the construction of dams, regulators and reservoirs (Elp et al, 2006).

The Büyükçekmece Lagoon (Istanbul), an important reservoir, was blocked from the sea by the construction of dam gates. Over time, the fish population has changed, affecting migratory species such as *Acipencer gueldenstaedtii* and *Anguilla anguilla* (Saç and Özuluğ, 2017).

FISH PASSAGES

Fish passages are hydraulic structures designed to provide suitable flow conditions for fish to swim easily without being harmed by natural and artificial obstacles encountered along their migration routes. Various fish passages and numerous variations of these passages have been developed. There

are four main types of fish passages. These are: Pool type fish passages, Denil fish passages (Larinier type), Vertical slot fish passages and Culvert-type fish passages (DSİ, 2016; Üçüncü and Altındağ, 2012).

Pool-type Fish Passage: It is one of the oldest and most commonly used types of fish passage. This fish passage is usually constructed for species with strong swimming abilities and requires a large area.

Vertical Slot Fish Passages: These fish passages have a similar system to pool type fish passages. There are curtains placed at regular intervals along the passage, and these curtains direct the flow of water.

Denil Fish Passages: The Denil passage consists of a linear channel with regularly organised, short-interval plates. The plates inside are positioned at an angle against the direction of water flow. These passages adapt to changes in water level.

Culvert-type Fish Passages: These are channels constructed in elliptical, rectangular, circular, pipe-arch or square cross-sections, used to convey water from one side of the road to the other in areas where culverts are required for fish passage.

In addition to these four types of fish passes, fish ladders, fish lifts, fish ramps and trap-type fish passes also contribute to finding ecological solutions for fish migration.

CONCLUSIONS

Many species that cannot change their habitat to meet their needs, becoming trapped in specific areas and experiencing intense pollution and fishing pressure, face the threat of extinction from the ecosystem. To eliminate these negative effects, freshwater ecosystems must be considered as a whole, the physical, chemical and biological characteristics of aquatic environments must not be overlooked, and species-specific solutions must be developed.

Fish passages are structures that play an important role in ensuring the continuity of fish migration. It is believed that the construction of fishways as environmentally friendly structures can prevent the problems fish encounter during migration (Üçüncü and Altındağ, 2012; Silva et al., 2017, Bozkurt and Yüksel, 2017). Following detailed research in areas where fish passages are required, passages with the necessary characteristics to meet the need should be constructed and regularly monitored. Interdisciplinary coordination should be prioritised during the design of the passages. The efficiency of fish passages should be monitored regularly, the necessary data collected and the results reported. The information obtained will be useful to experts and managers in better managing fish stocks.

Avoiding pollution, preventing overfishing and poaching, and stopping invasive and alien species from being released uncontrollably into aquatic habitats will also help protect fish stocks. New structures can be built to protect natural migration routes, or existing structures (dams, weirs, canals, embankments) can be removed to allow the natural process to take its course.

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EFFECTS OF BLENDING DIFFERENT RATIOS OF COTTONSEED OIL WITH DIESEL FUEL IN A SINGLE-CYLINDER DIESEL ENGINE

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ABSTRACT

The persistent reliance on internal combustion engines, particularly within the transportation sector, continues to pose significant environmental challenges due to the emission of exhaust gases. This issue has intensified global efforts to identify and develop alternative fuels that can mitigate greenhouse gas emissions associated with fossil fuel consumption. Concurrently, the gradual depletion of petroleum reserves and volatility in fuel prices have introduced economic sustainability concerns. In this experimental study, the impact of blending conventional diesel fuel with 10% and 20% cottonseed oil by volume on engine performance and exhaust emissions was evaluated using a single cylinder direct injection diesel engine. The results demonstrated that the addition of cottonseed oil did not significantly affect engine power output or brake specific fuel consumption. However, a notable decrease in carbon monoxide (CO) emissions was observed, accompanied by an increase in nitrogen oxides (NO) emissions with higher biodiesel ratios. These findings indicate that cottonseed oil–diesel blends up to 20% by volume can be considered a technically viable partial substitute for conventional diesel fuel in compression ignition engines, with tradeoffs in emission characteristics.

Keywords: Energy, diesel engine, emissions, cotton seed oil

INTRODUCTION

The level of energy diversity and energy consumption is widely recognized as an indicator of a country's level of development. With the growing global population, meeting the increasing demand for energy and addressing associated economic factors have become critical challenges. In this context, the concept of sustainability requires balanced planning that considers energy production, resource diversity, costs, and environmental impacts. The continued use of internal combustion engines, coupled with the gradual depletion of fossil fuel resources, rising costs, and harmful emissions, has become a matter of growing concern for much of society. The use of biodiesel as an additive to petroleum-based diesel fuel in certain proportions is considered a viable alternative to mitigate these negative impacts [1–3]. Moreover, standard diesel fuel is known to be harmful to human health due to the emission of toxic oxides and its contribution to acid rain [4-5-6]. During the desulfurization process of diesel fuel, the removal of polar compounds results in a significant reduction in the fuel's lubricating properties [7]. These polar compounds typically form a protective layer or lubricating film between metal surfaces, thereby reducing wear and friction. The absence of such compounds in ultra-low sulfur diesel (ULSD) significantly decreases its lubricity [4]. Lubricity is particularly critical for diesel engine components such as fuel pumps and injectors, where proper lubrication is essential to prevent mechanical wear and maintain system efficiency [8]. To compensate for the loss of lubricity in diesel fuel, biodiesel has been increasingly used as a natural lubricity enhancer. Compared to mineral based

fuel additives, biodiesels derived from vegetable oils are considered environmentally friendly due to their biodegradability [9]. In this regard, several studies in the literature have focused on the use of cottonseed oil biodiesel. For instance, in a study conducted using a single cylinder, four stroke, direct injection diesel engine, different ratios of cottonseed oil were blended with diesel fuel to evaluate their impact on engine performance. When 10% cottonseed biodiesel was blended with diesel, the brake specific fuel consumption (BSFC) increased by 29.15%. However, average reductions in hydrocarbon (HC), smoke, and nitrogen oxide (NO) emissions were observed at rates of 12.89%, 4.12% and 8.84%, respectively, compared to standard diesel [10]. In another experimental study, diesel fuel was blended with cottonseed oil and 5% ethanol. The results indicated improvements in the fuel blend's physicochemical properties, engine performance, and reductions in harmful exhaust emissions. Additionally, a 7% decrease in kinematic viscosity was reported [11]. A more recent investigation examined an alcohol enhanced, water emulsified cottonseed oil biodiesel in a single cylinder diesel engine. Compared to conventional diesel, the emulsified blend improved combustion and significantly reduced exhaust emissions. Notably, the addition of 10% pentanol resulted in reductions of 14.3% in HC emissions and 12.8% in carbon monoxide (CO) emissions. Furthermore, a 1.4% decrease in brake specific fuel consumption was observed [12].

Distinguishing itself from previous studies, the present research experimentally investigates the effects of blending 10% and 20% cottonseed oil biodiesel by volume with standard diesel fuel on the performance and emission characteristics of a single cylinder, four-stroke diesel engine.

MATERIALS AND METHODS

In this study, a single cylinder, four stroke diesel engine, shown in Figure 1, was used at the Erin Motor R&D Center. To investigate the effects of the fuel blends on engine performance, two fuel mixtures were prepared by blending 10% and 20% cottonseed oil biodiesel by volume with 1 liter of standard diesel fuel. Before starting the tests, the engine was operated under no-load conditions until the radiator coolant temperature reached 82°C to ensure thermal stabilization. Once the coolant reached the desired operating temperature, engine tests were conducted at four different load conditions 25%, 50%, 75%, and 100% using a dynamometer. Initially, tests were carried out using standard diesel fuel. Subsequently, the same procedure was repeated for the fuel blends containing 10% and 20% biodiesel.

During the experiments, engine power, fuel consumption, and exhaust emissions specifically carbon monoxide (CO) and nitrogen oxides (NO)were measured simultaneously. Based on the collected experimental data, performance and emission characteristics were analyzed, and graphical representations of variations in power output, fuel consumption, and emission levels were produced.

Figure 1: Test Engine

Technical specifications and necessary information of the test engine are given in table 1.

Table 1: Technical Specifications of The Test Engine

General Characteristics	Technical Specifications

Engine type	Erin Motor Base Model
Number of Valves	4
Continuous Power (kw/rpm)	11.5 / 1500
Bore (mm)	108
Stroke (mm)	127
Combustion System	Direct Injection
Compression Ratio	14.6 : 1
Engine Cooling	Water
Weight (kg)	157

RESULTS AND DISCUSSION

Based on the experimental results, the effects of blending different proportions of cottonseed oil biodiesel with standard diesel fuel on engine performance and exhaust emissions were evaluated and illustrated through graphical analysis. When standard diesel fuel was used, the engine produced power outputs of 3.0 kW, 5.9 kW, 8.8 kW, and 11.4 kW under 25%, 50%, 75%, and 100% load conditions, respectively. With the addition of 10% and 20% cottonseed oil biodiesel, a slight decrease in engine power ranging from 0.1 to 0.2 kW was observed across all load levels. The effect of standard diesel fuel and the blended fuels (containing 10% and 20% biodiesel) on engine power output is presented in Figure 2.

These results indicate that the addition of cottonseed biodiesel to diesel fuel caused no significant change in engine power output. The observed power losses were minimal and within acceptable limits, suggesting that such blends can be used without substantial performance degradation.

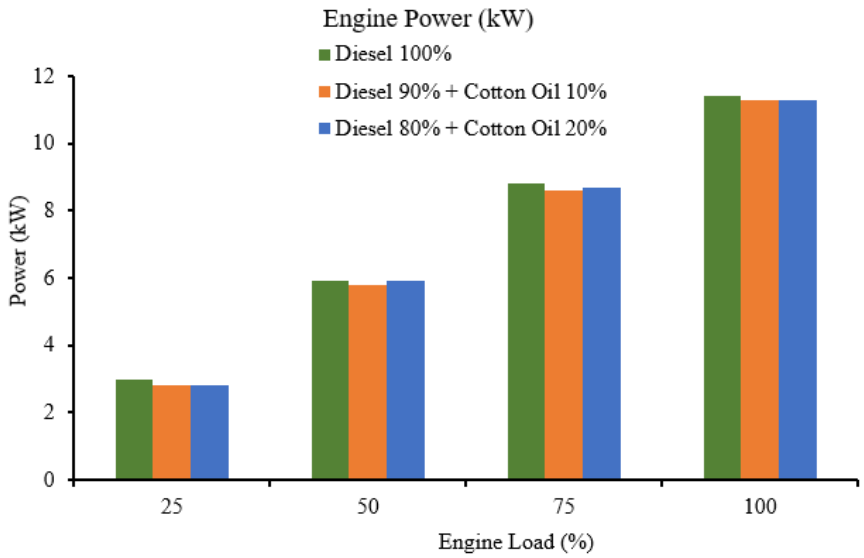

Figure 2 : Power variation according to the load on the engine

Furthermore, under all load conditions tested, the addition of 10% and 20% cottonseed oil biodiesel to standard diesel fuel resulted in a slight reduction in fuel consumption. As shown in Figure 3, fuel consumption decreased consistently across all load levels, with the blend containing 10% cottonseed oil demonstrating the most favorable results. This indicates that the 10% biodiesel blend offers improved combustion efficiency compared to both standard diesel and the 20% blend, likely due to better atomization and oxygen content in the fuel.

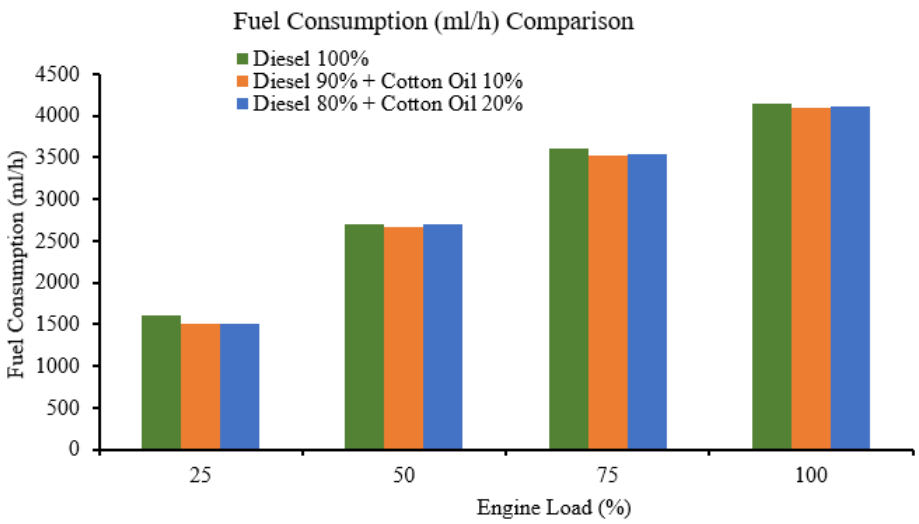

Figure 3: Change in fuel consumption as a function of engine load and fuel type

During the experiments, carbon monoxide (CO) emission levels at various engine loads were measured and are presented in Figure 4. It was observed that under higher load conditions, particularly at 75% and 100% engine load, the addition of 20% cottonseed oil biodiesel to standard diesel fuel resulted in a noticeable reduction in CO emissions. At other load conditions and blend ratios, CO emission levels remained nearly unchanged compared to standard diesel.

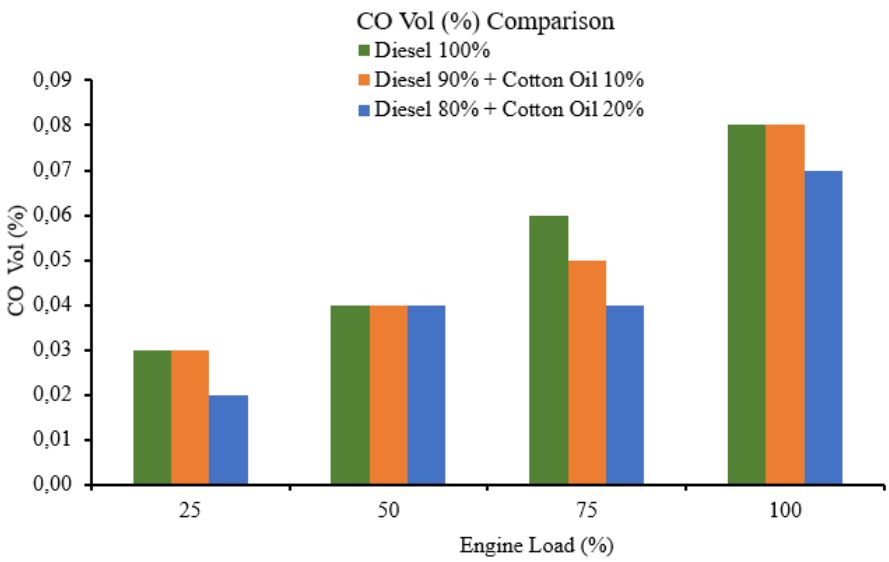

Figure 4: Change in CO in engine exhaust emissions as a function of load and fuel type

The nitrogen oxides (NO) emission levels measured under different load conditions for standard diesel fuel and blends containing 10% and 20% cottonseed oil biodiesel are presented in Figure 5. Across nearly all load conditions, the addition of cottonseed biodiesel resulted in an increase in NO emissions compared to standard diesel fuel. This rise in NO levels is consistent with the higher combustion temperatures and oxygen content associated with biodiesel blends.

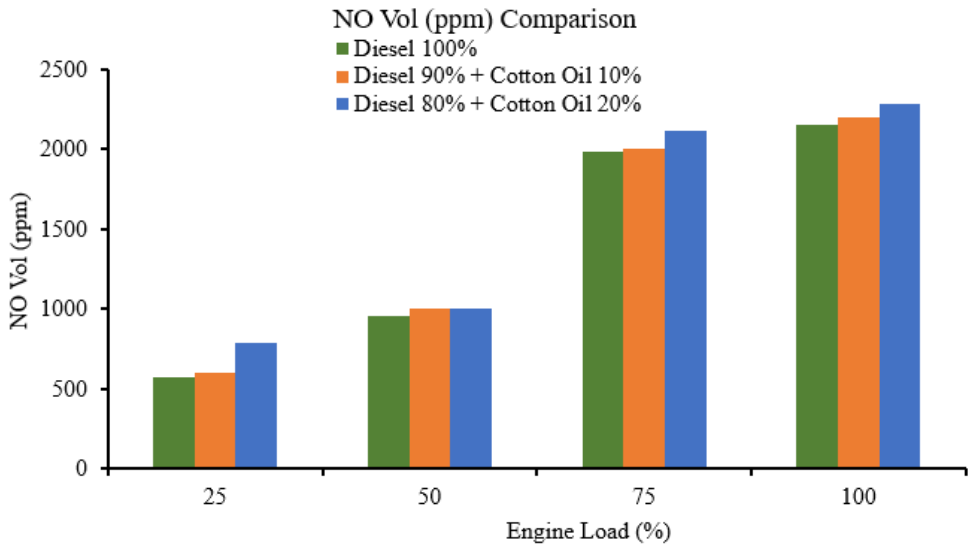

Figure 5: Change in NO in engine exhaust emissions as a function of load and fuel type

CONCLUSIONS

In this study, the effects of biodiesel blends containing 10% and 20% cottonseed oil by volume with standard diesel fuel on engine characteristics were investigated. Based on the experimental results, it

was found that blending cottonseed oil at these ratios led to a very slight reduction in engine power, with a maximum decrease of approximately 0.2 kW. However, under almost all load conditions applied to the engine, a reduction in the amount of carbon monoxide a harmful exhaust emission was observed, while a slight increase in nitrogen oxide (NO) emissions occurred. Additionally, a minor decrease in fuel consumption was detected. These results suggest that it is feasible to blend 10% to 20% cottonseed oil with standard diesel fuel for use in a single cylinder diesel engine without significant negative effects on engine performance.

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SOURCES OF INSTITUTIONAL SUPPORT FOR SUSTAINABLE MANAGEMENT OF TREES FROM THE GENUS *Tilia sp.* IN BOSNIA AND HERZEGOVINA

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ABSTRACT

The research of the three dominant linden species in Bosnia and Herzegovina as part of the DBU "MOE" 2021 scholarship program opened the door for a new perspective of these species in the Bosnian-Herzegovinian economy, primarily forestry as an industrial branch. Species from the genus *Tilia* in Bosnia and Herzegovina are characterized by a wealth of intra- and interpopulation diversity, and due to their good properties (primarily resistance to long-term drought) they are considered trees of the future. The goal of the wider research project is to assess the possibilities for raising linden nurseries in Bosnia and Herzegovina in the future, so that the country can provide high-quality, highly resistant planting material, which would be used in mass afforestation. Based on the review of transparent information of forestry companies in the country, it was learned that several companies are already growing linden trees in their nurseries. This article discusses how support could be provided to forestry experts, who could possibly promote the idea of more frequent planting of trees from the genus *Tilia* in Bosnia and Herzegovina in the future. Namely, Bosnia and Herzegovina as a country has a relatively complex administrative structure, however, this should not be an obstacle to sustainably managing forests of interesting genetic importance throughout the territory of Bosnia and Herzegovina. Namely, research conducted as part of the MOE scholarship (DBU.de, 2021) (if the report of this research is reviewed) indicates that linden, especially the species *Tilia tomentosa*, should be viewed as a potential tree of the future. Since the facts indicate that linden spreads in almost all habitats in Bosnia and Herzegovina and other countries of the Western Balkans, a logical question arises: "Why not support innovative ideas in order to find sustainable solutions for the wood market on the Balkan Peninsula?". Although it was relatively difficult to obtain detailed information on the popularity of linden in nursery production based on the data available on the websites of forestry companies, it is noticeable that the websites of forestry companies are relatively transparent and aesthetically adequately arranged. Forestry companies in Bosnia and Herzegovina transparently provide contact information, so that it is possible to establish contact and inquire about cultivation, protection and sale, as well as prices of planting material. This article relies on a literature review method, as well as an overview of available relevant information on the work of forestry companies and the species that are represented in nursery production for filling forest crops, planting plantations, but also for planting in alleys, on river banks and in floodplains. The main results of the research indicate that the functioning of forestry enterprises in Bosnia and Herzegovina is sufficient to sustainably manage species of the genus *Tilia*, especially considering that the Governments of both entities are willing to support the Master Plans for Afforestation and that there is an interest in carrying out the necessary afforestation of forest land with adequate tree species, as well as supplementing forest crops with valued commercial tree species.

Keywords: *Tilia*, Plant nursery, Sustainable Management, state organisation, governmental support

1.INTRODUCTION

In Bosnia and Herzegovina, according to Yugoslav sources (Herman, 1971), which are the most detailed on the subject of linden, there are relatively large areas of linden, which is considered a rare noble tree in many European countries. According to an oral statement by Professor Dr. Mikic (Oral Communication, 2016), there are two large linden sites on the European continent, namely Croatia (with linden dominance on the Bilogora mountain) and Serbia (with large linden complexes on the Fruska Gora mountain). In Bosnia and Herzegovina, there are relatively large linden forest stands in the Cazinska Krajina area, with a distribution center in Bosanski Petrovac and Sanski Most, while relatively large forests of the thermophilic species *Tilia tomentosa* extend in the Neretva River canyon (Herzegovina). Bosnia and Herzegovina is a country with a very complex administrative structure, which is closely correlated with all segments of the functioning of the state, and the administrative structure of the country directly reflects on the management of forest ecosystems. However, the existence of a complex state administration is not and does not have to be a reason for unresponsive forestry, and it is possible to establish an integrated system of management supervision of tree species of special importance, with the aim of preserving important highly resistant trees of the future for future generations.

The results of the Second Large-Scale Forest Inventory in Bosnia and Herzegovina (Lojo et *al*., 2008), whose methodology was developed by prominent forestry experts in the country, indicate significant potential for future forest land reforestation with appropriate tree species, appropriate to the given forest ecosystems and soil conditions (its quality and history). Some of the forest land can be planted with species of the genus *Tilia*, as indicated by research within the MOE scholarships (DBE.de, 2021), within which a report was published indicating the importance of conservation and cultivation of linden trees, as well as the preservation of the genetic pool of existing linden forest stands in Bosnia and Herzegovina. In addition to this research, there is additional research indicating the importance of preserving linden for the future, in relation to protecting habitats from negative climate impacts. Thus, the authors Vejzagić, Ballian and Memisević-Hodzić (2021), in their study of the morphological diversity of *T. tomentosa* trees in Bosnia and Herzegovina and Serbia, state that this species is significant in the context of climate change, while Vejzagić (2022) also points to a similar significance, describing the potential connection between climate change and the appearance of multiple bracts in the fruits of silver linden (in the short communication on discovery of multibracteate linden in Mostar).

The institutions of the Federation of Bosnia and Herzegovina and the Republika Srpska can significantly help preserve the linden gene pool, not only in Bosnia and Herzegovina, but also in the wider region consisting of the Western Balkan countries. Thus, the authorities in Bosnia and Herzegovina can support the preservation of both linden forests and individual linden trees, which are important for genetic diversity (protection from illegal logging and any fragmentation of linden habitat). The importance of the Government of the Federation of Bosnia and Herzegovina and the Government of the Republika Srpska is also reflected in the support for the potential future production of seeds and high-quality seedlings resistant to drought and temperature fluctuations, which could help preserve populations of beneficial insects, such as bees, as well as protect the climate of local cities from overheating. Taking into account the research of Pigott (1992), who described the linden in Europe and presented information on pollen maps of Europe, according to which the linden was the dominant tree in the past, as well as the statements of Finschi et *al*. (2003) who explain what happened in the past with the genus *Tilia* in Europe due to the onset of the Ice Age, and if we add to the context of consideration the fact that forestry companies are obliged to do everything possible in cooperation with the academic community and authorities to ensure sustainable forest management, it is noted that

there is a need to investigate the framework of potential support for sustainable linden management in Bosnia and Herzegovina. Since there is no previous knowledge about this problem (scientific articles in Bosnia and Herzegovina), and since forest management elaborates of individual forest companies are extremely large documents, which are difficult to analyze in a short period of time, there was a need to collect basic information about which forestry companies are engaged in linden cultivation in Bosnia and Herzegovina, and whether there are documents that support the idea of linden production and planting in Bosnia and Herzegovina. The research was also guided by the fact that the countries of the Western Balkans chronically suffer from a lack of statistical data (Mueller and Hofmann, 2022), and care was taken to try to collect basic information about the problem under study.

2.MATERIAL AND METHOD

Research obtained basic information from different forestry companies in Bosnia and Herzegovina, that are existing and functioning at the moment, but also whose management data are transparently available at the web-sites. Since the simple review method of the given information was used, the main or key terms in the website search related to: 1) territory of management, 2) main services and production aims, 3) data about the plant nurseries and 4) data about the linden production. The report data of this review work should provide background information on where the potential for wider use of linden could be explored in the future.

However, since the organization of forestry companies in Bosnia and Herzegovina is complex in accordance with the division of responsibilities into different administration units, this indicates the need to conduct basic research whether the Governments of Federation of Bosnia and Herzegovina and Republika Srpska do have certain framework for the future afforestation, concretely whether the afforestation with linden trees is has already been planned. For the purposes of this exploration, the readings of two documents, “Republika Srpska Afforestation Master Plan” (Govedar and Mataruga, 2021) and “Afforestation Manual” (Visnjic, 2024) (provided on the website of the Association of Forestry Engineers and Technicians of Bosnia and Herzegovina), were analyzed. The aim of the readings was to identify whether linden species had been planned for the afforestation in the Federation of Bosnia and Herzegovina and Republika Srpska.

Some additional data about the forest companies, such as human resources or monitoring capabilities, were added in the results, since they could indicate the potential of individual enterprise level and the institutional support together to keep linden forest resources sustainable. Finally, to be emphasized is that the research did not use data from the forestry enterprise elaborates (which consist potentially more detailed data on the amount of linden trees planned for afforestation, in the frame of Plans of afforestation), but the research focused on the publicly available data from the web source.

Studied were the websites contents obtained from the following forestry companies: 1) Public company “Forestry economic society of Zenica-Doboj Canton” Zavidovici L.l.c., 2) Cantonal public company “Sarajevo-forests“ L.l.c. Sarajevo, 3) Public company "Forests of Tuzla Canton" J.s.c. Kladanj, 4) Forestry economic society “Central Bosnia forests” L.l.c. Donji Vakuf, 5) Forestry economic society “Herzeg-bosnian forests” L.l.c. Kupres, 6) Forestry economic society “Una – Sana forests” L.l.c. Bosanska Krupa, 7) Public company "Bosnian-herzegovinian forests" e.o. Sarajevo, 8) Public company "Forests of Bosnian Drina" L.l.c. GORAZDE, 9) Forestry “Central Neretva” J.s.c. Mostar, 10) Public company „Herzegovina-Neretva forests“ L.l.c. Mostar, 11) Forestry "PRENJ" J.S.C. Konjic, 12) "FORESTRY LJUTA" L.l.c. Konjic, and 13) Forestry company "FORESTS OF REPUBLIKA SRPSKA" J.s.c. Sokolac.

After reviewing basic data about the companies, basic information related to the possibilities of improving the sustainability of linden stands and/or their individual trees, through the existing

framework of institutional support and company capabilities, was clarified by the information sources from the institutional level (governmental supported documentation).

3.RESULTS AND DISCUSSION

The functioning of forestry companies in Bosnia and Herzegovina is to a certain extent supported by municipal (individual) and cantonal, but also Republika Srpska institutions, either through the means of those institutions directly, or through funds related to them and their services (offices), such different cooperations between forest companies and foreign organisations, who support afforestation projects in Bosnia and Herzegovina. There are different types of institutional support for the work of forestry companies in Bosnia and Herzegovina, about which a lot of information can be found on the official websites of forestry companies. Companies in general take care to publish information about partnerships and important projects on their websites. In the context of the analysis of potential addresses for seeking support in the future, when companies would seek help to harmonize their operations with the parallel negative consequences of climate change on forestry as an industry, and what also refers to support for the establishment of nursery production of resistant species of trees, from the available information it is known that support can be provided by the institutions in Republika Srpska and Federation of Bosnia and Herzegovina.

3.1. Individual forestry companies' data

The first company analysed is the P.C. "Forestry economic society of Zenica-Doboj Canton" Zavidovići L.l.c.. According to the transparent content on the website, this company deals with the cultivation and protection of forests, production planning and hunting. This company has been managing Cantonal forests since 2006. There are several business units (*Olovo*, *Vares*, *Visoko*, *Kakanj*, *Zenica*, *Zepce*, *Zavidovici*, *Maglaj*, *Tesanj*, and *Business unit "Natural Monument TAJAN Zavidovici"*), which are united in five wider forestry areas (*Gornjebosansko*, *Olovsko*, *Krivajsko*, *Kakanjsko* and *Natron-Usorsko-Ukrinsko*). This area manages forests and forest land on an area of 183,389.20 hectares, of which 69.12% are forests with natural regeneration, 15.22% are vegetative origin forests, 9.18% are forest cultures and plantations, and 6.48% are bare and unproductive areas. The mined areas cover 21,946.40 hectares. Seed and nursery production is very successful. Seed production of silver fir, Norway spruce, Serbian spruce, green Douglas fir, Scots pine, Austrian pine, European larch, European beech, ash, sycamore, Norway maple, Scots elm, small-leaved linden, sessile oak and chequer is stable. The nurseries owned by this company are: "Olovo", "Ajdinovici", "Zepce", "Zavidovici" and "Tesanj". (SPDZDK.ba, 2025)

In Sarajevo Canton is a center of the company C.P.C. "Sarajevo-forests" L.l.c. Sarajevo, which manages the forests and forest land of Sarajevo Canton. The activities of this company are: 1. Forest cultivation, 2. Forest protection, 3. Forest exploitation, 4. Construction and maintenance of forest transport infrastructure, 5. Bioengineering, 6. Environmental projects, 7. Hunting and hunting tourism, 8. Nursery production, and 9. Various projects and collaborations. The company was founded on November 24, 1999 with the main goal of ensuring the protection of forests and forest lands in the Canton area, and to this day it fulfills the mission and vision of the company. The area of forestry is divided into two sectors, of which the first sector, which is responsible for cultivation, protection and use of forests, includes: 1. Service for cultivation and protection of forests, 2. Service for planning and analysis, 3. Service for preparation and design, 4. Service for transport and maintenance of machinery, 5. Service for maintenance of forest communications and facilities, 6. Service for hunting and tourism, 7. Service for other forest products, 8. *Srednje* Forestry Administration, 9. *Hadzici* Forestry Administration and 10. *Trnovo* Forestry Administration, *while* the other sector has jurisdiction over

ecology and the management of protected areas, which is known as Service for Protected Areas. There are two nurseries, in Tarcin and in the settlement of Sedrenik. The two nurseries have a total area of 28,538 m2, and the total capacity of these two nurseries is 700,000 seedlings. (SARAJEVO-SUME.ba, 2025)

Forestry company P.C. "Forests of Tuzla Canton" J.s.c. Kladanj is a cantonal forestry company engaged in the following activities: -Forest cultivation, -Forest protection, -Seed and nursery production, -Planning and production, -Hunting, and -Non-timber (secondary) forest products (sale). The cantonal company has over 530 employees, and in addition to the Directorate, there are four business units: Forestry "KONJUH" Kladanj, Forestry "SPRECKO" Zivinice, Forestry "MAJEVICKO" Srebrenik, and Forestry "Vlasenicko" Turalici. The area manages forests and forest land on an area of 44,713 hectares. The nursery production is good developped, and the "Budim potok" nursery is characterized by the successful production of the following species/tree *generas*: silver fir, Norway spruce, blue spruce, Scots pine, Austrian pine, European larch, *Juniperus spp.* and *Thuja spp*. (JPSUMETK.ba, 2025)

Forestry company F.E.S. "Central Bosnia forests" L.l.c. Donji Vakuf deals with the following activities: -Production, -Seed production and nurseries, -Ecology and protection, and -Forestry. Within this company there are 12 forestry units, which are named after the names of municipalities (*Jajce*, *Dobretici*, *Donji Vakuf*, *Travnik*, *Bugojno*, *Novi Travnik*, *Vitez*, *Gornji Vakuf - Uskoplje*, *Fojnica*, *Busovača*, *Kreševo* and *Kiseljak*). Twelve smaller units are included in four wider forest management areas: Forestry “Gornjevrbasko”, Forestry “Srednjevrbasko”, Forestry “Lasvansko” and Forestry “Fojnicko”. The company has three nurseries: “Bugojno” Nursery, “Busovaca” Nursery and "Slimena" - Travnik Nursery. The nurseries have the following trees in the production: Norway spruce, Scots pine, Austrian pine, Bosnian pine, ash, and as for other species such as silver fir, Douglas fir, European beech, sycamore and oak, the organization of collection and production is ongoing. (SBKSUME.ba, 2025)

Next for the analysis was the F.E.S. "Herzeg-Bosnian forests" L.l.c. Kupres company, which performs the following activities: -Forest cultivation and other activities in forestry, -Forest exploitation, -Auxiliary services in forestry, -Hunting and service activities related to hunting, -Collection of uncultivated forest fruits and products, except forest assortments, -Processing of seeds for plant nursery material *etc*. The work within this company is carried out in the following business units: Directorate, Forestry *Bosansko Grahovo*, Forestry *Drvar*, Forestry *Glamoc*, Forestry *Kupres*, Forestry *Tomislavgrad*, Forestry *Livno*, and Work unit *RASADNIK*. The "Przine" nursery within this company has a very successful production of the following trees: Austrian and Scots pine, Norway spruce, silver fir, *A. platanoides*, *A. pseudoplatanus*, *A. negund*o, *F. excelsior*, *S. aucuparia*, *S. aria*, *C. betulus*, *T. platyphyllos*, *P. avium*, *M. sylvestris*, and *P. pyraster*. (HBSUME.ba, 2025)

Forestry company F.E.S. "Una - Sana forests" L.l.c. Bosanska Krupa is a cantonal company that performs the following activities: -Utilization of forests, -Timber wholesale trade, -Forestry (citizen) services, -Forest cultivation and protection, -Hunting, (trapping) and care of wild animals including appropriate services, -Forest cultivation and protection, -Production of other wood products, -Wood retail trade, -Cork and wicker production. The company has the following filiales: *Bihac*, *Bosanska Krupa*, *Bosanski Petrovac*, *Buzim*, *Cazin*, *Kljuc*, *Sanski Most*, and *Velika Kladusa*. This company manages 172,687.14 hectares of forests and forest lands. In addition to operations within the units, the activity is also carried out in the "Cazin" nursery. (USSUME.ba, 2025)

In the eastern part of Bosnia and Herzegovina is the center of the forest company P.C. "Forests of Bosnian Drina" L.l.c. GORAŽDE. That cantonal forestry company in the Bosnian-Podrinje Canton for its main activities has: -Planning and production, -Forest cultivation and protection, -Additional activities (SUMEBPK.ba, 2025).

Forestry "Central Neretva" J.s.c. Mostar is a forestry company based in Mostar, in the Herzegovina-Neretva Canton. The main activity of the company is the utilization and cultivation and protection of forests. The company has been operating for about 25 years and has 34 employees. (SUMARSTVOMOSTAR.ba, 2025)

Forestry company P.C. "Herzegovina-Neretva forests" L.l.c. is located in Mostar and it participate in the following activities: -Cultivation and protection of forests, -Use and sale of all products of forests and forest land, -Construction and maintenance of forest communications and facilities, -Protection, maintenance and improvement of the general useful functions of forests, -Management of protected areas and forests with special purpose, -Planning in forestry, -Seed production, -Nursery production, and -Hunting and fishing and Tourism in forestry. The company has several units, namely: *Capljina*, *Jablanica*, *Konjic*, *Prozor-Rama*, *Mostar* and *Citluk*. (SUMEHNZK.com, 2025)

Forestry "PRENJ" J.s.c. Konjic is a company registered in the municipality of Konjic, and it participates in the following activities: -Forest cultivation, -Forest protection, -Forest utilization, -Construction and maintenance of forest transport communication, and -Nursery production. The nursery "Boracko jezero" has 2.60 ha surface. (SUMARSTVOPRENJ.ba, 2025)

"FORESTRY LUTA" L.l.c. Konjic is a company whose activities are: -Production, -Cutting and export of forest wood assortments, -Sales, and -Transport. (SUMARSTVO-LJUTA.ba, 2025)

In Sarajevo there is a center of P.C. "Bosnian-Herzegovinian forests" e.o. Sarajevo, the forestry company that can be singled out as an example of a company that provides support to all the aforementioned forestry companies, and whose professional assistance in reviewing and revising project documentation and forest management fundamentals is a huge professional help and support to all forestry companies' demands (BHSUME.com, 2025). This is thanks to its different departments from which every one provides professional service of writing, reviding and supporting forestry elaboration documentation related to Forest management, Forest exploitation, Forest breeding, Forest protection and all sorts of forestry economic analyses.

Large forestry company F.C. "FORESTS OF REPUBLIKA SRPSKA" a.d. Sokolac is a comprehensive forestry company in the Republika Srpska, whose activities are: -forest utilization, -service activities in forestry, wood wholesale trade, and -Wood retail trade. The company manages 1,000,608.56 hectares of forests and forest lands, of which: - forests with natural regeneration reaches 469,164.64 hectares, - degraded forests reaches 14,528.97 hectares, - coppice forests reaches 226,336.88 hectares, - forest cultures reaches 48,687.63 hectares, - areas suitable for afforestation and management reaches 154,846.61 hectares, -areas unsuitable for afforestation reaches 68,374.82 hectares, -usurpations reaches 18,669.01 hectares. The volume of total wood mass amounts to 202,426,428 m3, of which deciduous forest wood participates with up to 122,429,391 m3, and conifers make up to 80,997,037 m3. The annual yield amounts to 5148250 m3. (SUMEREPUBLIKESRPSKE.org, 2025)

The business units of this company are: Forestry "Banja Luka", Forestry "Ostrelj-Drinic", Forestry "Panos", Forestry "Birac", Forestry "Gradiska", Forestry "Doboj", Forestry "Zelengora", Forestry "Cemernica", Forestry "Vrbanja", Forestry “Majevica", Forestry "Lisina", Forestry "Botin", Forestry "Jahorina", Forestry "Prijedor", Forestry "Ribnik", Forestry "Sjemec", Forestry "Romanija", Forestry "Drina", Forestry "Maglic", Forestry "Borja", Forestry "Visocnik", Forestry "Vucevica", Forestry "Gorica", Forestry "Treskavica", Forestry "Klekovaca-Potoci", Forestry "Milici", Forestry "Rudo", "Karst Management Center" with headquarter in Trebinje, "Research-development and project center" with headquarter in Banja Luka, "Center for seed and nursery production" with headquarter in Doboj, Forest house "Ognjiste" with headquarter in Pale, and "Directorate of Public Enterprise" with headquarter in Sokolac. The nursery production is well developed and stable, and the

company has the following nurseries: "Stanovi" and "Usora" in Doboj, "Stupine" Nursery in Nevesinje, "Bisina" Nursery in Sekovici, "Gornja Puharska" Nursery in Prijedor, Trebinje Nursery (*Cupressus spp.*, *P. pinaster*, *P. halepensis*, *P. nigra*, *P. heldreichii*, *Thuja spp.*, *Cedrus spp.*, *B. sempervirens*, *P. granatum*, *L. nobilis*, *Lavandula officinalis*, palm trees, *F. ornus*, *Euonymus spp*.), and the Nursery "Petkovaca" in Brcko (50 hectares surface). (SUMEREPUBLIKESRPSKE.org, 2025).

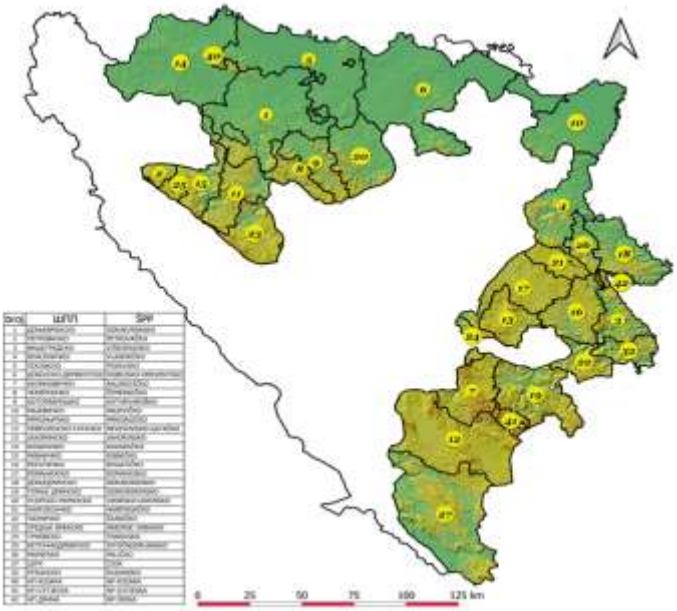

Figure 1. Forests of Republika Srpska, with clear administrative borders among Srpska's Forestry units/area. Source: https://sumerepublikesrpske.org/index.php/strana-1/rg-niz-ci

3.2. Institutional support in the form of afforestation approving documents

Forestry companies in the Federation of Bosnia and Herzegovina and Republika Srpska are encouraged to implement afforestation programs on their territory of management. Special care for the noble species of trees in the regional forest ecosystems should be anyway taken in the drough-affected areas, since they are mostly affected by climate, and so the forestry companies should take measures and plan activities to concretize protection measure, so that the forestry production does not suffer, as warned Kumar (2021).

The assumption that lindens can compete with oaks in Europe in terms of their biodiversity should be an incentive for increasingly frequent further molecular research and the search for hybrids in existing natural linden stands (populations). In order to preserve the linden trees in Bosnia and Herzegovina, and conserve the naturally occurring species with their subspecies and varieties for the future, it is necessary to learn in detail the behavior of linden trees within the species and between species, as well as the behavior of several linden species within one mixed stand. In this direction, it would be possible to go with a survey method, where the possibilities within forestry companies in the Republika Srpska and the Federation of Bosnia and Herzegovina could be investigated. If there are capacities (a sufficient number of employees) for additional monitoring of the behavioral patterns of linden trees in relation to changed environmental conditions, observing insects species that might appear (macroscopic identification on the terrain) and tracking whether the leaves and fruits of linden trees deviate from their typical appearance, there are then fundamentals for an integrated high quality

health condition and vitality parameters observations. The typical appearance of lindens growing in the territory of the former Socialist Federal Republic of Yugoslavia was described by Herman (1971), and that literature source should not be neglected in the process of systematic monitoring of behabioral patterns of linden complexes in the context of climate change.

Institutional support to forestry companies in the Republika Srpska and the Federation could, therefore, help further fragmentation of linden forests, and potential planning and financing of nursery production. Each forestry company is located within the territory of Republika Srpska and Federation (nurseries in Brcko District should be investigated in the future), which is characterized by different climatic, hydrological, relief and vegetative characteristics. It is important to note that through the review of transparent information on the activities of forestry companies and the species they grow for afforestation purposes, it was noticed that there is linden cultivation, both in the continental part of Bosnia and Herzegovina) (Zavidovici area) in the west-southern Mediterranean parts (Kupres) of the country. It has been observed that there is an interest in buying linden seedlings, considering that this is a very attractive species for ornamental purposes and park planting, but the question arises whether and to what extent there is interest in planting this species as part of the afforestation program, which needs to be analyzed separately in the future. In a meanwhile the forestry companies exploit lawful support in the institutional documentation, such “Republika Srpska Afforestation Master Plan” (Govedar and Mataruga, 2021) and “Afforestation Manual” (Visnjic, 2024) (provided on the website of the Association of Forestry Engineers and Technicians of Bosnia and Herzegovina).

According to the data of the Federal Forestry Administration (FMPVS.gov.ba, 2025), the support of the Federal Forestry Administration has as part of its scope of work the collection of data and the maintenance of a database on the development of all forests in the Federation of Bosnia and Herzegovina, including their inventory. The federal administration, therefore, helps forestry companies that manage state-owned forests by preparing a draft of the Forestry Program of the Federation of Bosnia and Herzegovina. In addition, this institution determines the monitoring of the dynamics of the creation of forest management bases, which are documents that include all important information on forest management, (so forest stands as well as individual trees), which grow on public forest land. The provision of expert assistance for the development of forest management bases for forest management areas and forestry economic units in scope of companies *is* one of the most important assistances of this federal institution, but in addition, the development of plans and programs for extended biological reproduction of forests and programs and plans for afforestation of karst and bare forests is ensured. Establishing and maintaining registers and records of facilities for the production and processing of forest seeds and producers of forest trees and shrubs are under the authority of the institution. Monitoring the health status of forests, carrying out the work of the reporting and forecasting service and monitoring the condition and degree of forest damage is a very important function of institution, which can help in monitoring the health and condition of the habitus of the remaining linden trees, which may have the potential for cloning, i.e. more mass clonal nursery production, or which may be interesting from the aspect of seed collection for the necessary production of healthy and resistant linden seedlings. Since these trees may have commercial use in the future, and the production of linden wood for the production of furniture can potentially develop over time, it is important to familiarize all the stake-holders interested in protection of remaining linden trees and their stands with the described function of the Federal Forestry Administration, but also governmental offices in Republika Srpska. Namely, monitoring the economic condition of forestry industry, based on data obtained by cantonal administrations in the Federation of Bosnia and Herzegovina and from the Republika Srpska Governmental administration, as well as monitoring the prices of forest wood assortments and secondary forest products, including informing the public about the vitality condition of forests and the development of forestry branche, enables forestry companies to contact the Forestry

Administration of the Federation of Bosnia and Herzegovina and Government of Republika Srpska at any time, in order to propose possible solutions for their local market, the needs of their partners - customers, but also to suggest species for planting in the future. The development of an adequate solution plan in front of each forestry company is the product of its engineers, i.e. experts in forestry and various fields, which means the adequacy of those proposals for consideration and support by the Federal Administration and Srpska's Government. According to the obtained data, it is clear that forestry institutional based legislation is really made by forest experts and the full implementation could serve as an example how complex administration can in final provide a healthy framework for forestry laws implementation, when all legal criteria are met.

The preparation and creation of programs in the field of forestry, which are financed or co-financed from the dedicated fund of the Federation of Bosnia and Herzegovina, is closely correlated with the preparation and implementation of scientific research activities and the transfer of knowledge in the field of forestry. New knowledge useful for forestry must, in a certain way, be forwarded to the Federal Forestry Administration, so that it has insight into the solutions offered by forestry scientists, who may work in federal forestry companies (engineers, Masters and *PhD*s of Forestry). The preparation of expert opinions for the establishment and revision of forest management areas is the domain of the Federal Administration (FMPVS.gov.ba, 2025). Also, in order to protect an area with an existing high-quality stand, it is necessary to propose it to the Federal Forestry Administration. The reason for this is that the preparation and giving of opinions for the declaration of protected forests *and* forests with a special purpose is carried out in accordance with the powers and support of the named Administration. The organization of consultations and the provision of professional assistance to cantonal administrations and cantonal forest management societies is one of the functions of the Federal Administration, on which companies can always rely, therefore, frequent communication of forestry companies is significant and useful compared to sporadic ones. This kind of communication partially meets the recommendation of Kumar (2021). The same complex task in a form of huge support to Republika Srpska forestry units in giving the governmental office for forestry, which also supports the exchange of new scientific knowledge, ideas and solutions for existing or potential problems and helps developing cooperation between companies and institutions, as well as in terms of inter-units cooperation and strengthening awareness of the importance of protecting forests from the negative consequences of climate change. The Federal Forestry Administration and Government of Republika Srpska are supporting scientific achievements and experts recommendations from faculties for forestry (Faculty of Forestry in Banja Luka, Republika Srpska, and Faculty of Forestry in Sarajevo, Federation of Bosnia and Herzegovina), whose research projects draws the resources from the support of the Federal Ministry of Agriculture, Water Management and forestry companies themself, and Government of Republika Srpska. Strengthening the bond between Ministries, forestry faculties and forestry companies increase the capacity to help the development of wider forestry regions. According to the Association of Forestry Engineers and Technicians (USIT.ba, 2025) of the Federation of Bosnia and Herzegovina (in Sarajevo), the support of the Croatian Forestry Society and the Faculty of Forestry of the University of Sarajevo is also very significant, not only in terms of scientific and professional assistance , but also in joint lobbying to find the best solutions for forestry as a whole and to promote solutions in the society, academic and business community. On the other hand, Faculty of Forestry in Banja Luka is host to Forest institute, which gathers forestry experts from the country and the Region, in aim to promote forest studies results and lobby for implementation of good scientific practices in the forestry of Republika Srpska (SF.UNI.BL.org, 2025).

In Republika Srpska, support is provided to the company F.C. "Forests of Republika Srpska" J.s.c. Sokolac, which is further implemented individually by all its individual business units, in accordance with the needs of those units. Due to climate differences and the presence of different

ecological-phytocenological areas in Bosnia and Herzegovina, there are certain differences in the focus of forest restoration support between the Federal Ministry of Agriculture, Water Management and Forestry and the Ministry of Agriculture, Forestry and Water Management of the Republic of Srpska. For example, the percentage representation of conifers and deciduous trees is different in Federation of Bosnia and Herzegovina and Republika Srpska, so there are certain differences in the amount of planting material for forest restoration and differences in the tree species, too. For example, the focus of nursery production within the business units of the "Forests of the Republika Srpska" company for the units located in the southern municipalities is focused on species that are designated as Mediterranean, while in the case of the northern municipalities, focus in production is more on continental species. In the Federation of Bosnia and Herzegovina, for example, the need to restore forests using Mediterranean species is in close correlation with the needs of the southern and west-southern cantons, on whose territory grow the species that require Mediterranean habitat and cultivation conditions. Therefore, there are certain differences related to the flow of information such as what are the needs of forest units regarding their climate and ecological conditions. Because of the complexity of the administration in Bosnia and Herzegovina, there are also certain specifics that play a huge role in the information flow and sometimes even can affect the speed of decision making processes, i.e. whether information from business units through forestry companies must go through the processing procedure first in the cantonal institutions, and then continue its journey to the competent ministry, or (as is the case in the Republika Srpska) that information travels directly, in the sense that it does not undergo processing at some mediate levels, but can continue its journey faster to the Ministry of the Republika Srpska. Ways of supporting forestry companies with all their units, can be found on the organized and transparent pages of the Ministry in the Republika Srpska. (VLADARS.rs, 2025, FMPVS.gov.ba, 2025)

Document "Republika Srpska Afforestation Master Plan" (Govedar and Mataruga, 2021) treats even *Tilia* species, by giving recommendations on breeding, planting, planting density index and percentage participation in the mixed forest stands. Recommendations for the linden planting are made also for the forest types, taking into consideration the habitat and vegetation characteristics for different forestry areas.

Precise data from this afforestation master plan are even given by individual *Tilia* species, as follows (Table 1).

Table 1. Surface for afforestation with *Tilia* specimens in hectares (according to Mataruga and Govedar, 2021)

Species	Dinarids (*ha*)	Mediterranean region (*ha*)	Pannonian region (*ha*)	Illyrian-Moesian region (*ha*)	*Total* surface for Republika Srpska (*ha*)
Tilia cordata	605,08	710,47	13,01	147,96	1.476,52
Tilia platyphyllos	441,83	754,06	13,55	6,86	1.216,30
Tilia tomentosa	301,06	1.208,87	177,76	23,16	1.710,85

At this moment it is unknown (when analysing forestry companies website content) how much

of the recommended (calculated) plant material from *Tilia* genera have been planted, but it is obvious that detailed calculations exist in accordance to climate and habitat requirements.

Similar recommendations but more general, were published in Bosnia and Herzegovina in 2024 ("Afforestation Manual" by Visnjic, 2024, - provided on the website of the Association of Forestry Engineers and Technicians of Bosnia and Herzegovina), consisting detailed lists of species (non)resistant to drought, frost and temperature turbulences. Light requirements are also taken into considerations, while afforestation soil preparation process and preparation of the planting material was also described in detail. So, it is possible to conclude that governmental supported legislation and documents supporting afforestation programmes are completed by professional and fulfilling forest experts and Forestry professors works.

4.CONCLUSION

The institution support of the Government of the Federation of Bosnia and Herzegovina and the Government of the Republika Srpska could significantly help in the future, if the forestry companies decide to request the help of institutions for mitigating climate change. In accordance with the reviewed literature that describes the ecological-phytocenological areas in the country, and transparent information provided by forestry companies that manage area with small-, large- and silver-leaved linden forests, an analysis was performed in order to preliminarily suggest which forest company and under what conditions could be considered for potential feasibility study on mass linden production. In accordance with the natural habitat characteristics of the plant habitats and respecting the requirements of the species from the genus *Tilia*, which were described for the area of the former Socialist Federal Republic of Yugoslavia by the author Herman, and adding the findings of the linden research presented in the DBU.de report, which indicates a high degree of variability within and among linden populations, the recommendation of conducting wider research on establishing linden nursery for mass afforestation purpose in the future, in order to combat the negative consequences of climate change, could be given to Zavidovici-Teslic region (since natural conditions for growing linden in the nurseries already gave good results, as seen in the transparent information about nursery activity in the given company). It is interesting that the pollen maps of Europe, according to which linden pollen dominates in all habitats of the continent, raise a legitimate question whether linden cultivation in a nursery with various natural conditions in Bosnia and Herzegovina can result in quality linden trees that can adapt to all habitat conditions. Institutional support for the research of this possibility as well as various variants can be sought in the financing of scientific-research projects by forestry funds that belong to the Federal, that is, the Ministry in charge of forestry in the Federation of Bosnia and Herzegovina, and in the Republika Srpska Governmental institutions, that also could support scientific research and needed expertise. Areas such Una-Sana Canton also represents an interesting location for linden nurseries respecting the fact about existence of wider linden forest stand structures there, where the focus is on *T. tomentosa*. Successful production of *T. platyphyllos* in the Kupres, as seen from the website data collection of their active forestry company, gives hope that wider production of this species could be expected also in the future, with upcoming of higher temperatures and more frequent heat waves. Potential for further afforestation in Republika Srpska is giving optimism that the *Tilia* trees can provide better climate conditions for the local cities areas, what could result in the sustainable development of support of local forest management, forestry as industry and perseverance of local climate and biodiversity, as main fighter in mitigating climate change.

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SELECTION OF FOREST TREE SPECIES FOR SUSTAINABLE FORESTRY PRODUCTION IN THE FUTURE - CASE STUDY: PRICE OF LINDEN PLANTING MATERIAL AS A FACTOR IN AFFORESTATION SPECIES SELECTION

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A B S T R A C T

The article provides results on quantitative and qualitative analysis of data related to potential of linden trees to be used in the future as one of the commercial species in afforestation and plantations funding. Qualitative research of the forestry companies' bulletins in Bosnia and Herzegovina, consisting data on appearance of different, climate change process related pathogens, reveal real danger for the future of tree plantations in the country. In addition to this, a quantitative analysis on plantings price, as a potential decisive (limiting) factor in choosing commercial species for afforestation, was conducted. For the purpose of explaining behavior of the market prices relations of main competition - Oak and European beech wooden products, the linear regression function was used. Main results indicated clearly that the price of planting material is not and cannot be the limiting or crucial factor in deciding for the introduction of linden in the commercial forestry production in Bosnia and Herzegovina. Next results even in that, *that* the linden is more favored species when comparing duration of the production cycle, and that the species is less affected by diseases caused by climate change in comparison to Oak and European beech. Main linear regression results show that there is an extremely strong linear relationship between the prices of wooden products made from European beech and those made from Oak, suggesting the regression model is highly accurate and explains nearly all variations in oak wood prices based on beech wood prices. Approximately 40 scientific papers, books, reports, and forestry ministry related websites were used in aim to approach the problem and understand the importance of biology and ecology of the species in the context of forestry economic analysis related to prices on the market. The goal was to gather relevant data on the genus *Tilia*, its production potential, high immunity, resistance to negative effects of global warming, and the history of linden tree planting in Europe. This article is part of a broader interdisciplinary research project that combines forestry, forestry economics, forest ecosystem management, and city climate protection. The research results indicate that a careful planning strategy for future production, which must withstand climate change, is essential. This research is therefore designed as a foundation for future recommendations regarding linden research in the country. Linden, as a potential tree of the future and a species resistant to harmful environmental influences, could replace biomass that is threatened by the detrimental effects of deteriorating habitat changes, primarily in the form of soil drying in natural habitats and reduced efficiency in the production of commercial tree species such as oak and beech. In addition to its favorable effects on insects and the overall ecosystem, linden is also an attractive tree, with potential for carbon storage and broader use in furniture and construction timber production. Therefore, this article should serve as a guide for creative problem-solving techniques in the field of a large-scale production of industry wood.

Keywords: Tilia, linden, drought resistance, wood market, afforestation, commercial plantation, plant material price, limiting factor, quantitative analysis, qualitative analysis.

1. INTRODUCTION

Climate change threatens to jeopardize the sustainable economic flows of forestry as an industrial branch (Kumar, 2021). The need for the production of safe quantities of wood mass, as well as other forest products, is growing. Society, which includes numerous stakeholders who expect forestry companies to fulfill their requirements in terms of selling the highest quality wood mass in the shortest possible time, also demands respect for biological principles of forest management (Manrique & Franco, 2020). This means that forestry companies must pay attention to the sustainable use of forest resources, which are increasingly endangered by the harmful consequences of climate change, in their management of forests and valuable plant species, from the aspect of preserving the gene pool and biodiversity. Climate change threatens the local economy, especially in small and poor countries and developing countries, where the lack of statistical data is very often a problem (Mueller & Hofmann, 2022). Namely, having adequate sources of information is crucial for developing strategies for long-term afforestation (establishing forest tree cultures), which ensures continuity of production and market stability in terms of supply of forestry products (McHenry, 2012). According to the available information from the latest national inventory of forests on large areas in Bosnia and Herzegovina, the methodology of which was developed by experts from the Faculty of Forestry in Sarajevo, there are about one million hectares of areas in Bosnia and Herzegovina that currently do not have sustainable plant communities in terms of forest stands, and these areas are dominated by either bare land or land with low or poor vegetation (Lojo et al., 2008). Afforestation of large areas of land can have not only beneficial effects on the local economy, in terms of the future of forestry production at the level of smaller local communities, but can also have extraordinary and protective effects on the local climate, in terms of ensuring favorable temperatures in areas affected by intense drought, reduced levels of retained water and generally improving the local climate of cities. Of particular importance in the context of raising forest cultures is the participation of planting resistant tree species in drought in terms of maintaining populations of beneficial insects, which find shade and resources for life support in forest tree communities (Visnjic, 2002). This Paper will present information on the benefits of raising plantations in Bosnia and Herzegovina, in terms of supplementing forest production. The need for innovative solutions in climate change mitigation is becoming an imperative for today's forestry companies, which are increasingly cooperating with the academic community and taking into account the proposals and recommendations of forestry experts and scientists when it comes to plans for the future, sustainable monitoring of the health of forest stands, as well as calculating all possible challenges in terms of supplying society with an adequate amount of wood mass, without disrupting the balance of the ecosystem or worsening local climate conditions. The most important commercial tree species in Bosnia and Herzegovina include Oak (*Quercus sp.*), European beech (*Fagus sylvatica*), hornbeam (*Carpinus sp.*), white and black pine (*Pinus spp*.), Douglas fir (*Pseudotsuga sp*.), fir (*Abies sp.*), and spruce (*Picea sp*.), while many other species, such as noble broadleaf trees, are more respected in the sense of managing biodiversity, insects population viability, honey production, *etc*. (Mekic et al., 2006). One of the tree species with particularly unexplored potential for wider use in forestry is linden (*Tilia sp*.), which is prohibited for felling in certain parts of Europe, or its trees are extremely protected. However, this Paper will discuss the hidden potential of this species for wider use in the future, given the results obtained in recent years in the context of the genetics and distribution, or history, of this species. This paper will focus on a linear regression analysis, which should preliminary illuminate the path for future research, in terms of the cost-effectiveness of considering linden as a tree of the future for the timber market in Bosnia and Herzegovina.

2.MATERIAL AND METHOD

2.1. Research Design and Study Area

The research is planned to include analyses of the prices between different seedling materials and identifying whether the density in planting plays an important role in choosing species for afforestation. Quantitative analyses should include interpretation of regression model usage in analyzing variations in prices of first-class wooden products of Beech and Oak, to check whether this model can be used in the future to identify rentability of mass production of higher number of Linden wood products (for furniture industry, construction etc.). In order to create a clear picture of the real impact of planting spacing (afforestation density) on the number of seedlings per hectare required for planting, and the relationship to the simple cost of planting (manual planting with a stick per individual seedling), only two-year-old linden, oak and beech seedlings will be taken into account, which would be planted on land of the same quality class (let's assume that it is the first quality class). Ignoring other factors that affect the increase in planting costs, which relate to soil preparation for planting, fertilizing seedlings, hoeing seedlings, removing weeds, thinning the culture, cutting branches and cleaning thickets, the Paper will focus only on approximating the picture of deciding on a particular tree species regardless of the price of seedlings and planting density, in relation to the benefits of planting that are potentially found in the length of the production cycle (Mekic, 2006). The reason for avoiding the unnecessary presentation of plantation establishment costs related to these activities lies in the fact that costs are calculated per hectare on a flat rate basis, regardless of whether in practice a worker working on an area of one-hectare processes, for example, 600 or 2000 trees during the performance of these tasks. It will not analyze the reasons for such a calculation in this Paper, but will base it on the price of seedlings. For the purpose of leading the discussion on gotten results, the following keywords are used in the search for scientific papers: 1) forest tree pathogens, 2) linden pathogens, 3) oak pathogens, 4) spruce pathogens, 5) tree mortality, 6) forest drying, 7) forest and climate changes, 8) sustainable forestry, 9) wood production challenges. The papers are obtained from: 1) ResearchGate, 2) Google Scholar and 3) Web site of Journal of Faculty of Forestry in Banja Luka. Other sources are: 1) Website of Federal ministry for forestry in Bosnia and Herzegovina and 2) Master plan of afforestation supported by the same type of Ministry office in the entity Republika Srpska. This paper should present the results of quantitative and qualitative analysis. Namely, in order to obtain a preliminary picture of the profitability of considering linden (*Tilia sp*.) as a tree of the future for planting plantations in Bosnia and Herzegovina, a ***linear regression*** analysis will be conducted, which will examine whether regression model is enough accurate and does it explains nearly all variations in Oak wood prices based on European beech wood prices. Price of linden seedlings can be a decisive factor in the choice of tree species for planting plantations. But if lower prices on linden wood produces articles that affects choice of tree species in afforestation, if the bonity class of the wood is same or similar, then regression model on comparing prices variations of potential wood products in the future could bring adequate results if compared to variations in Oak and European beech products. According to the previous knowledge of forestry companies, planting linden was abandoned and always resorted to planting beech, oaks, or coniferous tree species such as pines or spruce, not only because these are the most frequently planted tree species, but also because the prices of seedlings are favorable. However, this research design should also help in explaining how the price of linden seedlings relates to the prices of beech and oak seedlings and to allow making recommendations on future plantation plans funded on real benefits of planting linden (respecting climate change facts).

2.2. Research Objectives with Hypothesis

Considering that the Bosnian-Herzegovinian forest management is abandoning the planting of linden trees due to the discussable cost of linden seedlings, the aim is to investigate whether there is a basis for considering the price of planting material as a limiting factor for the selection of linden trees for cultivation, by comparing the prices of planting material, according to the average prices of seedlings in Bosnian-Herzegovinian nurseries (Govedar & Mataruga, 2021). In addition, the importance of linden tree cultivation is reflected in the possibility of introducing this tree as a substitute in the production of certain products (for example, wood for furniture, consumer wood) in which European beech and Oak have so far dominated. Taking into consideration that prices of wood products rise in accordance to wood quality class (European beech and Oak are considered to represent this class on the bosnian-herzegovinian market), it will be conducted the linear regression which should explain whether the regression model is accurate enough to explain nearly all variations (if possible) in oak wood prices based on beech wood prices. This should help to understand how the prices of wooden products of other species of trees (wood types) behave based on the bonity (quality wood based price for a first bonity of linden trees, and any other first class wood trees) (Cabaravdic, 2010). Considering that linden is a softer quality wood, it is necessary to conduct this preliminary research and consider the option of introducing linden wood into mass production, in order to timely start developing a future strategy for growing trees from substitute tree species for the current commercial tree species. Therefore, our goal is to investigate: 1) Does the price of linden (*Tilia sp*.) planting material represent a limiting factor for afforestation and establishment of linden plantations for the future, and does planting density or lack of knowledge of the species have more of an impact on not considering mass planting of linden in Bosnia and Herzegovina?, 2) Also, efforts will be made to determine whether there are dangers or risks to production in the context of climate change, in terms of pathogens emerging due to increased temperatures and the recurrence of forest fires in larger areas of forests and forest lands. Accordingly, the research hypothesis is tried to be proven, according to which:

H: *"The price of planting material is statistically not decisive for the choice of tree species for afforestation, what supports the regression model of wooden products prices trend in the context of prognosing rentability of linden products increase on the market."*.

2.3. Structure of the Paper

Structure of paper follows methodology that should approach advanced knowledge on interpreting qualitative and quantitative data supporting research hypotheses. In this case, the Paper focuses on analyses of real data in the real market conditions in Bosnia and Herzegovina, retrospecting to: 1) Market needs for wooden products, 2) Market prices of most important wooden articles sold, 3) Interpretation of further climate change correlated factors in sustainable production of biomass.

2.4. Data Collection

When considering the way of data collection, the next fact was taken into account: forestry logic assumes that deciduous tree species are planted on lands of those quality classes that correspond to more demanding deciduous tree species (what is in forestry known as first bonity class). Because of this, it was imagined to use relevant data showing real time prices of the planting material and wooden products in the real market in Bosnia and Herzegovina. It is also important to emphasize that the quantitative analysis will rely on the assumption that only the prices of two-year-old linden, beech and oak seedlings are taken into account in the calculations, while the cost of planting each individual seedling (manual planting with a stick) is calculated at 0.50 BAM per seedling. The data to be used in the quantitative analysis are taken from the planting and afforestation protocol from the

Afforestation Master Plan (Govedar and Mataruga, 2021), which is valid in Bosnia and Herzegovina, and mathematical calculations will be made regarding the number of seedlings per hectare, for three tree species: beech, oak and linden. The fixed price (cost) of manual planting with a stick will always be 0.50 BAM. Data on constant variables (tree species) and diverse variables (price of seedling, price of planting) will be entered into a *"Microsoft Excel"* table, where a regression analysis will be run. Data on the length of the *minimum*, *maximum* and *typical* production cycle duration will be taken from official guidelines in the field of Forest Management and Silviculture, for the conditions of Bosnia and Herzegovina (Lojo et al., 2008). Data related to prices of 100 different wooden products of Oak and Beech wood has been collected with the help of product bulletins of forest companies in Bosnia and Herzegovina, and their partners - different firms that are producing furniture, construction facilities and interier-exterier equipment. Regression analysis will be conducted in the ***"Microsoft Excel"*** (Cabaravdic, 2010). Part of the quantitative analysis which will consider the duration of the minimum, maximum and typical production cycle in beech, oak and linden forests, should be linked in a way of correlating results of a linear regression analysis on wooden products prices in Bosnia and Herzegovina, and also making a correlation to the qualitative analysis, but in a way that will try to explain how the influence of pathogens caused by climate change may play a role in deciding on the type of tree to be used in the establishment of plantations, that should in the same time represent one of the key factors in creating rentable prices of its wood products in the future.

2.5. Quantitative Analysis

Calculating the planting density (example given for *Tilia*) will be one of the first things to be done. It is worth noting that the number of seedlings per hectare is calculated as follows:

1 ha = 10 000 m2 => 10 000 : (X m * X m) = number of seedlings per 1ha

E.g.: the density of linden planting is 2 m * 2 m, in this case:

10 000 : (2 m * 2 m) = 2500 seedlings per 1ha (Mekic, 2006)

In addition to these, results of the linear regression, graphically shown results (chart type) and tables will be presented in order to follow up testing prices variations of wooden products made in Bosnian Herzegovinian firms from Oak and Beech wood. The duration of the minimum, maximum and typical period of the production cycle of linden, beech and oak stands will be presented after calculating simple mathematical percentages, on how longer or shorter the production cycle of certain tree species relates to other commercial species.

2.6. Qualitative Analysis

Unlike the quantitative analysis, the qualitative analysis will determine the presence and type of pathogens that attack linden, oak and beech trees due to increased temperatures, evaporation of moisture from the soil and forest fires, and will describe how these pathogens may affect production in the future. The data used for the qualitative analysis will be collected from reports by forestry academic experts in Bosnia and Herzegovina (FMPVS.gov.ba), who have identified and confirmed which forest tree pathogens are involved, where they occur, in what way and what damage they cause to forest production. This data will be the result of a review of written sources of literature, available on the official websites of forestry companies and within the framework of the forest units' draft studies (document "Afforestation Plan" and document "Forest Protection Plan"). The qualitative analysis, which refers to the interpretation of the danger of forest fires and increased tree mortality due to the appearance of various pathogens, the spread of which is associated with the intensification of climate change, aims to provide a closer picture of the vulnerability of beech and oak in the future of forest production, in comparison to linden, which does not show symptoms of vulnerability that could lead to drying out, additional fragmentation or extinction.

3.RESULTS AND DISCUSSION

3.1. Review of the Literature with Main Theoretical Results

The annual permitted volume of cut timber in Bosnia and Herzegovina is slightly more than 7 million cubic meters. This timber is provided on an area of forests and forest lands of about 1.2 million hectares, where tall and coppice forest stands grow, most of which are located within the administrative borders of the entity of Republika Srpska (Lojo et *al*., 2008). Important guidelines for future production of forest timber and the establishment of plantations are contained in the so-called "Master plans for afforestation" of the Federation of Bosnia and Herzegovina and the Republika Srpska, which are written with the help of information from individual forestry companies from all over Bosnia and Herzegovina, which manage forests and plantations that are not privately owned. The results of the comparison of areas with high (generative origin) forests and wood stocks in the period between the two national inventories on large areas in Bosnia and Herzegovina show worrying phenomena. According to these data, the level of forest cover in Bosnia and Herzegovina has indeed increased compared to the period of the first national inventory of forests on large areas (1964-1968), and in the period of the second national inventory on large areas (2006-2009) there was an increase in the percentage of forest cover from over 50% to over 60% (Lojo et *al*., 2008). However, these are areas that have become overgrown with plant vegetation in rural areas after the population moved out due to the war in Bosnia and Herzegovina (1992-1995). Due to the abandonment of private estates and agricultural areas, mainly young plant communities have appeared, which, due to the slowing down of the population's return to their properties, have the potential to grow into larger forest complexes. The devastating fact of the analysis comparing the results of two forest inventories indicates a reduction in the area under high forests (forests of seed origin or short "generative origin") by about one hundred thousand hectares. Although the number of residents in the country decreased due to increased population migration during the war and in the post-war period, due to the difficult economic situation, the needs of society as a whole for wood and forestry products are growing significantly from year to year (Mekic, 2006). Experts and students of the Faculty of Forestry in Sarajevo have been working on developing an application that can be used to report forest theft, which partially helps the process of monitoring the forest fund. A particular problem in the post-war forestry of Bosnia and Herzegovina is illegal logging, in which the leading trees are Oak, European beech, pine, spruce, fir, acacia, maple and ash, but also wild cherry, wild pear and walnut have not been spared either. The increase in prices of forest wood assortments on the market is dictated by the demand for commercial tree species, where volatility in the prices of beech and oak wood is observed in certain years, such as periods characterized by the occurrence of long-term droughts and the absence of fruiting in natural forests (Avdibegovic et *al*., 2007). European beech (*Fagus silvatica*), Oak (*Quercus sp*.), pine (*Pinus sp*.), Douglas fir (*Pseudotsuga sp.*), chestnut (*Castanea sp*.), walnut (*Juglans sp*.), fir (*Abies sp*.), spruce (*Picea sp*.), maple (*Acer sp*.), wild cherry (*Prunus sp*.), juniper (*Juniperus sp*.), poplar (*Populus sp*.) and willow (*Salix sp.*) continue to be the most attractive tree species, not only in afforestation, furniture production, firewood production (mostly oak, beech, hornbeam), veneer production, the establishment of parks and alleys, the establishment of flood protection belts in coastal areas (mostly poplars, willows), and other tree species are less emphasized in afforestation, and are mainly used in beekeeping, horticulture and landscaping private properties. Finding a solution for sustainable wood production in Bosnia and Herzegovina is of crucial importance in the context of climate change, especially taking into account that drought is endangering oak and beech forests, which poses linden as a drought resistant species in the focus of economic analysis.

3.2. Findings on Theoretical Results Explaining the Previous Research on Plantations Fundings to Secure Biomass for the Wood and Furniture Market

The history of planting tree plantations on larger areas in Bosnia and Herzegovina has not been sufficiently researched (Govedar and Mataruga, 2021). Certain oral data on the establishment of chestnut and linden plantations exist in the area of the Cajnice municipality in Bosnia and Herzegovina, the origin of which is linked to the expansion of the Austro-Hungarian monarchy in the territory of Bosnia and Herzegovina. In general, more intensive planting of trees for the needs of furniture production and additional quantities of wood began to take place during the 1920s, when certain studies or results of research on Bosnian and Herzegovinian forestry were already published in local documents. The exploitation of Bosnian and Herzegovinian wood during the annexation of the Austro-Hungarian monarchy in Bosnia and Herzegovina, in addition to exports to other parts of the monarchy, largely enabled the construction of roads, railways and other infrastructure in Bosnia and Herzegovina, and the planting of plantations took off mainly at the beginning of the 20th century (Mekic, 2006). However, during the 1960s, certain trends were also present in the former common state, the Socialist Federal Republic of Yugoslavia, when pine plantations became extremely popular. Namely, there was a great need for pine wood in Europe, especially in the northern European countries, and the production of furniture from coniferous wood experienced an expansion. The wood market in some countries promoted coniferous wood more than hardwood (f.e. Oak and European beech), but hardwoods still remained the focus of firewood production. Solid Oak and beech wood remained primarily in the wood products market for furniture production, and the sale of veneers “L” (glued veneer) and “F” (peeled veneer) became one of the most current topics of economic research by local forestry companies and wood processors and furniture manufacturers for homes, offices and schools. The most famous furniture company that buys and processes wood for the purpose of making extremely high-quality furniture is SIPAD (Avdibegovic et *al*., 2007) from Sarajevo, Bosnia and Herzegovina. The company employed tens of thousands of workers, and furniture was exported to many countries in Europe and the world. Depending on the hardness qualities of the wood, it is possible to make different objects or semi-products. Each forest area, which is characterized by the presence of large complexes of natural high forests, has certain tree species that can be called primary or dominant in these areas. The transport of wood over greater distances has greatly influenced the total costs of hauling and processing wood mass, and products made from this wood have certainly had a higher price. However, with the establishment of plantations in different areas, as well as by supplementing forest cultures, it is possible to reduce costs and thus influence the total price (Mueller and Hofmann, 2022). Certainly, the design of a strategy for mass afforestation in the future should be thoroughly analyzed and evaluated from various aspects, and one of them is finding a solution for selecting species that are estimated to have primacy in the future in the production of furniture and other products (DBU.de, 2021).

3.3. Theoretical Findings on Bosnian-herzegovinian Actual Issues Regarding Climate Change context

The biggest problem that arises for forestry in Bosnia and Herzegovina in the context of climate change is the drying of forests, both due to attacks by calamitous insects whose inoculums (centers of infection) appear after forest fires, and due to the drying of individual trees due to the complexity of the health status of trees (Andrianjar et *al*., 2021). Namely, the weakest trees in plant communities are usually the first to become infected with certain pathogens. Micro-localities of habitats that are affected by soil drying do not only become poorer in terms of reducing the amount of moisture in the soil, but the problem is also complicated by the evaporation of useful chemical elements and compounds from the soil. The evaporation of useful chemical elements from forest soil can ultimately

cause an imbalance in the proportions of organic and inorganic matter in the soil, which can result in a disruption in the development of vegetative and reproductive parts of plants. In addition to high air temperatures, the long-term absence of adequate moisture in the soil, and the occurrence of forest fires on large areas can irreversibly damage the habitat (primarily the quality of the soil, its chemical and physical properties) and even result in the succession of forest tree species. It is known that so-called pioneer tree species, such as those from the *Populus* and *Betula* genera, usually appear first on burned soils, which can only later, in the future, help improve the soil quality and repopulate previously existing tree species in that habitat (Kaul et *al*., 2010). Therefore, one of the challenges for forestry production is preserving the quality of soil in forest complexes and striving to improve the quality of forest soil, both through bio-technical measures of planting adequate tree species, removing (felling) infected trees, and through various land reclamation measures (depending on profitability, financial resources and goals of the forestry company) (Kumar, 2021). A special challenge in the context of forestry is the care of so-called honey-bearing species in forest ecosystems, which are important for biodiversity and the preservation of insect populations. An example of a honey-bearing species is linden (*Tilia spp.*), which is widely used in apiculture, which is based on beekeeping, honey production for the local and regional market with honey and products related to beekeeping, but also products that have a wide use in pharmacy and medicine (Kusiak et *al*., 2022). The honey market is one of the most important agricultural markets in Bosnia and Herzegovina, where honey has been traditionally used for centuries in everyday nutrition, alternative and official medicine (teas, tinctures, pharmaceutical raw materials) but also in gastronomy (sale of honey-related products in service industries), which is why linden is listed as one of the focus trees in planning future plantations on private properties (Bartsha, 1995., Barker et *al*., 2016, Barker et *al*., 2022). Finding also implicate appearance of pathogens on the forest species *Picea* (spruce wood), *Pinus* (pine wood), *Fagus* (beech wood) and *Quercus* (oak wood) which cause drying of the trees, what was in detail reported by Mekic (2006), who also named forest area around Vares as an example of pine forests drying. Something similar was spotted in the pine standings in the Konjic area (Ovcari), after the thermophilous pathogen *Viscum album* (white mistletoe) infected many trees. Exactly these pathogens, with some additional fungi and bacteria, cause increased mortality ratio.

3.4. Presentation of Quantitative Results

In this subchapter of the Paper, quantitative results will be presented first, followed by qualitative results. The quantitative results relate directly to the analysis of planting material prices and how the price of planting material of the commercially dominant species of beech and oak compares to the proposed substitute tree species, namely linden. The results will be immediately followed by a discussion, which should help clarify the results.

3.4.1.Context of Planting Density and How it Affects Species Choice

Analyzing data on recommended planting densities of forest tree species within the "Afforestation Plan" documents, which are an integral part of technical-technological (implementation) projects of forestry companies (which are part of broader *Forest Management Regulatory Studies*, which are adopted for a period of at least 10 years/10 years from the production period of the forest), the following results were obtained: **I. -** The recommended planting density for the European beech (*Fagus silvatica*) tree species in Bosnia and Herzegovina is 4m x 4m, which means that the distance between neighboring beech trees is at least 4 meters in all directions, if maximum wood production is to be achieved with minimal damage to the physiological development of the trees, **II. -** The recommended planting density for the oak tree species (*Quercus petraea*) in Bosnia and Herzegovina is 3m x 3m, which means that the distance between neighboring oak trees is at least 3

meters in all directions, if maximum wood production is to be achieved with minimal damage to the physiological development of the tree. It must be noted here that in Bosnia and Herzegovina, two types of oak dominate in oak wood production, namely *Quercus petraea* (sessile oak) and *Quercus robur* (pedunculate oak). In cases where the soil is richer in water, pedunculate oak fits better into the production agenda, since it is a tree species that tolerates the presence of groundwater, wetland ecosystems and generally a higher percentage of moisture, without having negative consequences for the quality of the wood produced, **III. -** The recommended planting density of lime trees (*Tilia sp*.) is 2m x 2m, which means that the distance between neighboring lime trees is at least 2 meters in all directions, if maximum wood production is to be achieved with minimal damage to the physiological development of the trees. It is worth noting that three main types of lime trees grow naturally in Bosnia and Herzegovina, namely the small-leaved lime tree (*Tilia cordata*), the large-leaved lime tree (*Tilia platyphyllos*) and the silver-leaved lime tree (*Tilia tomentosa*). Since the silver-leaved linden tree, due to its habit, has the potential to occupy a larger space, and that, proportionally to its much larger leaves (compared to the small-leaved and large-leaved linden trees), it can absorb a higher concentration of CO2 from the air and produce a larger wood mass, it is assumed that a higher planting density of 2m x 2m can be calculated in some other (future) analyses, even up to a planting density of 7m x 7m, which could greatly reduce the cost of purchasing planting material, which in that case becomes more than 300% more cost-effective (cheaper) compared to the cost of purchasing planting material if the planting density of 2m x 2m (which would be more suitable for the small-leaved linden tree) were to be maintained. Next table shows how the planting density relates between the three types of linden.

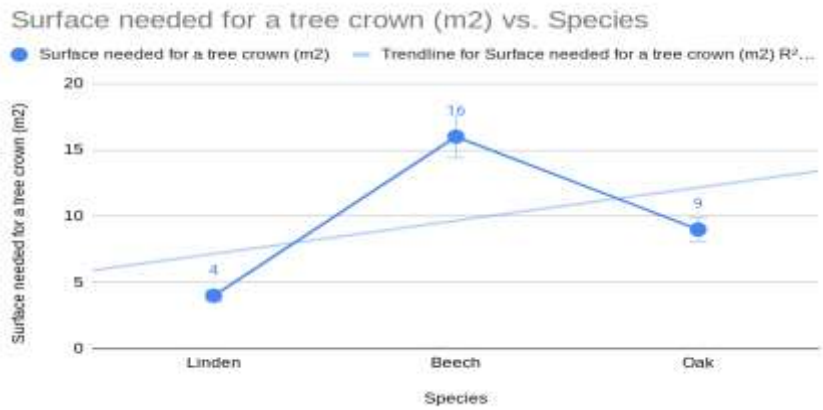
Table 1. Comparison between trees planting density among three suggested species.

Species	Planting Density	Trees per Hectare
Linden (*Tilia spp.*)	2 meters x 2 meters	2500
European Beech (*F. silvatica*)	4 meters x 4 meters	625
Oak (*Quercus spp*.)	3 meters x 3 meters	667

It is observed that, according to the given planting density, the most trees must be used to plant one hectare of forest land with quality planting material (2500 plants). Based on the mathematical relation (Mekic, 2010):

Percentage increase = [(Number of linden trees - Number of beech trees): Number of beech trees] x 100

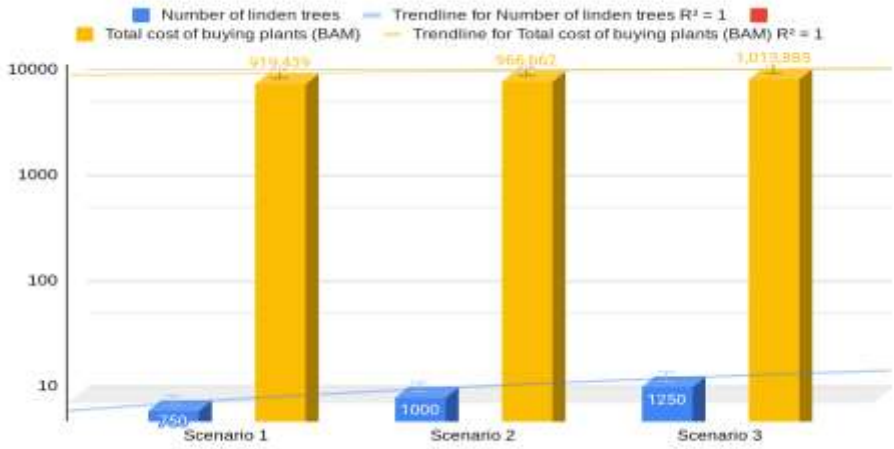
calculation arrives at the result of 300% more linden trees than beech trees, which are needed to plant one hectare of forest land. In general, guided by this planting density for all three tree species, it is observed that the most space is needed for the beech crown, and the least for the linden crown (graph 1).



Graph 1: Proportion of forest soil surfaces needed to handle the healthy tree crown area (in square meters) for each tree species.

The difference between the number of beech trees required in relation to the number of oak trees required for afforestation is not large, and amounts to 42 trees per hectare. This is 6.56% (with the value of R2 = 0.172) more oak trees than beech. If we focus further on quantitative analyses, we will notice differences in the costs of purchasing oak, beech and linden seedlings, which can give us forests experts an idea of how the cost of purchasing seedlings can affect the choice of species for afforestation if the forestry engineer decides to establish a culture that would be of a mixed character, i.e., in which a certain percentage of linden, beech and oak trees would be planted. In forestry and in general in the establishment of plantations (cultures) of forest trees for the production of a wide range of wood products, cultures consisting of one species (so-called pure or monospecific tree cultures) and mixed cultures (multispecific forest tree cultures) can be established (Lojo et *al*., 2008). Accordingly, a forestry engineer can influence the economic profitability and even save on the costs of establishing forest plantations. For example, if the density of linden planting (2m x 2m) is a major economic problem for a forestry company, because for afforestation or establishment of a culture consisting of linden trees, as much as 300% more plants need to be planted than if it were done only with beech (or oak), and the price of linden and beech seedlings is identical (0.50 BAM), while oak seedlings are somewhat more expensive (0.70 BAM). In order to establish a sustainable tree plantation from the aspect of climate change mitigation, a forestry engineer may propose, for example, a proportion of planting linden and beech together, in the ratio of 20% to 80%. In that case, 20% of the forest land area on one hectare would be planted (20% of the area of one hectare is 2000 m2, which is divided by 4m2 of space per linden tree crown) with linden seedlings, the purchase cost of which would be "500 linden seedlings * price of one seedling", and 500 beech seedlings would be planted (the remaining 80% of the area of one hectare is 8000 m2, which is divided by 16m2 of space per beech tree crown), which would make the total number of linden and beech seedlings in one mixed culture 1000. From the aspect of ecology and biology, and referring to dendrochronological research, i.e. the history of the settlement of European habitats by forest tree species, this proportion of species, i.e. their trees in the establishment of cultures, would have relatively sound foundations for the growth and development of a healthy and competitive mixed forest stand, because these are two species that can equally well thrive in a shared habitat (Mekic, 2006). What would be left for forest experts to consider is certainly the method of mixing, or rather, the mixing of trees (whether linden trees, which are fewer in number, would be planted individually or in groups next to beech trees, which are more numerous). Research into competition would be interesting for forest scientists, and perhaps a certain evolutionary pattern of behavior of these two species in the case of sharing a common space could be observed. If the recommendations of German experts (Heinrichs et *al*., 2021), which were previously written for

the conditions of Romanian forestry, were to be accepted, that linden trees should be planted together with oaks on lands where there is potential for this, and in order to successfully mitigate climate change, the proportion of linden trees compared to the proportion of oak could be greater, and perhaps even equal to the proportion of oak. For example, if one adult tree (full form of developed crown) of linden requires 4 m2, and one adult tree of oak (full form of developed crown) requires 9 m2, then: **a. -** in scenario 1 (example) around ca. 30% of linden trees (3000 m2 : 4 m2 = 750) + 70% of oak trees (7000 m2 : 9 m2 = 777.77) comes to the figure of 1527.77 linden and oak trees for afforestation of one hectare, **b. -** in scenario 2 (example) around ca. 40% of linden trees (4000 m2 : 4 m2 = 1000) + 60% of oak trees (6000 m2 : 9 m2 = 666.66) comes to the figure of 1666.66 linden and oak trees for afforestation of one hectare, **c. -** in scenario 3 (example) around ca. 50% of linden trees (5000 m2 : 4 m2 = 1250) + 50% of oak trees (5000 m2 : 9 m2 = 555.55) comes to the figure of 1805.55 linden and oak trees for afforestation of one hectare. When taking into account, for all three reforestation scenarios according to the recommendations for Romania (Radoglou et al., 2009), the prices of planting material according to the Master Plan for Reforestation in Bosnia and Herzegovina, 0.50 BAM for linden and 0.70 BAM for oak, we arrive at the following results (graphs 2).



Graph 2: Costs for buying the total plant material for planting 1 hectare decreases with higher proportion of linden trees in the mixture proportions between linden-oak mixture scenarios.

To understand how the cost of buying trees aimed for funding a plantation changes, as the percentage of linden trees increases in the mixture, it is necessary to observe that in all the three scenarios linden trees take a new 10% of the total proportion (at the same time oak decreases equivalently). By adopting the second scenario, linden percentage increases:

40% -30% = 10%,

so for each 10% increase in the linden tree percentage, the cost increases by ca. 4.66% or 47,223 BAM (for value R2 = 1.0). So, it can be observed that the mathematical relation:

Percentage = (Cost increase: Total cost) x 100

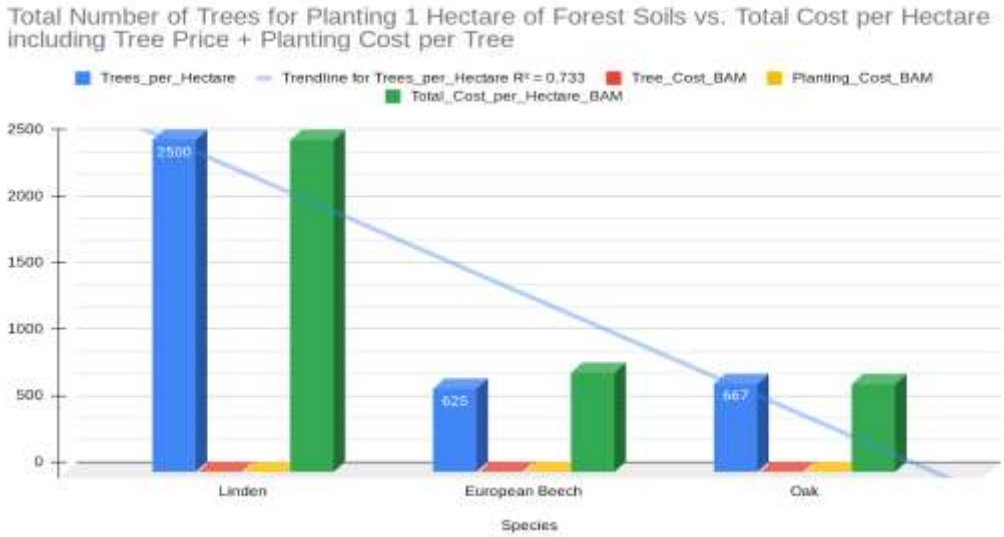
(Lojo et al., 2008) was used.

However, the presented results of the mathematical analysis of percentages and proportions and the progressive growth of the cost of purchasing seedlings in all three scenarios remain the responsibility of the forestry engineer or forestry company, who should decide what the needs of society and stakeholders are in terms of the assortment, quantity and quality of wood products or plantations. Therefore, these three scenarios represent simple models for calculating the cost of purchasing seedlings if different models of mixed planting of forest tree seedlings were to be considered. It is worth noting that the prices of beech seedlings are lower than the prices of oak seedlings, and that one beech tree requires 16 m2 of space compared to an oak tree that requires 9 m2.

This would certainly significantly affect the cost of planting in terms of the cost of purchasing seedlings, but due to the lack of recommendations from current studies in Europe, scenarios for planting mixed beech and linden cultures are not considered, due to competition. Due to the past of Europe, which until the beginning of the Ice Age was characterized by large expanses of linden, whose habitats were taken over by linden after the cold snap, it is observed that linden pollen (Pigott, 2002, Klug et *al*., 2000) is present everywhere. A justified research question arises as to whether linden planting material would enter into sharp competition with beech trees. On the other hand, there is already research indicating that the presence of linden in oak stands improves the growth of oak forests, which is why scenarios for planting mixed stands of linden with beech will not be considered in this Paper.

3.4.2. Prices of Individual Seedlings and Production Cycle Duration of Tree Species

In this case, a simple percentage calculation was performed, in order to investigate whether the price of the seedling itself statistically has a significant influence on the selection of the tree species for afforestation. Since the introductory considerations of the Paper state that the calculation is made with two-year-old seedlings of three broadleaf tree species that can equally grow and develop on land of the same quality and credit rating class, and that the price of seedlings taken from the Afforestation Master Plan (Govedar and Mataruga, 2021) in force in Bosnia and Herzegovina is the same for beech and linden (0.50 BAM) and slightly higher for oak (0.70 BAM), it will be analyzed whether the price of the seedlings themselves can statistically represent a decisive factor for the selection of the tree species. By comparing prices and durations of production cycles of Oak, Linden and Beech, we come to next results:

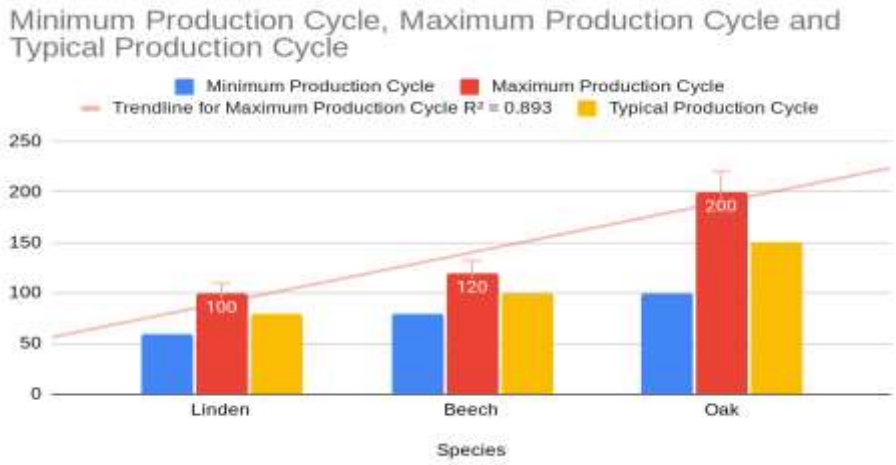


Graph 3: Trend of planting cost decrease (increase) in comparison to total number of trees planned in the afforestation of 1 hectare

By comparing it to the duration of production cycles among three species, we come to next graph:

Following the gotten data from Forestry Elaborate Methodology by Lojo et *al*. (2008), Linden plantations have shortest production cycle duration (60 years minimum, 100 maximum, 80 years typical), while beech is being planted for minimally 80 years, maximally 120 years and typically for

100 years. Having oak plantations being planted for minimally 100 years, maximally 200 years, and typically 150 years, Linden seems to be most rentable species for cultivating, with its minimal production period 100 percent shorter than beech typical production cycle duration and more than 300 percent shorter than maximal oak production cycle duration.



Graph 4: Trend of percentage increase in production cycle in three plant species.

3.4.3. Regression Analysis on Wood Article Prices of Beech and Oak in Bosnia and Herzegovina

By conducting the regression analysis in the "Microsoft Excel", it came to the next results:

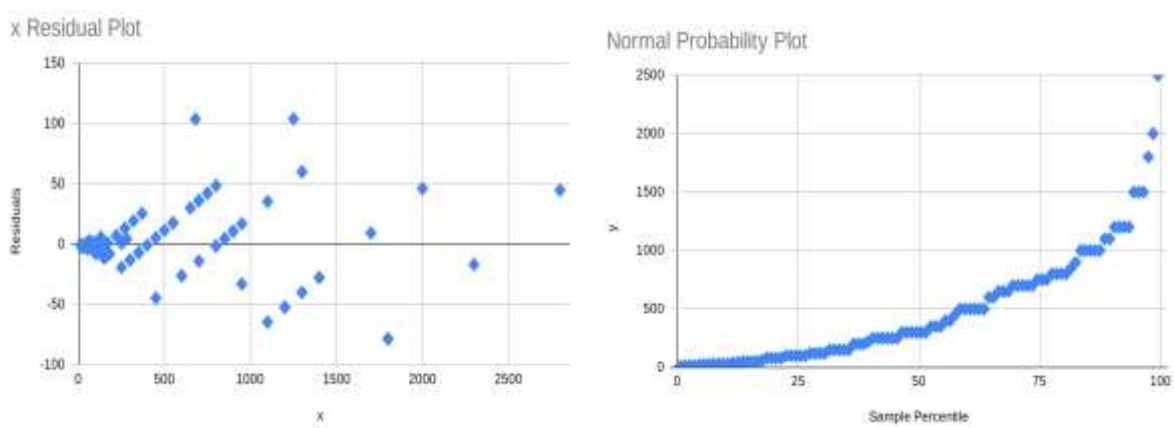
Table 2. Statistical analysis results on beech-oak prices in Bosnia and Herzegovina

Multiple R	0.9991258016
R Square	0.9982523675
Adjusted R Square	0.9982347146
Standard Error	28.92027093
Observations	100

ANOVA						
	df	*SS*	*MS*	*F*	*Significance F*	
Regression	1	47296623.17	47296623.17	56549.0639	0	
Residual	99	82801.82501	836.3820708			
Total	100	47379425				
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*

Intercept	0					
x	0.87686350	0.003687391	237.8004708	0	0.869546918	0.88418008

When explaining strength of the relationship, it is possible to observe next: a) Multiple R of 0.9991 means that the correlation coefficient is showing almost a perfect linear relationship, b) R^2 (R-squared) of 0.9983 is meaning that 99.83% of the variation in the dependent variable (oak wood prices) can be explained by the independent variable (beech wood prices), while c) Adjusted R^2 of 0.9982, as slightly lower than R^2, is still very high, confirming the strong explanatory power of the tested model, which is similar to the Cabaravdic (2010) models explained in comparing deciduous and conifer species products for construction. Explaining the model significance by conducting ANOVA analysis means next: a) F-statistic of 56,549.06 is extremely high, indicating that the model is statistically significant, and b) Significance F (p-value) of 0 means there is almost no chance that the relationship is random, which compares to Avdibegovic (2007) statements for perfect forest market models. Explaining the regression coefficients it comes to next interpretations: a) Intercept of 0 means that the model does not include an intercept term (which means if beech prices were 0, oak prices would also be 0), b) Slope (x coefficient) of 0.8769 means that for every unit increase in beech wood price, the oak wood price increases by 0.8769, c) Standard Error of x coefficient of 0.0037 is pretty small, indicating a precise estimate, d) t-Statistic of 237.8 is very large, meaning the coefficient is highly significant, and e) P-value of 0 means that the predictor variable is highly significant. Explaining the model accuracy and residual analysis means next: a) Standard Error of 28.92 is the average deviation of the actual prices from the predicted prices, b) Residuals - most of them are small, but a few larger residuals (e.g., -78.35, 103.92) suggest possible outliers, and c) Normality of residuals shows in a way that they appear relatively small and balanced between positive and negative values, suggesting a good model fit., which again agrees with Cabaravdic (2010) findings in scope of explaining error values in the monograph. As a conclusion it came to next: 1) The used regression model is highly accurate and explains nearly all variations in oak wood prices based on beech wood prices, 2) The coefficient (0.8769) shows a strong proportional relationship, and 3) The residual analysis suggests minor outliers, but the overall fit is excellent.



Graph 5 (a - left, b - right): Relation between *x* residual plot and normal probability plot

3.5. Presentation of Qualitative Results

3.5.1. Identification of Main Pathogens in the Context of Planting Under the Droughts

On the basis of Federal Ministry office for forestry administration, based on the previous reports of the federal forestry companies, main illnesses affecting the sustainable production of Oak, Beech and Linden forests are: a) disease of grey leaf in Oak species, which causes drying and destruction of the oak plantations, b) disease of the black core in European beech, that devastate beech ecosystems and destroys complete plantations up to 90 percent of juveniles, and c) wilting disease in Linden, which appears as a result of combined natural and anthropogenic factors such as fertilization. The sources announced that mass drying of the beech and oak forests had been identified in certain parts of the country, but that it is still under control. Source announces no mortality cases in Linden. Compared to Kumar (2021) it is visible that forest pathogens represent everywhere in the world a real danger for forest production, because they combine with negative effects of wild forest fires in the large ecosystems and suppress natural juvenile populations to fight. Most of the diseases correspond to those described by Avdibegovic (2007), as main risks for the forestry firm's management. It is very important to point out to the wider academic community the importance of afforestation in the country, especially in those habitats where there is a potential for planting tree species that would be highly resistant to high temperatures and long-term droughts. Consideration of the price of planting material, possibly a large number of seedlings per hectare of area, as well as a smaller planting distance (density) should not be a decisive factor in limiting future cultivation, cultures (plantation) supplementation and afforestation to the most frequent tree species so far, but rather that it is necessary to give a chance to the application of current research results and the search for solutions for climate change mitigation. In this analysis, we should not lose sight of the harmful consequences of climate change, which includes an increased level of tree mortality in forests, and the appearance of pathogens and harmful insects, the spread of which is correlated with increased temperatures and the frequency of forest fires.

3.5.2. Knowledge Gaps in Understanding Potential of Healthy Linden Plantations in the Context of Drying Typical Commercial Species Such Oaks and Beech

A complex study of the morphological, phenological and genetic characteristics of three species of lime tree in Bosnia and Herzegovina has shown that the genus *Tilia* is very important for the process of climate change mitigation (Vejzagic et *al*., 2021). In earlier studies in Bosnia and Herzegovina, linden (in literature also named as “lime”) tree has been characterized as a tree of the future, due to its high resistance to drought, but also due to its distribution in almost all habitats where forests naturally grow. Certain linden tree populations in Bosnia and Herzegovina are characterized by different morphological characteristics of their fruits, which enable them to be dispersed by wind over greater distances, due to the greater number of bracts (Vejzagic, 2022). Linden trees were studied much more extensively on the European continent about 100 years ago, but the study of this tree finally intensified again in the 2010s. A major contribution to linden research and the analysis of pollen maps of Europe was made by the author Pigott, who pointed out the potential for linden to return to ancient habitats, since linden pollen is widespread throughout Europe, including in European forest habitats where linden currently dominates (Pigott, 1969, 1991, 1992, 1997, 2002). The onset of the Ice Age led to significant changes in the proportion of forest tree species in Europe, as well as to glacial retreats of entire plant populations (Klug et *al*., 2000). However, thousands of years later, researchers are witnessing an intensification of climate change, which brings with it high temperatures and long-term droughts, which necessitates the need for in-depth research on species that once, before the onset of the Ice Age, had primacy in forest plant communities (Logan et *al*., 2015). A great contribution to the study of linden, as well as its application in the establishment of plantations, the establishment of gardens, planting alleys and parks, was made by Scandinavian countries, followed by Germany, the Netherlands and the United Kingdom, where the largest number of studies on linden (Baxandall, 1995, Becker, 1920, Bengtsson, 2005, Binder, 2016, Christensen, 1981, De Jaegere et *al*., 2016, Engler,

1969, Huntley and Birks, 1983, Hyde and Williams, 1945, Karling, 1931, Lindberg, 1956, 1957, Svoboda, 1953, 1957, Wicksell and Christensen, 1999) come from. The potential of linden for planting in the future is supported in the deciduous forests of Europe, such as Romania, where it is possible to plant linden with oaks and beech (Heinrichs et *al*., 2021). A Ukrainian study has shown that linden trees are extremely long-lived and much more resistant to diseases and temperature turbulence. If seedlings costs and sustainable afforestation strategy are met, the potential of sustainable biomass production for future, including rentable prices of wooden products for society, the potential crisis on the wood market could be prevented. Unfortunately, negative reports on drying in the Oak and Beech stand structures are not bringing positive news to the wood and furniture market, which implies additional potential for the healthy linden plantations to be used more intensively in the future.

4.CONCLUSION

Respecting the quantitative and qualitative results gotten in the research, it is possible to identify several crucial findings: - forest species have to be chosen in scope of the afforestation lans not only on the fundament of expenses for buying seedlings or planting material, but also by respecting the benefits from planting cultures with drought resistant trees, - in comparison to first two commercial species in bosnian wood market, which are oak and beech, the linden tree can also be produces on a high bonity class soil, and with enough biomass produces in the cultures it an provide similar quality of certain wood products for the society, - lenght of the production cycle is crucial in the context of the climate change, because focusing on production of linden, whose minimal duration of the production cycle is more than 300 percent shorter than maximal oak production, can prevent drying of forest plantations caused by drought resistant pathogens, and help provide sustainable and production with less risk of drying, - regression model can be used to compare prices of same or similar quality wooden products of linden, since it is shown to be ideal in analyses of variation of prices between oak and beech products. Respecting the gotten results, we failed to reject the null hypothesis of the research that "*The price of planting material is statistically not decisive for the choice of tree species for afforestation, what supports the regression model of wooden products prices trend in the context of prognosing rentability of linden products increase on the market.*". It is needed to conduct further investigation on the linden wood products market and to explore further danger for the linden plantation funding, if any indicators for the health of the species exist.

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ASSESMENT OF INCEPTISOL AND ENTISOL SOIL CLASSES COMMONLY FOUND IN TURKEY IN TERMS OF FERTILITY

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ABSTRACT

Inceptisols and Entisols each possess unique attributes concerning soil fertility. The management of these soils through appropriate agricultural practices such as the application of organic amendments, monitoring nutrient levels, and understanding soil texture and composition can play a crucial role in optimizing agricultural outputs. Given their moderate fertility levels, Inceptisols can be enhanced further through strategic fertilization, while Entisols, being less fertile, typically require more intensive management practices to improve
their productivity. In summary, while both Inceptisols and Entisols present distinctive fertility profiles affected by their parent material and organic matter content, Inceptisols exhibit a greater potential for fertility improvements through management practices. This underscores the need for targeted soil management strategies to balance nutrient availability, particularly in areas where either soil type predominates.

Keywords: Inceptisol, entisol, soil properties, fertility

INTRODUCTION

Semi-arid climates are regions with limited rainfall and frequent dry periods. Semiarid climates are ecosystems where annual precipitation is mostly in the range of 300–600 mm. Inadequate water resources and high evaporation directly affect the physical and chemical structure of the soil. These effects lead to a decrease in organic matter, soil erosion, and mineral decomposition. Furthermore, soil with low water-holding capacity are common in these climates. Oxidation processes are frequent in the soil profile, threatening sustainable production. In Turkey, the semiarid zone is concentrated in Central Anatolia, Eastern Anatolia, and Southeastern Anatolia. Entisol and Inceptisol soils are

common in these regions. Inceptisols and Entisols are two significant soil orders that exhibit distinct characteristics influencing their fertility and agricultural potential. Understanding their fertility status is vital for effective land management, which is particularly critical in agricultural practices where nutrient availability directly impacts crop yield. In this review fertility status of inceptisol and entisol soils were discussed.

CHARACTERISTICS OF INCEPTISOLS AND ENTISOLS

Inceptisols are soils with limited horizon development, are pedologically young, and have low levels of mineralogical weathering (Soil Survey Staff, 2014). Inceptisols are generally young, underdeveloped soils and constitute approximately 17% of the world's land surface (USDA, 2014). The formation of these soils is shaped by the interaction of environmental factors such as climate, parent material, and vegetation. Inceptisols, which generally develop on alluvial sediments and in humid-temperate climates, are characterized by low carbonate content and neutral to slightly alkaline pH (Buol et al., 2011; Brady & Weil, 2010). Physically, these soils, which have a mostly loamy-sandy texture, exhibit good drainage. Clay content can range from 35–50%, which increases water retention capacity while limiting permeability (White et al., 2007). Inceptisols are generally rich in essential nutrients such as nitrogen, phosphorus, and potassium. However, organic matter levels vary depending on environmental conditions. Soil organic matter not only supports microbial activity but also enhances structural stability. These soils are densely populated by microorganisms such as bacteria, fungi, and protists. These organisms play an important role in humus formation and nutrient cycling (van der Heijden et al., 2008). The spatial distribution of different soil orders at the global scale is shown in Figure 1.

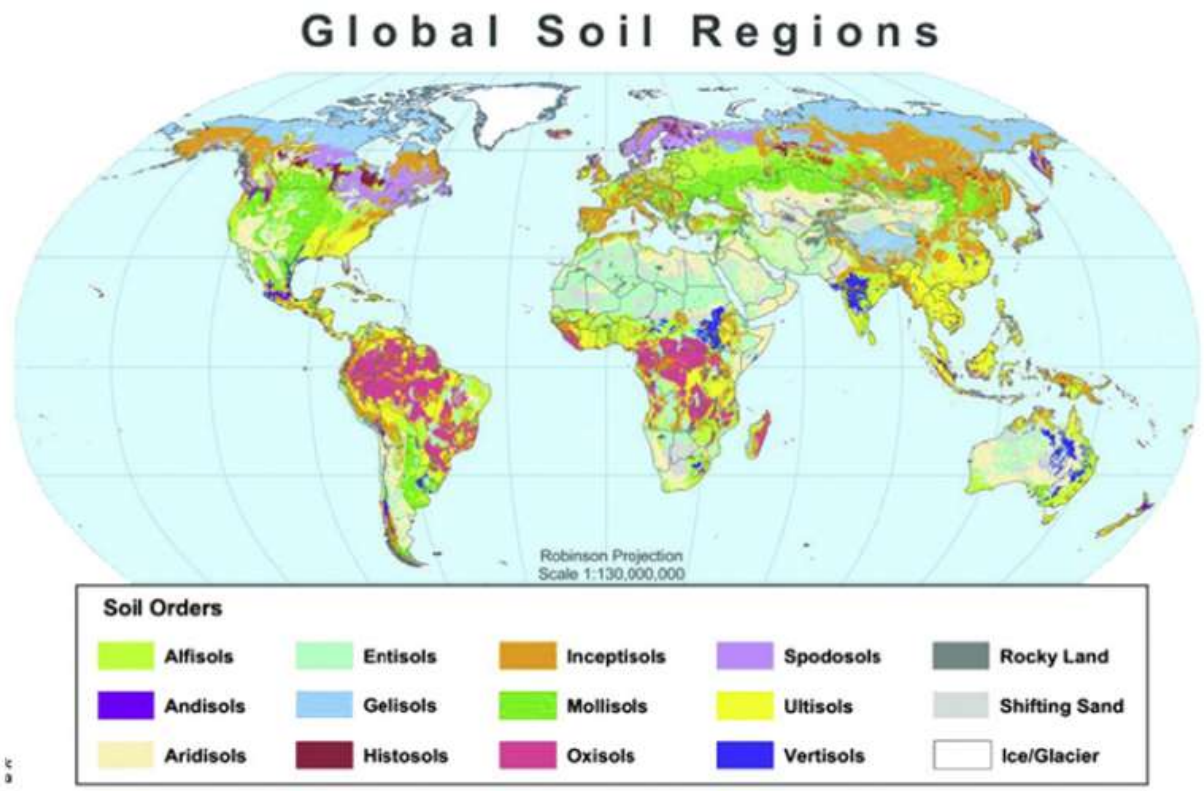

Figure 1. Global Classification of World Soils: A Mapping Focused on Entisols and Inceptisols (Eswaran and Reich, 2005).

Inceptisols are recognized for their relatively young and poorly developed profile, often featuring a significant horizon with minimal weathering. Inceptisol soils have poorly developed horizons and are generally characterized by stony and young profiles (Figure 2). They typically possess more nutrients compared to the more weathered Entisols, which are characterized by their young, unaltered state. Research has shown that Inceptisols, especially those studied in regions such as Aceh and Kerala, exhibit higher levels of critical nutrients such as nitrogen, phosphorus and potassium than Entisols, thereby presenting favorable conditions for crop production (Sharma., 2020; Nafiah & Prasetya, 2019). Vegetation improves soil physical structure by increasing organic matter accumulation through root release and detritus. Inceptisol soils have significant potential for sustainable agricultural production. However, they require careful land management due to limiting factors such as poor horizonization and salinity (Eswaran et al., 2003). The fertility status of these soils can be affected by various inputs, including organic fertilizers and mineral amendments. For instance, the application of biochar and other organic materials has been observed to increase soil organic matter and nutrient contents, resulting in enhanced fertility of Inceptisols (Kolambani & Widowati, 2022). Additionally, the use of vermicompost and microbial inoculants has shown promising resultsin improving the biological and chemical characteristics essential for plant growth (Lubis & Sebayang, 2024; Muktamar et al., 2023). Conversely, Entisols are characterized by lower fertility owing to their relatively young profiles and minimal horizon development. They often exhibit coarse textures leading to high infiltration rates and low water retention ability, which considerably limit their productive capacity (McLeod et al., 2021). Despite these challenges, studies indicate that Entisols can be improved through the application of biofertilizers, mycorrhizal associations, and organic amendments that enhance nutrient availability for crops like patchouli (Syafruddin et al., 2022). Furthermore, Entisols show variable fertility depending on their parent material, with certain instances indicating that alluvial Entisols can be quite fertile, while those on steeper slopes or sandy areas tend to have low productivity due to low organic matter accumulation (Syafruddin et al., 2022). In terms of micronutrient dynamics, both soil types exhibit distinct mobility patterns for heavy metals. For example, the mobility factor of nickel (Ni) is higher in Inceptisols compared to Entisols, which demonstrates their differing capacities to retain and mobilize various elements based on their mineralogical and chemical nature (Patra et al., 2021). Additionally, while Entisols might have deficiencies in certain macronutrients, some studies report higher mobility for specific micronutrients like zinc (Zn) in different land use systems, highlighting the need for careful management to mitigate nutrient deficiencies in both soil types (Gowthamchand et al., 2023). Entisols are young and underdeveloped soils; they have low organic matter content and are susceptible to erosion. Inceptisols, on the other hand, contain more organic matter in the upper horizons and are structurally more developed (Türkeş, 1999). Entisols are young soils that do not show significant horizon development (Figure 3).

Figure 2. General view of inceptisol soil (https://www.nrcs.usda.gov/conservation-basics/natural-resource-concerns/soil/inceptisols).

Figure 3 General view of entisol soil (https://www.nrcs.usda.gov/conservation-basics/natural-resource-concerns/soil/entisols)

NUTRIENT AVAILABILITY AND MANAGEMENT STRATEGIES

Despite their generally favorable fertility, Inceptisols often face challenges related to nutrient management, primarily nitrogen deficiencies (Rizwan & Harahap, 2022). Studies indicate that the addition of both organic and inorganic fertilizers, along with balanced nutrient management strategies, can significantly improve the chemical characteristics of Inceptisols, increase crop yields, and reduce reliance on synthetic fertilizers (Rizwan & Harahap, 2022; Lubis & Sebayang, 2024). For instance,

the application of combinations of organic fertilizers such as chicken manure has been found to effectively enhance the fertility of Inceptisols (Rizwan & Harahap, 2022; Kolambani & Widowati, 2022).

Moreover, findings from Corrêa et al. indicate that fluid mineral fertilizers can be particularly effective for increasing carbon stock and optimizing soil nutrient dynamics in Inceptisols due to their relatively lower degree of weathering compared to more developed soils (Corrêa et al., 2019). Therefore, integrating different fertilization methodologies can significantly impact the nutrient profile, leading to enhanced agricultural productivity (Lubis & Sebayang, 2024, Ghosh & Debnath, 2010).

CONCLUSION

Semiarid climate regions present special challenges in terms of soil fertility due to environmental factors such as low and irregular rainfall regime, high evaporation rate and limited water resources. Entisol and Inceptisol soils are commonly found in this climate zone. In order to ensure sustainable agricultural production, it is necessary to develop appropriate management and reclamation strategies for these soils. Increasing soil organic matter, reducing erosion, and improving water management are among the keyways to increase productivity, particularly in Entisol-characterized lands. In Inceptisol soils, integrated land management practices are gaining importance for the preservation and sustainable use of existing potential. Furthermore, a combined evaluation of national and international soil classification systems demonstrates the need for combined use of multi-source data to map and manage soil diversity in Turkey. Consequently, the accurate classification, monitoring, and management of Entisol and Inceptisol soils in semiarid conditions is a scientific and strategic necessity for establishing sustainable agricultural policies.

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ULTRASONIC EXTRACTION and ANTIOXIDANT ACTIVITY of Elaeagnus umbellata FRUIT

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ABSTRACT

Natural antioxidants are gaining increasing importance in functional food and pharmaceutical applications. *Elaeagnus umbellata* fruit is rich in phenolic and flavonoid compounds and has high antioxidant potential. In this study, the effect of water content (%), lactic acid/choline chloride ratio, and temperature (°C) factors on antioxidant capacity was analyzed using DPPH (2,2-diphenyl-1-picrylhydrazil) radical scavenging capacity, FRAP (Ferric Reducing Antioxidant Power), TPC (Total Phenolic Content) and TFC (Total Flavonoid Content) analyses, and optimized using Response Surface Methodology (RSM). The results of the analyses showed that the highest antioxidant activities were obtained at a water content of 28.28 %, a lactic acid/choline chloride ratio of 3, and a temperature of 70 °C. Under these conditions, DPPH, FRAP, TPC, and TFC values were measured as 6.51 mg TE/ g, 21.88 mg TE/ g, 23.09 mg GAE/ g, and 4.15 mg QE/ g, respectively. The results indicate that phenolic and flavonoid compounds are temperature-sensitive and that optimal extraction occurs at medium-high temperatures. Furthermore, FRAP and DPPH analyses confirm that the extract possesses high free radical scavenging and reducing capacities. This study demonstrates that *E. umbellata* fruit is a potent natural antioxidant source under optimized conditions and can be used in functional food and pharmaceutical applications.

Keyword: *Elaeagnus umbellata, Antioxidant activity, Optimization,*

INTRODUCTION

Medicinal plants have been used for both food and therapeutic purposes throughout history in various civilizations (Jamshidi-Kia et al., 2017; Bhat et al., 2023). *E. umbellata* Thunb., a plant commonly found in the Northwestern Himalayas, is notable for its rich bioactive compound content (Chauhan et al., 2023; Bhat et al., 2023). It is also widely used in traditional medicine for its antioxidant, antimicrobial, anti-inflammatory, anticancer, and antidiabetic effects (Nazir et al., 2021). Today, research on the effects of consuming natural compounds on human health has increased, and it is crucial to better understand the potential of plants such as *Elaeagnus umbellata*. *E. umbellata* holds significant potential for the pharmaceutical and functional food industries due to its broad bioactive compound profile. Current studies support the protective and therapeutic effects of this plant against diseases, but emphasize the need for more detailed investigation of the mechanisms of its active compounds.

The fruits of *E. umbellata* are rich in polyphenols and anthocyanins with high antioxidant capacity (Ozen et al., 2017), and the plant's fruits and leaves also contain high amounts of polyphenols, flavonoids, carotenoids, alkaloids, and saponins (Khattak, 2012). Therefore, they provide protection against free radical damage and play a role in preventing diseases associated with oxidative stress (Nazir et al., 2021; Bhat et al., 2023). Additionally, the flavonoids and phenolic compounds found in the plant support the immune system by exhibiting anti-inflammatory effects (Bhat et al., 2023). Furthermore, its high lycopene content suggests that this plant could be an important resource in the prevention of cancer and cardiovascular diseases (Patel, 2015; Ozen et al., 2017). Pharmacological studies have revealed that *E. umbellata* possesses anticancer activity, particularly that ellagic acid components may inhibit tumor growth (Vignera et al. 2022). Furthermore, studies have shown that *E. umbellata* extracts lower blood glucose levels in diabetic models, and that this effect is related to the inhibition of free radicals by the phenolic and flavonoid compounds it contains and the increase in insulin sensitivity by suppressing the α-glucosidase enzyme. (Nazir et al. 2018).

In this study, the effects of ultrasonic power, extraction time, and sample amount on the DPPH radical scavenging capacity, FRAP, TPC and TFC of *E. umbellata* fruit extracts were investigated. The results obtained using RSM reveal the significant effects of these variables on antioxidant properties.

MATERIAL AND METHOD

Material

The fruits of *Elaeagnus umbellata* were obtained from local producers in Trabzon, Türkiye. They were dried at 50 °C until a constant weight was achieved. The dried samples were ground using a mortar and subsequently used for extraction. All chemicals employed in the study were purchased from Sigma.

Extraction

The extraction procedure was performed with modifications based on the method described by Alishlah et al. (2019). For extraction, 1 g of sample was weighed into 50 mL falcon tubes, followed by the addition of 20 mL of the prepared DES solutions at the ratios specified in the experimental design. The mixture containing the sample and solution was then placed in an ultrasonic water bath (20% ultrasonic power) for 30 min, considering the temperature parameters defined in the experimental design. After the incubation period, the samples were centrifuged at 3500 g for 45 min. The supernatants were collected and filtered using coarse filter paper. The prepared extracts were stored at −20 °C until further analysis.

Experiment design and optimization

Experimental conditions were established using the Box–Behnken response surface methodology, with the experimental design generated using Minitab 18.1 software (Table 1). Extraction was performed according to the procedure described in the "Extraction" section. The extracts were evaluated for TFC, DPPH, FRAP, and TPC which were employed as response variables to optimize extraction efficiency.

Three independent factors were investigated: water content (20–40%) (X1), molar ratio (lactic acid/choline chloride, 1–3) (X2), and temperature (30–70 °C) (X3). A total of 15 experimental runs were implemented under these conditions. Statistical analyses were carried out at a significance level of $P < 0.05$, and the adequacy of the extraction model was assessed using analysis of variance (ANOVA).

Table 1. Experimental variables based on water content, temperature, and molar ratio

Water content (%)	Molar ratio (lactic acid/choline chloride)	Temperature (°C)
40	2	30
20	2	30
30	1	30
30	3	30
30	2	50
40	3	50
40	1	50
30	2	50
20	3	50
20	1	50
30	2	50
20	2	70
40	2	70
30	1	70
30	3	70

DPPH radical scavenging activity assay

The decrease in optical density was associated with DPPH radical uptake. For analysis, 100 µL of the ethanol extract was mixed with 2000 µL of 0.2 mM DPPH solution, vortexed, and incubated for 30 min at ambient temperature. The absorbance was then recorded at 517 nm, and the results were expressed as Trolox equivalents (mg/g) (Kumaran and Karunakaran, 2007).

Ferric reducing antioxidant power assay

A fresh FRAP working solution was prepared by mixing 25 mL of acetate buffer, 2.5 mL of TPTZ solution, and 2.5 mL of $FeCl_3 \cdot 6H_2O$ solution from the stock reagents. The mixture was preheated to 37 °C prior to use. Subsequently, 150 µL of the extract (0.1 mg/mL) was reacted with 2850 µL of the FRAP reagent for 30 min in the dark. The formation of the ferrous–tripyridyltriazine complex was quantified at 593 nm, and the results were expressed as mg Trolox equivalents per g of sample (Upadhyay et al., 2010).

Total phenolic content

The TPC of the extract was quantified using the Folin–Ciocalteu reagent following the procedure described by Gülcin et al. (2002). Briefly, 0.1 mL of the extract was diluted with 46 mL of distilled water in a volumetric flask. Subsequently, 1 mL of the Folin–Ciocalteu reagent was added, and the mixture was thoroughly mixed. After a 3-min incubation, 3 mL of 2% Na_2CO_3 solution was introduced, followed by intermittent shaking and a 2-h resting period. Absorbance was recorded at 760 nm, and the results were calculated based on the gallic acid calibration curve and expressed as mg gallic acid equivalent (GAE)/g.

Total flavonoid content

The aluminum chloride colorimetric method was employed to determine the flavonoid content of the fruit extracts. In this assay, 1 mL of the ethanol-diluted sample was combined with 1 mL of 2% $AlCl_3$ in methanol. Following a 15-min reaction period, absorbance was recorded at 430 nm. Flavonoid content was calculated using a calibration curve prepared with quercetin standards of known concentrations, and results were expressed as mg quercetin equivalents (QE) per g of sample (Djeridane et al., 2006).

RESULTS AND DISCUSSION

Effect of experimental conditions on TPC, TFC, FRAP and DPPH

According to the DPPH radical scavenging capacity results, the lowest and highest values were recorded as 1.33 mg TE/g and 6.42 mg TE/g, respectively. According to the ANOVA results, the model was found to be highly significant ($P < 0.05$), with $R^2 = 97.50\%$. The highest DPPH values were observed in combinations of high temperature levels, medium-high water content, and medium lactic acid/choline chloride ratios. In contrast, the lowest DPPH values were recorded at low temperature, low water content, and low lactic acid/choline chloride ratio levels. The combined increase in water content and temperature factors created a general upward trend in DPPH values. Similarly, the quadratic effect of the lactic acid/choline chloride ratio contributed to an increase in antioxidant capacity at certain levels. Furthermore, the interaction between water content and temperature factors was found to be statistically significant ($P < 0.05$), indicating that optimal antioxidant activity can be achieved under certain combinations (Figure 1).

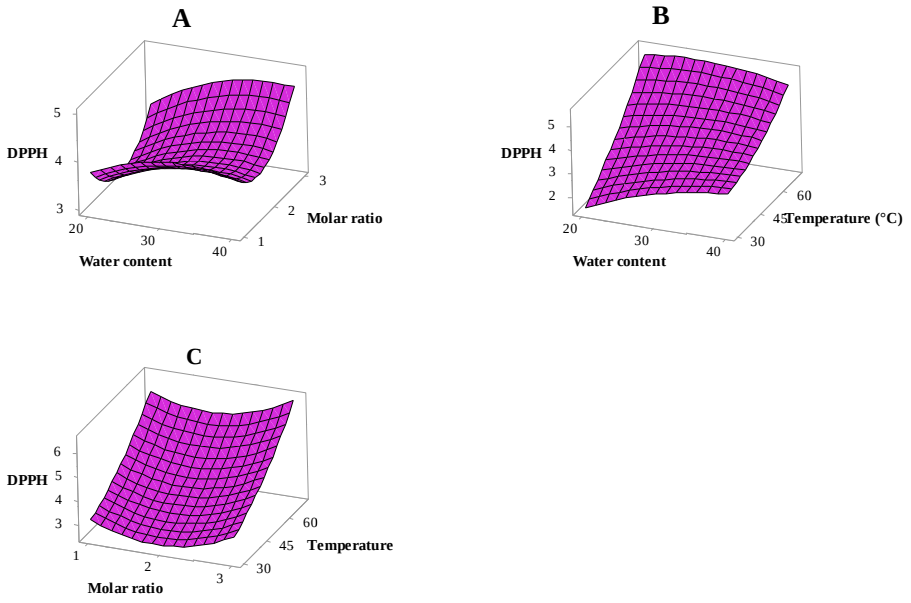

Figure 1. Variation of DPPH with independent variables Water content–Molar ratio (A), Water content–Temperature (B), and Molar ratio–Temperature (C). Water content (X1, %), Molar ratio (X2, lactic acid/choline chloride), and Temperature (X3, °C).

FRAP analysis revealed values ranging from 7.67 mg TE/g to 22.27 mg QE/g. Statistical analysis via ANOVA indicated that the model was significant ($P < 0.05$), with a coefficient of

determination (R^2) of 91.97%. The highest FRAP responses were achieved under conditions of elevated temperatures, medium–high water content, and medium lactic acid/choline chloride ratios. Conversely, the lowest FRAP values were associated with low temperature, low water content, and low lactic acid/choline chloride ratios, highlighting the strong influence of these extraction parameters on antioxidant activity. The combined increase in water content and temperature has created a general upward trend in FRAP values. Similarly, the quadratic effect of the lactic acid/choline chloride ratio has contributed to an increase in antioxidant capacity at certain levels. However, raising the temperature to very high levels has caused a decrease in FRAP values beyond a certain point (Figure 2).

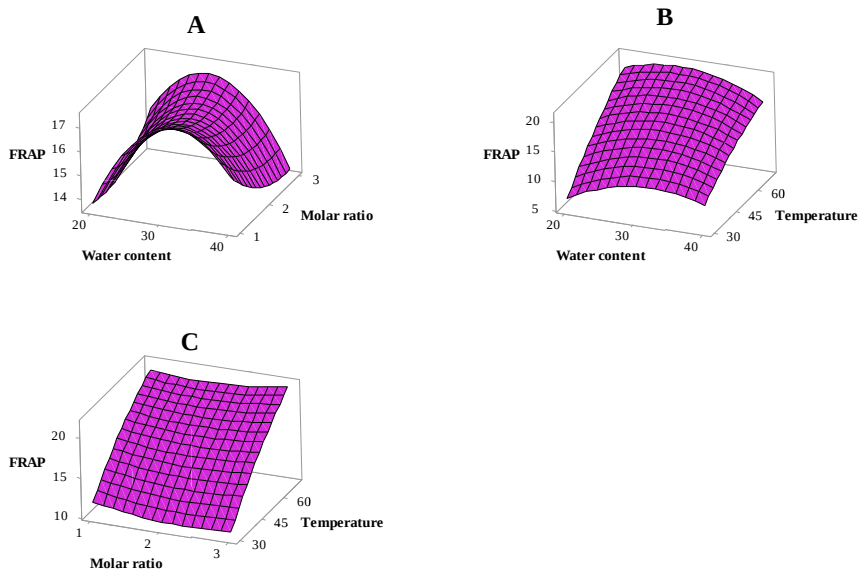

Figure 2. Variation of FRAP with independent variables Water content–Molar ratio (A), Water content–Temperature (B), and Molar ratio–Temperature (C). Water content (X1, %), Molar ratio (X2, lactic acid/choline chloride), and Temperature (X3, °C).

In terms of total phenolic content, the lowest value was 11.20 mg GAE/g, while the highest value was 24.09 mg GAE/g. According to the ANOVA results, the model was found to be significant ($P < 0.05$), and $R^2 = 92.42\%$ was calculated. The highest TPC values were observed in combinations of high temperature levels, medium-high water ratio, and medium lactic acid/choline ratio. In contrast, the lowest TPC values were recorded at low temperature, low water ratio, and low lactic acid/choline chloride ratio levels. The quadratic effect of the lactic acid/choline chloride ratio contributed to an increase in the TPC value, while the quadratic effects of the water ratio and temperature showed a downward trend in the TPC value at certain levels. Furthermore, the two-way interactions between the factors produced measurable changes but were not statistically significant (Figure 3).

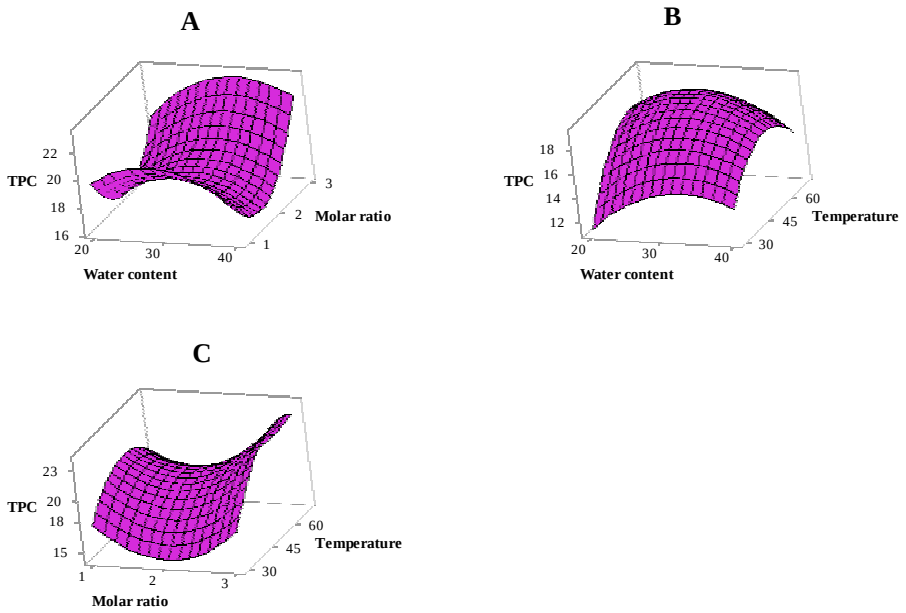

Figure 3. Variation of TPC with independent variables Water content–Molar ratio (A), Water content–Temperature (B), and Molar ratio–Temperature (C). Water content (X1, %), Molar ratio (X2, lactic acid/choline chloride), and Temperature (X3, °C).

In the total flavonoid content analysis, the lowest and highest values were determined to be 2.81 mg QE/g and 4.27 mg QE/g, respectively. According to the ANOVA results, the significance level of the model was borderline ($P > 0.05$), and R^2 was calculated as 87.49%. The highest TFC values were observed in combinations of high temperature levels, medium-high water ratio, and medium lactic acid/choline ratio. In contrast, the lowest TFC values were recorded at low temperature, low water ratio, and low lactic acid/choline chloride ratio levels. The interactions between moisture content and temperature, and between lactic acid/choline chloride ratio and temperature contributed to changes in TFC values. Additionally, the quadratic effect of water ratio and lactic acid/choline chloride ratio showed a tendency to decrease TFC values at certain levels. These results are consistent with the significant positive effect of temperature on TFC (Figure 4).

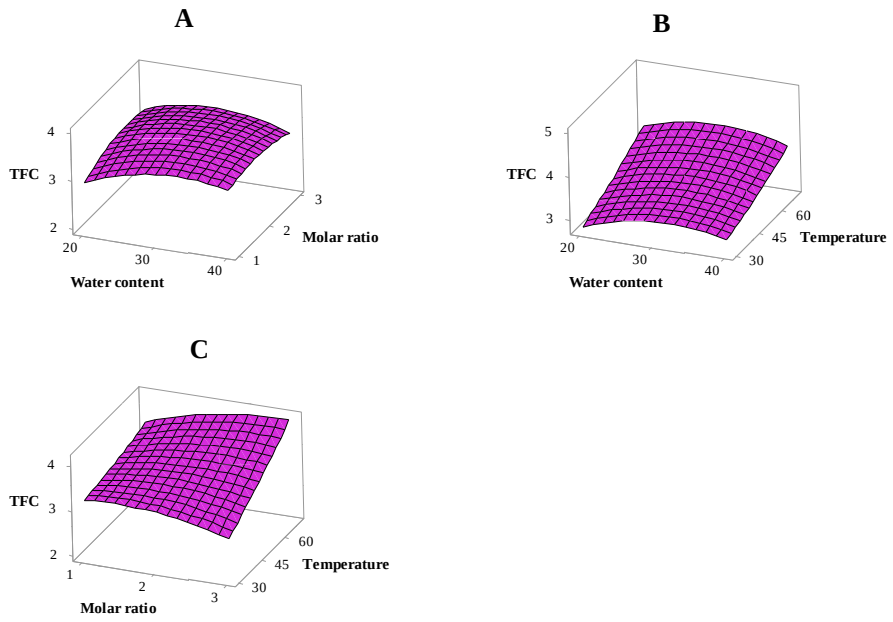

Figure 4. Variation of TFC with independent variables Water content–Molar ratio (A), Water content–Temperature (B), and Molar ratio–Temperature (C). Water content (X1, %), Molar ratio (X2, lactic acid/choline chloride), and Temperature (X3, °C).

In general, the combined increase in water content, lactic acid/choline chloride ratio, and temperature factors has led to an upward trend in antioxidant and phenolic content across all analyses. Specifically, combinations of high temperature and medium-to-high water content have resulted in maximum values. However, exceeding certain levels has caused a decrease in some parameters, highlighting the importance of optimal conditions.

Optimization Results and Factor Effects

In this study, the antioxidant capacity of extracts obtained from *E. umbellata* fruit was evaluated using FRAP, DPPH, TPC, and TFC analyses, and the extraction conditions were optimized using RSM.

The analyses showed that the optimal antioxidant responses were obtained at a water content of 28.28 %, a lactic acid/choline ratio of 3, and a temperature of 70 °C. Under these optimized conditions, the model predicted values of 6.51 mg TE/ g for DPPH, 21.88 mg TE/ g for FRAP, 23.09 mg GAE/ g for TPC, and 4.15 mg QE/ g for TFC. The corresponding experimental values were 6.45 mg TE/ g (DPPH), 21.50 mg TE/ g (FRAP), 22.55 mg GAE/ g (TPC), and 4.05 mg QE/ g (TFC), indicating a strong agreement between the predicted and experimental data. These results, supported by high R^2 values and narrow confidence intervals, demonstrate the model's strong predictive ability and reliability.

The DPPH radical scavenging capacity (6.51 mg TE/ g) and FRAP value (21.88 mg TE/ g) obtained indicate the extract's high free radical scavenging potential and strong reducing power. These values are close to the upper limit of the target range set at the beginning of the study and demonstrate that the optimization of antioxidant activity has been successfully achieved.

Additionally, these results indicate that both total phenolic and flavonoid compounds are temperature-sensitive, and optimal extraction occurs at medium-high temperatures. In particular, the

high TPC values indicate that the plant's polyphenolic structure can be extracted with maximum yield at around 70 °C. This supports the notion that phenolic compounds exhibit a certain heat resistance, but that excessive temperatures can cause degradation.

Additionally, it has been emphasized that extraction parameters (particularly temperature and solvent ratios) play a critical role in the yield of these compounds (Spigno et al., 2007; Mokrani and Madani, 2016; Shi et al., 2022). The literature reports that *E. umbellata* fruits extracted using 80 % ethanol have phenolic contents ranging from 1037–1260 mg GAE/100 g dry matter and possess strong antioxidant activity (Roscha et al., 2023). Similarly, Khattak (2012) reported the phenolic content of *E. umbellata* fruits to be 23.3 mg GAE/g and the flavonoid content to be 3.6 mg QE/g on a dry weight basis. The predicted values obtained from the model (DPPH: 6.45 mg TE/g, FRAP: 21.50 mg TE/g, TPC: 22.55 mg GAE/g, and TFC: 4.05 mg QE/g) were found to be highly consistent with the experimental data, further confirming the reliability and predictive accuracy of the optimization model. These findings indicate that the applied DES-based ultrasonic extraction conditions effectively yield phenolic compounds and that *E. umbellata* fruit is a potent natural antioxidant source.

This study has demonstrated that the antioxidant capacity of extracts obtained from *E. umbellata* fruit can be significantly increased under optimized extraction conditions, indicating that it could serve as a valuable natural antioxidant source for functional food and pharmaceutical formulations.

CONCLUSIONS

This study has demonstrated that ultrasonic extraction parameters play a critical role in enhancing the antioxidant properties of extracts obtained from *E. umbellata* fruit. Under conditions optimized using RSM, it was determined that total phenolic and flavonoid compounds, as well as DPPH and FRAP values, were obtained at maximum levels. In particular, the results obtained at around 70 °C show that polyphenolic structures can exhibit high stability at certain temperature ranges, but excessive temperatures can lead to degradation. The data obtained indicate that *E. umbellata* fruit can be evaluated as a powerful natural antioxidant source in functional food and pharmaceutical product development studies; it also provides a scientific basis for sustainable and healthy product design.

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EVALUATION OF THE POTENTIAL FOR RAINWATER HARVESTING IN THE AGRO-INDUSTRY: A CASE STUDY OF THE TABLE OLIVE INDUSTRY

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ABSTRACT

The objective of this study is to analyze the potential of a rainwater harvesting (RWH) as an alternative water source for the industrial sector. The table olive industry was chosen for this analysis, with two distinct companies, Firm I and Firm II, serving as case studies. Firm I, with an annual production capacity of 1350 tonnes of natural black, black, and green olives, has a roof area of 2333 m². Annual water consumption is calculated to be 8797 m^3 while the total harvestable rainwater is approximately 1250 m^3. Based on these figures, it is estimated that RWH could meet 15% of Firm I's total water requirements. Firm II produces 1500 tonnes of natural black olives annually and has a larger roof area of 2885 m². The annual water consumption is 2419 m^3, which is less than Firm I due to its smaller-scale, natural olive production profile. The annual harvestable rainwater is calculated as 1718 m^3. Thus, the runoff available from the rooftop of Firm II is insufficient to meet the annual water demand. However, the harvested rainwater can still supply approximately 70% of the firm's total water requirements. The results revealed that rainwater harvesting will not be sufficient to meet all the water requirements of the facilities examined, but it can provide significant water savings.

Keywords: Rainwater harvesting, Water reuse, Water scarcity, Table olive industry, Precipitation,

INTRODUCTION

Rainwater harvesting (RWH) is a viable solution for water scarcity, offering benefits such as an additional water supply, flood mitigation, and a reduced environmental footprint (Oral et al., 2025). While its success is well-documented in urban settings like office buildings, schools, and supermarkets, its use in industry remains limited. However, harvested water is suitable for a variety of industrial uses, including process water, cooling water, garden irrigation, and firefighting (Bayırtepe and Dolgen, 2024). Using harvested rainwater for non-potable purposes can significantly reduce a company's reliance on municipal water supplies, leading to lower water costs. Furthermore, rainwater's natural softness and lack of chemical additives make it ideal for many applications, including irrigation and toilet flushing. Implementing RWH also enhances a company's sustainability profile and corporate social responsibility, which can improve its reputation among environmentally conscious consumers and stakeholders.

A typical rainwater cistern system (RWCS) consists of four basic components: a collection (catchment) area, a conveyance system, storage, and treatment facilities (Dolgen et al., 2006). The most common collection area is a building's roof, though open fields can also be used. The efficiency and quality of collected water are influenced by the roof's effective area and construction material.

While any roofing material is acceptable for non-potable uses, water intended for drinking should not be collected from roofs with asphalt shingles, and lead flashing should be avoided. The conveyance system, typically composed of gutters and pipes, transports rainwater from the roof to storage. Both pipes and roof surfaces should be made of chemically inert materials, such as wood, plastic, aluminum, or fiberglass, to prevent water contamination (Thomas and Martinson, 2007). Water is ultimately stored in a cistern or tank, which should also be constructed from an inert material like reinforced concrete, fiberglass, or stainless steel. Storage tanks can be integrated into a building's structure or built as a separate unit. They are commonly designed as surface or above-ground tanks for roof collection and as sub-surface or underground tanks for ground catchment systems. The storage capacity of the cistern is a crucial design factor. Insufficient capacity can lead to water shortages during low-rainfall periods, while excessive capacity can increase construction and operating costs, potentially making the system economically unfeasible. To promote the use of RWH technology, governments can play a vital role. This includes offering financial incentives to users and establishing standards and codes for the construction, operation, and maintenance of these systems to ensure public health and safety.

This paper evaluates the potential for RWH in the olive sector in Türkiye. This focus is justified because: (i) olive oil and table olives are important Turkish products, (ii) olive farming is widespread in water-stressed regions of Türkiye, such as the Aegean, Marmara, and Southeastern Anatolian regions, and (iii) olive harvesting and processing periods coincide with high precipitation. To conduct this analysis, two table olive production companies in the Manisa-Akhisar region of Türkiye is chosen as a case study. Data on water consumptions are obtained from the companies. Precipitation data is provided from the Turkish State Meteorological Service. The volume of the harvested rainwater from a rooftop is calculated considering the roof surface area, the average rainfall, and a runoff coefficient that accounts for system efficiency. And, results are discussed considering the water consumption and harvestable rainwater volumes, potential uses and barriers to adopt RWH systems.

MATERIAL AND METHOD

Study Area

The study was conducted in Akhisar, a town in the southwestern region of Türkiye known for its high production of green, black, and natural black olives. This area was selected for analysis because the Akhisar is known for its intensive olive farming. The research focused on two distinct companies, Firm I and Firm II, both of which are located within the Akhisar Olive Specialized Organized Industrial Zone (AZIOSB).

Figure 1. Location of the Akhisar Olive Specialized Organized Industrial Zone (AZIOSB)

This zone, a strategic development, consists of over one hundred factories with varying sizes and production capacities. The geographic location of the examined factories is illustrated in Figure 1. Firm I, with an annual production capacity of 1350 tons of natural black, black, and green olives, has a roof area of 2333 m². Firm II produces 1500 tonnes of natural black olives annually and has a larger roof area of 2885 m².

Rainfall Analyses

The study utilized 94 years of monthly rainfall data (1930–2024) for the Manisa province, obtained from the Turkish State Meteorological Service. The analysis, summarized in Table 1, shows that the region receives an average annual rainfall of 743.8 mm over an average of 86.2 rainy days. As Table 1 illustrates, rainfall is at its highest in December, with 137.5 mm, while August receives the lowest amount, with 9.3 mm.

Table 1. The precipitation data for the Manisa Province

Month	**Average Number of Rainy Days**	**Average Monthly Total Rainfall (mm)**
January	13.23	127.4
February	11.11	108
March	9.89	77.8
April	8.96	55.1
May	6.76	38.9
June	3.39	20
July	1.05	9.7
August	0.83	9.3
September	2.28	19.4
October	5.72	51.8
November	9.42	88.9
December	13.57	137.5
Annual	**86.2**	**743.8**

Calculation

In the presented study, the volume of the harvested rainwater from a rooftop is calculated considering the roof surface area, the average rainfall, and a runoff coefficient. The basic formula given in the below was used in the calculations.

Volume of the harvestable water = Catchment area (m^2) x Annual rainfall (mm) x Runoff Coefficient

In the study, the roof top areas for Firm I and II was obtained from the company. Rainfall data obtained from the Turkish State Meteorological Service. Runoff coefficient which is a factor ranged between 0 and 1 consider the water loss due to evaporation, leaks, splash-over, and the material of the roof. For example, a smooth metal roof has a high runoff coefficient (often 0.9 or 0.95), while a more porous surface like asphalt shingles or a flat cement roof might have a lower coefficient (0.6 to 0.8). In the examined factories the roof material was metal therefore high runoff coefficient (i.e. 0.9) was used.

RESULTS AND DISCUSSION

The study analyzed the potential for rainwater harvesting at two firms, with rooftop areas of 2332 m^2 (Firm I) and 2885 m^2 (Firm II). Monthly harvestable rainwater volumes were calculated based on a formula detailed in the materials and methods section. To evaluate the feasibility of this water source, the firms' monthly water consumption data were also collected. A comparison of these two datasets for Firm I, shown in Figure 2, reveals that monthly water consumption consistently surpasses the available volume of harvested rainwater. The highest water consumption occurs in October, which is attributed to the beginning of the olive fruit collection season. The difference between water consumption and harvestable rainwater is significant due to very low precipitation during the summer. Annual water consumption is calculated to be 8797 m^3 while the total harvestable rainwater is approximately 1250 m^3. Based on these figures, actually, the runoff available from the rooftop of Firm I is insufficient to meet the annual water demand. Only the 15% of the total water requirement can be obtained from the rainwater. On the other hand, the monthly water consumption exceeding the harvestable rainfall also means that a smaller storage tank capacity could be sufficient which makes the investment economically feasible.

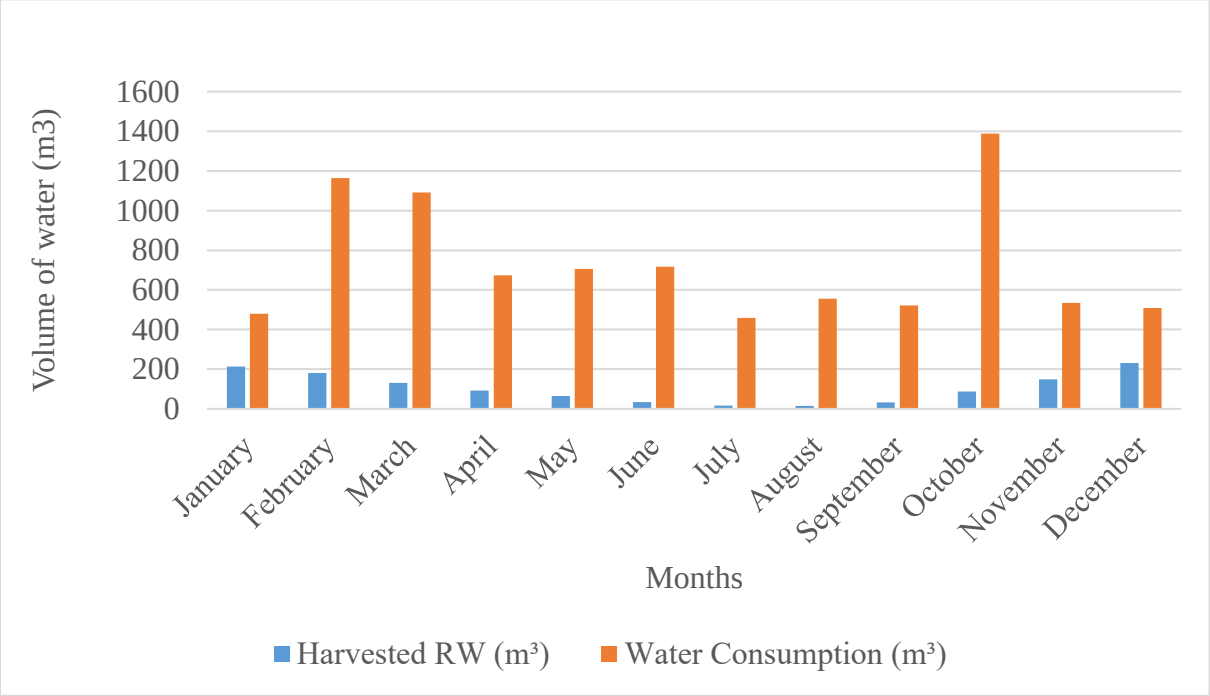

Figure 2. Water consumption and potential for harvesting rainwater at Firm I

Figure 3 shows Firm II's water consumption and harvestable rainwater data. The annual water consumption is 2419 m^3., which is less than Firm I due to its smaller-scale, natural olive production profile. While water consumption exceeds harvestable rainwater volumes from May through December, the harvested rainwater is sufficient to meet the firm's needs during the remaining months. Similar to Firm I, the highest water consumption is achieved in October, when the olive fruit collection season begins. Based on these figures, the runoff available from the rooftop of Firm II is also insufficient to meet the annual water demand. However, the harvested rainwater can still supply approximately 70% of the firm's total water requirements.

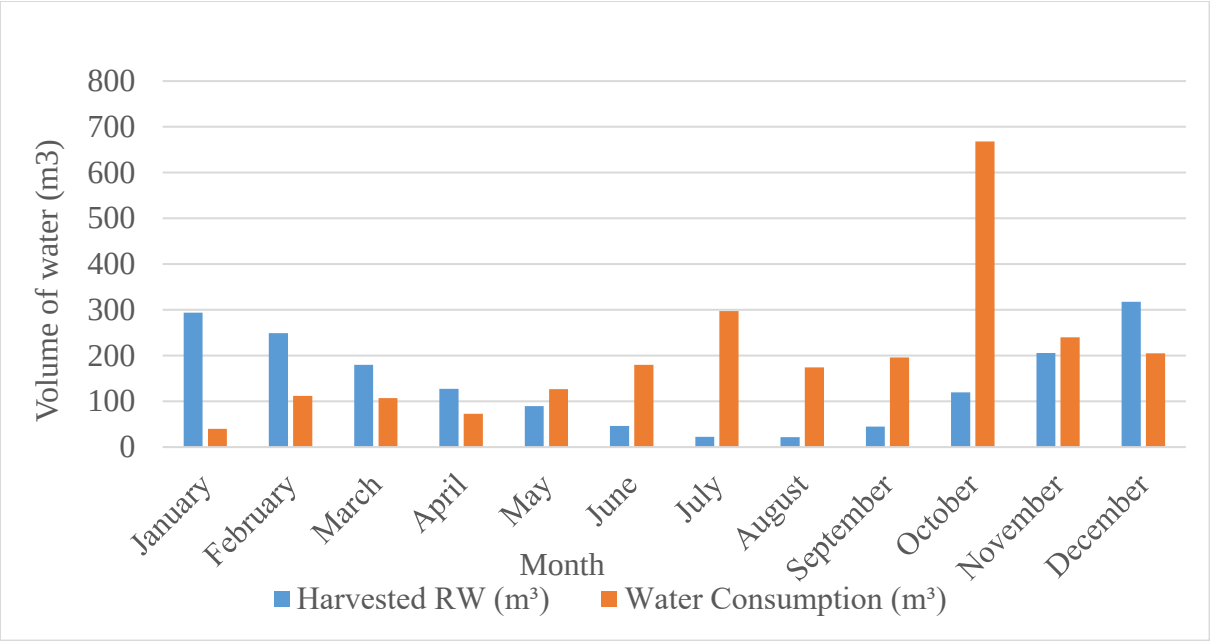

Figure 3. Water consumption and potential for harvesting rainwater at Firm II

High investment costs and relatively low water prices are often cited as the primary disadvantages of rainwater harvesting (RWH) systems. Our experience indicates that the payback period for these systems decreases as water prices increase. Additionally, if an industry's water consumption aligns with periods of high precipitation, the required storage tank volume can be reduced, thereby improving the investment's feasibility. In addition, the financial arrangements like low-interest loans and other financial support are beneficial for encouraging industries to invest in RWH systems.

The quantity of rainwater that can be collected, as well as its potential uses, are important considerations. This water can be used for various purposes, including landscape irrigation, toilet flushing, cooling water, and process water. Studies have shown that pollutant concentrations in roof runoff are highest at the beginning of a rainfall. The presence of high concentrations of pollutants in the first flush, relative to subsequent runoffs, is termed the 'first flush/first flush' phenomenon. A vortex filter separates suspended particles and pollutants from rainwater, originating from roof dust, through a centrifugal effect, especially during the initial minutes of rainfall, thereby ensuring minimal contamination of the storage tank. Therefore, rainwater harvested from rooftops is suitable for irrigation and toilet reservoirs, in general. However, its use as process water requires special attention to quality. Table olive production processes consist of picking of olives, de-bittering or lye step, rinsing, fermentation in brine, washing, packaging and pasteurization (Dölgen and Alpaslan, 2020). The collected rainwater is a viable option for washing olives and tanks, and rinsing as these tasks don't require high-quality water. For brine preparation, however, the hygienic quality of the stored rainwater is crucial. For this reason, we recommend using filtration and/or disinfection methods to ensure its safety.

Regulatory frameworks play a significant role in encouraging the widespread implementation of rainwater harvesting (RWH) systems. While a 2017 amendment mandated RWH for urban buildings with rooftops of 2000 m^2 or more, industries are not currently subject to such requirements. To address this, mandatory measures for industrial rainwater harvesting could be explored. Furthermore, offering incentives and benefits to facilities that improve water efficiency through RWH could be a powerful

motivator. Integrating RWH into broader initiatives like "green production" and "green industry" certifications would also enhance its adoption.

CONCLUSIONS

Rainwater harvesting (RWH) systems are substantial infrastructures to promote water efficiency. They offer multiple benefits, including flood mitigation, reduced reliance on municipal water, and lower water costs. While these systems are common in urban settings like homes, shopping centers, and schools, their use in the industrial sector is still limited. However, factories are ideal for RWH due to their large roofs and impermeable surfaces. This makes collected rainwater a promising resource for various industries, including construction, metal, coatings, and agro-industries that process agricultural products. Despite the benefits, high investment costs are a major barrier to adoption. To encourage wider use in the industrial sector, governments could offer financial incentives and integrate RWH into initiatives like "green production" and "green industry" certifications.

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FUNCTIONAL NOODLES ENRICHED WITH BELL PEPPER POWDER: EFFECTS ON COLOR, TEXTURE, AND COOKING QUALITY

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ABSTRACT

This study aimed to evaluate the effects of green and red bell pepper (*Capsicum annuum*) powders, in combination with celery (*Apium graveolens*) powder, on the quality characteristics of wheat-based noodles. Two control samples were prepared: one using only refined wheat flour and the other by substituting 10% of the wheat flour with celery powder. In enriched formulations, the celery-containing base was further fortified with 1%, 3%, or 5% green or red bell pepper powder. All samples were analyzed for raw and cooked color attributes (L*, a*, b*, Hue, SI), cooking performance (weight and volume increase, cooking loss), and cooked noodle firmness. The incorporation of red bell pepper powder significantly decreased lightness while increasing redness (a*) and SI, resulting in vivid orange-red noodles. Green bell pepper powder, in contrast, maintained higher lightness and shifted a* values toward the green spectrum, especially at higher substitution levels. Cooking yield increased with the addition of both vegetable powders, while firmness decreased proportionally. Cooking loss remained low in all enriched samples compared to the wheat flour control. Overall, the combined use of celery and bell pepper powders enhanced the nutritional profile and visual appeal of noodles without compromising cooking quality. The results suggest that vegetable powder blends can serve as functional and natural fortification agents in clean-label cereal-based formulations.

Keywords: Vegetable powder, Bell pepper, Celery, Noodles, Functional food, Clean label.

INTRODUCTION

In recent years, the development of functional foods and plant-based fortification strategies has gained significant attention in both academic and industrial fields. Particularly, the incorporation of dried vegetable powders into cereal-based products has emerged as a promising approach to enhance the nutritional value of foods while aligning with consumer demand for health-oriented alternatives (Zhao et al., 2018). Noodles, as a widely accepted traditional cereal product, offer an ideal matrix for such enrichment strategies.

Bell peppers (Capsicum annuum), originating from the Mediterranean region, are among the most important vegetables due to their vibrant color, distinctive flavor, and rich nutritional profile. Green and red bell peppers, representing different ripening stages of the same cultivar, are particularly notable for their high content of vitamin C, carotenoids (such as capsanthin and β-carotene), phenolic compounds, and dietary fiber (Marín et al., 2004; Howard et al., 2000). In addition to these common types, several other Capsicum annuum cultivars exist, including yellow, orange, purple, and brown

bell peppers, which differ primarily in their pigment composition and antioxidant profiles (Deepa et al., 2007). Among these, green, red, and yellow varieties are the most widely consumed globally.

Bell peppers are commonly utilized in the fresh produce market, canned and frozen vegetable mixes, and dehydrated forms such as powders and flakes. Their application spans multiple sectors including culinary use (e.g., salads, sauces, and stuffed dishes), functional food formulations, and natural colorant sources in the food industry due to their high pigment and antioxidant content (Zhang et al., 2021). When dried and ground into powder form, bell peppers serve as concentrated sources of bioactive compounds and can be incorporated into cereal-based products, snacks, and meat analogues to improve both nutritional quality and sensory appeal (Gonçalves et al., 2016).The inclusion of vegetable powders in noodle formulations may influence various quality parameters, including color characteristics, cooking yield, texture, and solid loss into cooking water. Previous studies have shown that such enrichment can alter both the technological and physicochemical properties of noodles, often resulting in increased water absorption, volume, and improved nutritional density (Wani et al., 2012; Bhasin et al., 2020).

In addition to bell peppers, celery (Apium graveolens) is another widely consumed vegetable valued for its distinctive aroma and high nutritional content. Celery is rich in dietary fiber, vitamin K, potassium, and bioactive compounds such as phthalides, flavonoids, and phenolic acids, which contribute to its antioxidant, anti-inflammatory, and hypotensive properties (Sharma et al., 2017; Liu et al., 2018). When processed into powder form, celery retains a substantial portion of its nutrients and phytochemicals, making it suitable for incorporation into cereal-based matrices. Celery powder has been used in food formulations not only as a natural flavoring and coloring agent, but also to improve fiber content and provide functional benefits (Zhou et al., 2020). Its inclusion in noodle formulations may affect the product's color, texture, and cooking quality due to its fibrous and aromatic nature. In composite formulations, the combination of celery powder with other vegetable powders, such as bell peppers, may offer synergistic effects in terms of both sensory characteristics and nutritional enhancement.

In this study, dried green and red bell pepper powders were incorporated into wheat noodle formulations at different levels. The effects of this enrichment were evaluated by analyzing raw and cooked color values (L*, a*, b*), cooked noodle firmness, cooking weight and volume increase, and cooking loss. The findings aim to provide insight into the functional potential of bell pepper powder as a clean-label, natural ingredient for enhancing noodle quality.

MATERIAL AND METHOD

Materials

Fresh green and red bell peppers (Capsicum annuum) were purchased from a local market in Konya, Turkey. The raw materials used in noodle production, including refined wheat flour, whole egg, and table salt, were also obtained from a local supplier.

Preparation of Bell Pepper Powders

Green and red bell peppers were washed thoroughly, deseeded, and chopped into thin slices. The slices were initially frozen at −80 °C for 2 hours and then freeze-dried using a laboratory freeze-dryer (Labconco FreeZone, USA). The dried pepper slices were ground using a laboratory grinder, and the resulting powders were passed through a 500 µm sieve to ensure uniform particle size (Figure 1).

Figure 1. Red and Green bell pepper powders

Noodle Production

In this study, eight different noodle formulations were prepared, including two control samples and six vegetable powder-enriched samples (Table 1). The first control sample (C) was produced using 100% refined wheat flour, while the second control (CC) included 10% celery powder by partially replacing refined wheat flour. For the vegetable-fortified formulations, noodles were prepared by incorporating 10% celery powder and partially substituting the remaining wheat flour with either 1%, 3%, or 5% red bell pepper powder (RC-1, RC-3, RC-5) or green bell pepper powder (GC-1, GC-3, GC-5). In all formulations, the total flour basis (including refined flour, celery powder, and pepper powder) was standardized to 100 g. Each dough was prepared by mixing 100 g of the respective flour blend with 40 g whole egg, 0.5 g salt, and 30 mL distilled water using a planetary mixer (Hobart N50, Offenburg, Germany) at low speed for 8 minutes. The dough was then rested at room temperature for 20 minutes. After resting, the dough was sheeted and cut into noodle strips using a manual pasta machine (Shule Pasta Machine, China). The noodles were then dried at ambient conditions (20–25 °C) for 72 hours before analysis. Representative samples are presented in Figure 2.

Table 1. Formulation codes and descriptions of noodle samples

Sample Code	Formulation Description
C	Control noodle with 100% refined wheat flour
CC	Control noodle with 90% refined wheat flour + 10% celery powder
RC-1	89% refined wheat flour + 10% celery powder + 1% red bell pepper powder
RC-3	87% refined wheat flour + 10% celery powder + 3% red bell pepper powder
RC-5	85% refined wheat flour + 10% celery powder + 5% red bell pepper powder
GC-1	89% refined wheat flour + 10% celery powder + 1% green bell pepper powder
GC-3	87% refined wheat flour + 10% celery powder + 3% green bell pepper powder
GC-5	85% refined wheat flour + 10% celery powder + 5% green bell pepper powder

Color Measurement

Color attributes (L*, a*, b*) of both raw and cooked noodle samples were measured using a colorimeter (Minolta CR-400, Minolta Co., Ltd., Osaka, Japan). Based on a* and b* values, hue angle and saturation index (SI) were calculated using the following formulas:

Hue angle = arctangent (b*/a*); when a*<0 and b*>0, 180° is added to the angle.
Saturation Index (SI) = $\sqrt{(a^2 + b^2)}$.

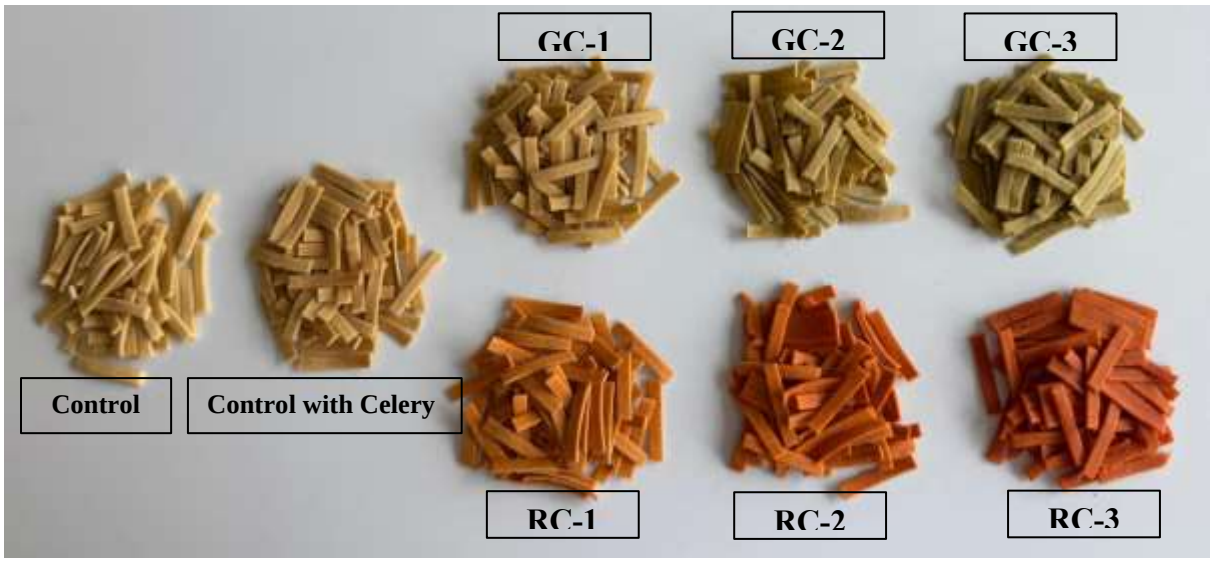

Figure 2. Control and bell pepper powder-fortified noodle samples.

Cooking Properties and Firmness

Water uptake, volume increase, and cooking loss were determined following the methods of Oh et al. (1985) and Özkaya and Kahveci (1990). Briefly, for water uptake, 20 g of noodles were cooked in 250 mL of boiling distilled water. The weight of noodles before and after cooking was recorded and expressed as percentage increase. For volume increase, the cooked and drained noodles were placed into graduated cylinders filled with distilled water, and the volume of displaced water was recorded. Cooking loss was determined by drying the filtrate obtained from the cooking water in a drying oven at 135 °C until constant weight. Firmness of raw and cooked noodles was evaluated using a texture analyzer (TA-XT Plus, Stable Micro Systems, Surrey, UK), following AACC Standard Method No. 66-50 (AACC, 2000).

Statistical Analysis

Data were statistically analyzed using SPSS version 22.0 (IBM Corp., Armonk, NY, USA). Means were compared using Duncan's multiple range test at a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

Color Properties of Raw Materials

The color characteristics of the raw materials used in noodle formulation, wheat flour, red bell pepper powder, and green bell pepper powder, are shown in Table 2. Color attributes such as lightness

(L*), red-green (a*), yellow-blue (b*), Hue and SI provide important indicators for predicting their visual and functional contribution to final products. Wheat flour showed the highest L* value (94.72), reflecting its bright white appearance. It had a negative a* value (–5.02), and a moderate b* value (13.93). Red bell pepper powder exhibited the lowest L* value (53.59), the highest a* value (38.48), and a high b* value (49.69), indicating its strong red-orange. The Hue (52.25°) and SI (62.85) confirmed the intense, warm coloration dominated by carotenoids such as capsanthin and β-carotene, which are thermally stable and often used as natural colorants in food formulations (Howard et al., 2000; Marín et al., 2004). In contrast, green bell pepper powder showed a relatively high L* value (70.19) and a strongly negative a* value (–12.45), characteristic of green pigments like chlorophyll. Its SI (32.90) was lower than that of red pepper, reflecting its more muted but distinct green tonality. Similar values have been reported in studies evaluating the color profile of green vegetables after drying (Deepa et al., 2007; Zhang et al., 2021). These findings confirm that the choice of vegetable powder significantly impacts the color potential of enriched cereal products. Red bell pepper contributes vibrant, saturated hues, while green bell pepper provides a fresher, vegetal green tone.

Table 2. Color properties of raw materials

Powder type	***L****	***a****	***b****	**Hue**	**SI**
Wheat flour	94.72±0.16	-5.02±0.07	13.93±0.10	109.82±0.19	14.81±0.11
Red Bell Pepper	53.59±0.11	38.48±0.13	49.69±0.09	52.25±0.16	62.85±0.09
Green Bell Pepper	70.19±0.09	-12.45±0.13	30.45±0.05	112.24±0.11	32.90±0.10

Color Properties of Uncooked Noodle Samples

The color parameters (L*, a*, b*, Hue and SI) of the uncooked noodles are presented in Table 2. Color is one of the most critical sensory quality attributes affecting consumer perception and preference, and it is significantly influenced by the type and amount of vegetable powder incorporated into the noodle. Incorporation of red bell pepper (RBP) powder significantly ($p < 0.05$) affected all color parameters of uncooked noodles. As the substitution level increased from 0% to 5%, a marked decrease in lightness (L*) was observed (66.49 to 48.46), indicating a darker appearance with higher pigment concentration. Concurrently, redness (a*) values increased sharply from 4.29 to 28.93, reflecting the intense red contributed by carotenoids such as capsanthin and capsorubin, naturally abundant in mature red peppers (Howard et al., 2000). The b* values also increased slightly but remained relatively stable (31.08 to 43.45), enhancing the yellowish-red. Hue angle decreased from 82.14° to 54.95°, confirming the shift from yellow to red tones, while the saturation index (SI) increased significantly (31.38 to 50.38).

Table 3. Color properties of uncooked noodle samples[1]

Powder type	***Powder ratio (%)***	***L****	***a****	***b****	**Hue**	**SI**
Red Bell Pepper	0	66.49±0.27a	4.29±0.10d	31.08±0.77b	82.14±0.04a	31.38±0.78c
	1	60.25±0.24b	16.91±0.34c	41.18±1.41a	68.52±0.26b	44.25±1.44b
	3	54.90±1.00c	23.25±0.97b	41.25±1.74a	61.83±0.81c	49.29±1.87a
	5	48.46±0.67d	28.93±0.51a	43.45±0.62a	54.95±0.85d	50.38±0.28a
Green Bell Pepper	0	66.49±0.27a	4.29±0.10a	31.08±0.77b	82.14±0.04d	31.38±0.78b
	1	62.79±0.41b	2.66±0.04b	30.25±0.72b	84.96±0.06c	30.36±0.72b
	3	60.35±0.35c	0.33±0.24c	30.29±0.27b	89.38±0.46b	30.29±0.28b
	5	58.51±1.31d	-0.59±0.10d	33.89±±0.79a	90.99±0.16a	33.90±0.79a
Noodle type	**N**	***L****	***a****	***b****	**Hue**	**SI**

Red Bell Pepper	8	57.52±6.97a	18.16±9.62a	39.24±5.12a	66.86±10.50b	43.82±7.96a
Green Bell Pepper	8	62.03±3.18a	1.67±2.01b	31.38±1.66b	86.87±3.67a	31.49±1.63b
Wheat flour noodle		65.63±0.92	3.19±0.13	29.29±0.47	83.78±0.22	29.46±0.47

[1]Means followed by the same letter within a column are not significantly different ($p < 0.05$).

Green bell pepper (GBP) powder also significantly influenced noodle color, but in a contrasting manner. Lightness (L*) values decreased moderately with increasing GBP levels (66.49 to 58.51), while a* values gradually shifted from positive to negative (4.29 to −0.59), indicating a transition from slight redness to greenness. This reflects the presence of chlorophyll pigments typical of immature green peppers (Marín et al., 2004). The b* values remained within a narrow range (30.25–33.89), maintaining a consistent yellow. Hue angle increased significantly from 82.14° to 90.99°, while SI values ranged between 30.29 and 33.90, lower than those observed in red bell pepper-fortified noodles.

The average color values of red and green bell pepper-incorporated noodles are summarized at the bottom of Table 2. Red bell pepper noodles exhibited significantly lower L* values (57.52) compared to green bell pepper noodles (62.03), indicating darker color. They also showed higher a* (18.16 vs. 1.67), b* (39.24 vs. 31.38), and SI values (43.82 vs. 31.49), reflecting more intense red-orange coloration. In contrast, green pepper noodles had a significantly higher hue angle (86.87° vs. 66.86°), characteristic of green–yellow tones.

When compared to the wheat flour control noodle (L*: 65.63, a*: 3.19, b*: 29.29, hue: 83.78°, SI: 29.46), red bell pepper-fortified noodles showed a darker, redder, and more saturated appearance, while green bell pepper noodles were closer in lightness and hue, but still had elevated b* and SI values. These findings suggest that both types of bell pepper powder significantly altered the visual appearance of the noodles, with red pepper contributing to strong reddish tones, and green pepper offering subtle greenish-yellow hues, consistent with previous reports on color modification in vegetable-enriched pasta and noodles (Zhao et al., 2018; Bhasin et al., 2020).

Color Properties of Cooked Noodle Samples

Cooking significantly alters the color of noodles, particularly due to pigment stability, starch gelatinization, and protein–matrix interactions. The color parameters of cooked noodles enriched with red or green bell pepper powder are presented in Table 4. Incorporation of red bell pepper (RBP) powder significantly ($p < 0.05$) affected all color attributes after cooking. As RBP content increased from 0% to 5%, lightness (L*) decreased markedly (from 69.61 to 57.15), reflecting the intensification of color due to heat-stable carotenoids like capsanthin. The a* value, indicating redness, rose from 1.57 to 22.45, while b* value increased from 22.01 to 45.57, indicating enhanced red-yellow tones. Consequently, hue angle dropped from 85.92° to 63.79°, and saturation index (SI) rose from 22.06 to 50.80, showing more vivid and saturated color as RBP level increased. These findings suggest that red bell pepper pigments are relatively stable under cooking conditions and remain strongly visible in the final product (Howard et al., 2000; Bhasin et al., 2020). Green bell pepper (GBP) powder, in contrast, exhibited a different color behavior after cooking.

The L* value showed a slight decline at higher concentrations (69.76 to 65.86), indicating minimal darkening. The a* value, which was positive (reddish) at lower levels, shifted to negative (–1.36 at 5% GBP), signifying a greenish hue attributed to chlorophyll and its derivatives. The b* values remained around 22–24, and hue angle increased with GBP concentration, reaching 93.20° at 5%, reflecting movement toward the green–yellow quadrant. The SI values ranged from 22.06 to 24.22, much lower than those in RBP noodles, indicating duller but stable coloration. The thermal degradation of chlorophyll to pheophytin during cooking likely contributes to the muted green tone in GBP-fortified noodles (Marín et al., 2004). The control noodle (wheat flour only) exhibited the highest L* value

(70.42), low redness (a*: –0.98), and modest b* (23.37), giving it a pale yellowish appearance. It had a high hue angle (92.42°) and low saturation (SI: 23.39), indicating a relatively bland visual profile.

Table 4. Color properties of cooked noodle samples[1]

Powder type	*Powder ratio (%)*	*L**	*a**	*b**	Hue	SI
Red Bell Pepper	0	69.61±0.81a	1.57±0.05d	22.01±0.46d	85.92±0.09a	22.06±0.47d
	1	63.75±0.52b	12.98±0.46c	38.10±1.11c	71.18±0.27b	40.24±1.18c
	3	59.60±0.27c	19.45±0.43b	43.44±0.58b	65.87±0.44c	47.60±0.62b
	5	57.15±0.18d	22.45±0.12a	45.57±0.79a	63.79±0.34d	50.80±0.74a
Green Bell Pepper	0	69.61±0.81a	1.57±0.05a	22.01±0.46a	85.92±0.09c	22.06±0.47a
	1	69.76±0.51a	0.67±0.28b	22.07±2.23a	88.25±0.72b	22.08±2.23a
	3	67.78±0.99b	0.73±0.38b	22.47±0.18a	88.13±0.96b	22.48±0.19a
	5	65.86±0.49c	-1.36±0.24c	24.18±1.98a	93.20±0.41a	24.22±1.19a
Noodle type	**N**	***L****	***a****	***b****	**Hue**	**SI**
Red Bell Pepper	8	62.53±4.95b	14.14±8.37a	37.28±9.66a	71.68±9.03b	40.18±11.65a
Green Bell Pepper	8	68.25±1.77a	0.41±1.15b	22.68±1.58b	88.87±2.83a	22.71±1.59b
Wheat flour noodle		70.42±1.40	-0.98±0.14	23.37±1.14	92.42±0.47	23.39±1.14

[1]Means followed by the same letter within a column are not significantly different ($p < 0.05$).

RBP noodles showed stronger pigmentation, greater redness, and color saturation, confirming the dominance of carotenoid-based pigments. GBP noodles were similar to the control in terms of lightness but displayed a more greenish hue and slightly higher color stability than expected. The differences between RBP and GBP noodles, particularly in a* and hue values, clearly reflect the distinct pigment profiles of the peppers, carotenoids versus chlorophyll, and their respective thermal stabilities (Zhao et al., 2018).

Cooking Properties of Noodle Samples

Cooking performance is a critical quality parameter for noodles, as it influences consumer acceptance and functional integrity during preparation. The effects of red and green bell pepper powder incorporation on weight increase, volume increase, cooking loss, and cooked firmness are shown in Table 5.

Table 5. Cooking properties of noodle samples[1]

Powder type	*Powder ratio (%)*	Weight increase (%)	Volume increase (%)	Cooking loss (%)	Cooked firmness (g)
Red Bell Pepper	0	101.63±1.04b	84.44±6.29c	0.50±0.00b	1119.03±59.16a
	1	105.77±0.17b	122.22±0.00b	0.59±0.06b	904.77±26.66b
	3	106.10±0.57b	131.25±8.84ab	0.71±0.06ab	852.77±22.26b
	5	113.44±2.90a	160.71±15.15a	1.17±0.38a	672.13±21.46c
Green Bell Pepper	0	101.63±1.04b	84.44±6.29c	0.50±0.00c	1119.04±59.16a
	1	103.83±0.05b	114.38±4.62bc	0.64±0.02bc	896.98±36.71b
	3	106.56±2.21b	131.25±8.84ab	0.81±0.05ab	687.41±22.17c
	5	118.23±5.40a	167.86±25.25a	0.94±0.13a	629.42±33.65c
Noodle type	**N**				
Red Bell Pepper	8	106.74±4.70a	124.66±29.95a	0.74±0.32a	887.18±169.17a
Green Bell Pepper	8	107.56±7.20a	124.48±33.89a	0.72±0.19a	833.21±204.18a

Wheat flour noodle	94.99±1.16a	146.43±5.05a	4.27±0.19c	1196.13±9.65a

[1]Means followed by the same letter within a column are not significantly different ($p < 0.05$).

The addition of red bell pepper (RBP) powder significantly influenced the cooking properties of noodles. As the RBP ratio increased from 0% to 5%, weight increase rose from 101.63% to 113.44% ($p < 0.05$), indicating a higher water absorption capacity. Volume increase also increased markedly from 84.44% to 160.71%, suggesting a more porous and expanded noodle matrix. However, cooking loss also increased with higher RBP levels, ranging from 0.50% (control) to 1.17% (5%), possibly due to the leaching of water-soluble pigments and weakly bound solids. Meanwhile, cooked firmness decreased significantly with increasing RBP content (from 1119.03 g to 672.13 g), suggesting a softening effect in the noodle structure. This could be attributed to the dilution of gluten and fiber interference by pepper particles, which has been reported in similar vegetable-enriched pasta formulations (Bhasin et al., 2020). A similar pattern was observed with green bell pepper (GBP) powder. Weight increase rose from 101.63% to 118.23% with 5% GBP incorporation, while volume increase reached 167.86%, showing a stronger swelling tendency than RBP samples. These enhancements may be related to the hydrophilic nature of dietary fiber and carbohydrates in the pepper powder (Marín et al., 2004). Cooking loss also increased from 0.50% to 0.94%, though the rise was more moderate compared to RBP. Interestingly, firmness values decreased significantly as GBP level increased, from 1119.04 g to 629.42 g, which was even lower than the RBP-5 sample. This loss in structural integrity might be due to the weaker colorant-protein interactions and thermal degradation of chlorophyll during cooking (Howard et al., 2000). When averaged across all levels, RBP- and GBP-fortified noodles showed similar trends in cooking performance: both had higher weight gain (~106–107%) and volume expansion (~124%), but lower firmness (~833–887 g) compared to the wheat flour control (firmness: 1196.13 g, weight gain: 94.99%, volume increase: 146.43%).

Notably, the cooking loss of bell pepper–enriched noodles (0.72–0.74%) was substantially lower than the control sample (4.27%), which is likely due to the presence of stabilizing compounds (e.g., fibers, phenolics) that limit starch leaching. This finding is in line with reports suggesting that incorporation of vegetable powders may reduce cooking loss by reinforcing matrix integrity through fiber–starch interactions (Zhao et al., 2018; Bhasin et al., 2020). These results confirm that although firmness decreases with vegetable addition, improvements in water holding, reduced nutrient loss, and visual appeal may compensate for texture softening in functional noodle products.

CONCLUSIONS

The present study demonstrated that the inclusion of red or green bell pepper powder, in combination with celery powder, has a significant influence on the color and cooking quality of wheat-based noodles. Red bell pepper imparted a more intense red-orange hue, while green bell pepper contributed to lighter, greener tones. Both powders improved cooking yield (weight and volume gain) but led to a reduction in firmness due to fiber interference and pigment–protein interactions. Despite the softer texture, cooking loss remained low in vegetable-fortified noodles, highlighting the functional stability of the formulations. Compared to the wheat flour control, the enriched noodles offered superior visual characteristics and enhanced nutritional potential, suggesting their applicability in the development of health-oriented noodle products. These findings support the use of vegetable powders as clean-label ingredients in functional food design, aligning with current consumer preferences for natural, plant-based, and value-added products.

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EFFECTS OF FUNCTIONAL OILSEED POWDERS ON THE QUALITY ATTRIBUTES OF CRACKERS

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ABSTRACT

This study aimed to evaluate the effects of different oilseeds (sesame, black cumin, blue poppy seed, and flaxseed) and their incorporation at various substitution levels (0%, 3%, 6%, and 9%) on the quality characteristics of crackers. The crackers were evaluated for color, physical, textural, and sensory properties. The addition of oilseed flours significantly affected the product's lightness (L*), yellowness (b*), and hue angle depending on the type and level of incorporation. In terms of physical attributes, increasing oilseed content tended to decrease diameter and thickness while increasing spread ratio. Texture analysis showed that hardness values increased with higher substitution levels, particularly at 9%. Sensory evaluation revealed that oilseed enrichment at moderate levels (3–6%) improved taste, crispness, and overall acceptability, while high levels (9%) led to a slight decrease in sensory scores. Among the samples, 6% blue poppy and sesame additions yielded the most favorable sensory and structural outcomes. The results suggest that oilseed-enriched crackers can serve as a valuable source of functional ingredients and improve consumer acceptability without compromising product quality.

Keywords: Snack, Oilseed, Functional food, Bakery product.

INTRODUCTION

The integration of functional foods and health-supporting ingredients into daily diets has become a priority research area in modern nutrition trends (Martirosyan & Singh, 2015). Cereal-based products, in particular, stand out as suitable carrier systems for enrichment with functional ingredients and are among the products with high consumer acceptance (Naqash et al., 2017). In this context, oilseeds, sesame (*Sesamum indicum* L.), black cumin (*Nigella sativa* L.), flaxseed (*Linum usitatissimum* L.), and blue poppy (*Papaver somniferum* L.) are widely used in the development of grain-based products due to their high nutritional value and functional properties.

Sesame seed is particularly notable for its high oil (50–60%), protein (18–25%), dietary fiber, and lignan (sesamin, sesamolin) content, and its high antioxidant capacity (Namiki, 2007; Elleuch et al., 2011). Black cumin, with its volatile compounds such as thymoquinone and high phenolic content, exhibits strong antioxidant and antimicrobial properties; its use in functional foods has also been proven (Ali & Blunden, 2003; Ramadan, 2007). Flaxseed, particularly with its α-linolenic acid (ALA), lignans, and soluble, unsaturated dietary fibers, is widely used in functional foods (Kajla et al., 2015). Blue poppy, on the other hand, is preferred in the food industry for its high content of oil (40–50%),

protein (around 20%), and calcium, phosphorus, and iron minerals (Bozan & Temelli, 2008; Siger et al., 2017).

The primary purpose of using oilseeds in bakery products is to enrich their nutritional content (e.g., essential fatty acids, dietary fiber, protein, and phytochemicals) and enhance their functional properties (Altaf et al., 2021; Yilmaz & Toledo, 2005). Furthermore, these seeds enhance both sensory quality and functionality by improving the product's texture, aromatic profile, and shelf life. However, the direct addition of oilseeds to product formulations significantly impacts physical properties such as structure, color, spread, and texture (Metwal et al., 2011; Otondi et al., 2020).

Crackers, being snack products with low moisture content, a crunchy texture, and a long shelf life, are suitable carrier matrices for the addition of functional ingredients. The resulting product is both nutritious and convenient for consumption (Ganorkar & Jain, 2014). In the literature, it is reported that oilseed additive ratios in grain-based snacks generally range from 3–10%, and that these ratios are optimal for achieving a balance between sensory quality and technological suitability (Kaur et al., 2015; Canalis et al., 2019). In products such as crackers, levels of 3–6% provide a significant increase in nutritional value and offer acceptable levels without negatively impacting consumer taste.

In this context, this study aimed to evaluate the sensory, physical, and color properties of products obtained by adding sesame, black cumin, flaxseed, and blue poppy seeds at different ratios (0, 3, 6, and 9%) to cracker formulations. The obtained data will contribute to functional cracker development studies and shed light on the formulation of new, nutritious, and acceptable products that meet consumer demand.

MATERIALS AND METHODS

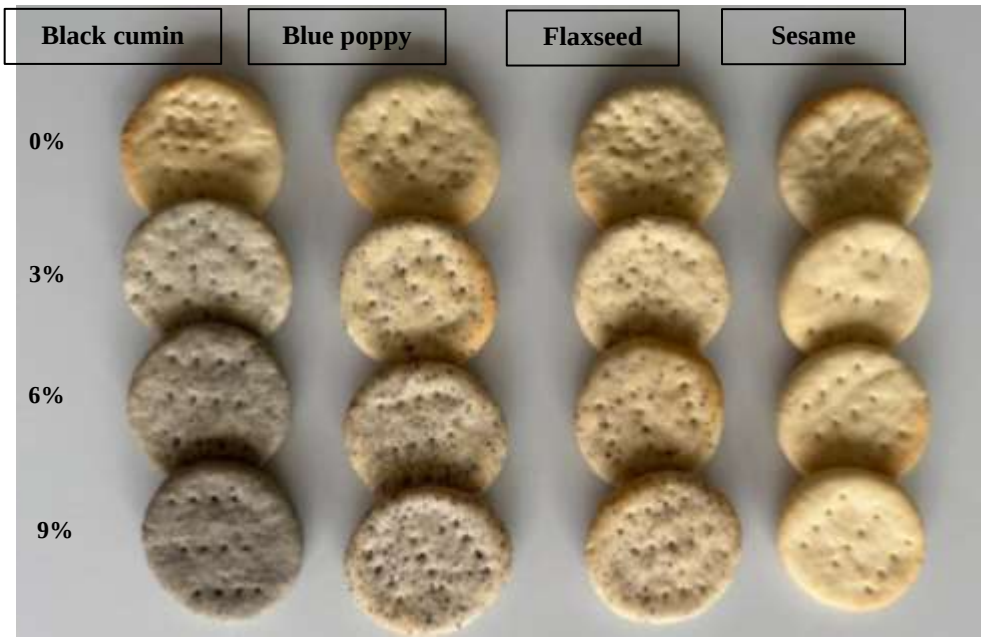
Materials

Refined wheat flour (type 550), powdered sugar, skimmed milk powder, sodium bicarbonate, shortening, salt, and vanillin were procured from a local retail market in Konya, Turkey. Oilseeds used in the study, sesame (*Sesamum indicum* L.), black cumin (*Nigella sativa* L.), blue poppy seed (*Papaver somniferum* L.), and flaxseed (*Linum usitatissimum* L.), were purchased from a certified organic supplier and ground in a laboratory mill (Retsch ZM200, Germany) to pass through a 500 µm sieve before use.

Preparation of crackers

Cracker doughs were prepared based on a standard low-sugar, low-moisture formulation suitable for crisp bakery products, with varying oilseed incorporation levels of 0%, 3%, 6%, and 9% (w/w) based on flour weight. The control group contained no oilseed powder. All dry ingredients were mixed in a planetary mixer (Hobart-N50; Hobart Canada Inc., Ontario, Canada) for 5 minutes, followed by the addition of water to obtain a firm but pliable dough. The dough was sheeted to a thickness of approximately 3 mm, cut into circular discs of 45 mm diameter, and baked in a laboratory oven (Profilo

HG1503T, İstanbul, Turkey) at 170 °C for 14 minutes. After cooling to 25 ± 2 °C, crackers were sealed in polyethylene bags and stored at room temperature until analysis.



Color analysis

The surface color of the cracker samples was measured using a Minolta CR-400 colorimeter (Minolta Camera Co., Ltd., Osaka, Japan). Results were recorded as L* (lightness), a* (red-green axis), and b* (yellow-blue axis) values. In addition, Saturation index ($(a^{*2}+b^{*2})^{1/2}$) and Hue angle (a*>0 and b*>0, if arctan [b*/a*]; a*<0 and b*>0, arctan [b*/a*] + 180°) values were calculated (Francis, 1998).

Physical properties

The diameter and thickness of the crackers were measured with a digital caliper (Mitutoyo, Japan, precision 0.01 mm) at five different points per sample, and the spread ratio was calculated as the ratio of diameter to thickness (AACC, 2000).

Texture profile analysis

The hardness of the crackers was measured 2 hours after baking using a Texture Analyzer (TA-XT2i; Stable Microsystems, UK) equipped with a three-point bending rig (HDP/3PB). Test conditions were: load cell: 30 kg, pre-test speed: 1.0 mm/s, test speed: 3.0 mm/s, post-test speed: 10.0 mm/s, test distance: 5 mm, and trigger force: 50 g. The maximum force recorded at break was reported as the hardness value (g).

Sensory evaluation

Sensory analysis was conducted with a panel of 20 untrained participants from Necmettin Erbakan University (Konya, Turkey) who regularly consume bakery products. Each panelist evaluated the cracker samples for color appearance, odor, taste, crispness, and overall acceptability using a 7-point hedonic scale (1 = dislike extremely, 7 = like extremely). Each panelist evaluated the coded samples in random order under daylight and ambient conditions.

Statistical analysis

All measurements were carried out in duplicate. The obtained data were analyzed using JMP statistical software version 10.0 (SAS Institute Inc., Cary, NC, USA). Analysis of variance (ANOVA) was performed to determine significant differences ($p < 0.05$) among the means, and comparisons were made using Tukey's HSD post-hoc test.

RESULTS AND DISCUSSION

Color Properties of Raw Materials

The visual quality of cracker products is strongly influenced by the color characteristics of the raw materials used in their formulation. Table 1 provides the L*, a*, b*, Hue, and Saturation Index (SI) values for the base flour and seed powders utilized in this study. The results indicated that all color parameters varied significantly depending on the material type ($p < 0.05$). Refined wheat flour exhibited the highest L* value (91.64), demonstrating its brightness and whiteness, attributes typically associated with traditional noodle products. Conversely, black cumin (33.85) and blue poppy (33.45) powders had the lowest L* values, reflecting their darker pigmentation, which can be attributed to their rich content of phenolic compounds and natural pigments (Sarikurkcu et al., 2015). Flaxseed and sesame powders displayed intermediate L* values (53.50 and 71.81, respectively), indicating moderate brightness levels.

In terms of the a* coordinate (red-green axis), values ranged from −0.36 in wheat flour to 6.08 in flaxseed powder. The elevated a* value in flaxseed suggests a dominant reddish-brown hue, likely related to the presence of lignans and tannins (Hu et al., 2007). Blue poppy showed a moderate a* value (3.24), while sesame and black cumin had relatively low values (0.69 and 0.82), suggesting less chromatic deviation toward red or green. Regarding b* values, which indicate yellowness, sesame powder had the highest value (19.52), followed by flaxseed (16.97), both of which contribute yellow-brown hues to the end product. Wheat flour had a lower b* value (12.50) despite its high lightness, whereas black cumin and blue poppy exhibited minimal b* values (2.78 and 3.31), aligning with their cool and subdued visual appearance.

The Hue angle, indicating the dominant shade of color, ranged from 45.54 in blue poppy to 91.63 in wheat flour. A lower hue angle in blue poppy reflects a tendency toward bluish-red tones, while higher values in wheat flour and sesame are indicative of neutral to yellowish hues (Pathare et al., 2013). The Saturation Index, which quantifies color intensity, was highest in sesame (19.54) and flaxseed (18.03), suggesting more vivid coloration, while black cumin had the lowest SI (2.90), reinforcing its dull and dark character. These findings highlight the distinct color attributes of seed powders, implying that their incorporation into noodle formulations is likely to produce noticeable changes in the visual attributes of the final product. This observation is consistent with previous literature indicating that the addition of naturally pigmented functional ingredients can significantly alter the visual appeal and consumer acceptance of cereal-based foods (Kähkönen et al., 1999; Pathare et al., 2013).

Table 1. Color properties of raw materials

Raw material type	***L****	***a****	***b****	**Hue**	**SI**
Wheat flour	91.64±0.03a	-0.36±0.01d	12.50±0.08c	91.63±0.02a	12.50±0.08c
Sesame	71.81±0.48b	0.69±0.10c	19.52±0.23a	87.98±0.31b	19.54±0.23a
Black cumin	33.85±0.78d	0.82±0.13c	2.78±0.18e	73.67±1.64c	2.90±0.21b
Blue poppy	33.45±0.37d	3.24±0.10b	3.31±0.13d	45.54±1.06e	4.63±0.14d
Flaxseed	53.50±0.43c	6.08±0.19a	16.97±0.24b	70.28±0.67d	18.03±0.22b

[1]Means followed by the same letter within a column are not significantly different ($p < 0.05$).

Color properties of crackers

Color is one of the most important quality indicators that directly affects consumer acceptability of bakery products (Pathare et al., 2013). The instrumental color values (L*, a*, b*, Hue angle, and SI) of the cracker samples produced with different seed types and varying additive levels are presented in Table 2. Among the different seed types, significant variations ($p < 0.05$) were observed in all color parameters of the cracker samples. Sesame-added crackers exhibited the highest L* value (77.71), indicating a lighter appearance, whereas black cumin crackers had the lowest lightness (L* = 63.85), resulting in a darker product surface. This variation may be attributed to the intrinsic pigment composition and thermal behavior of the seed materials during baking (Gandhi et al., 2022; Pawase et al., 2020). In terms of a* values, blue poppy and flaxseed crackers showed the highest redness values (1.25 and 1.15, respectively), while sesame (0.48) and black cumin (0.75) crackers exhibited significantly lower redness values. The observed differences in red/green color tones could be due to the seed skin pigments, including anthocyanins or other polyphenolic compounds, which may undergo complex Maillard and caramelization reactions during baking (Wang et al., 2022). Regarding b* values, which indicate yellowness, sesame crackers had the highest value (29.21), followed by flaxseed (24.82) and blue poppy (22.66), whereas black cumin crackers had the lowest (19.07). These findings suggest that sesame and flaxseed contribute more prominently to yellowish hues, potentially due to their higher oil content and carotenoid levels (Radwan et al., 2017). Hue angle and Saturation Index (SI) values were generally consistent with the L*, a*, and b* trends, with sesame crackers showing the most saturated and yellow-toned color appearance (Hue: 89.41, SI: 29.23), and black cumin resulting in a duller and less saturated appearance (SI: 19.08). When evaluating the impact of increasing additive ratios (0–9%), a general decreasing trend in lightness (L*) was observed. The control crackers (0%) had an L* value of 72.86, which slightly increased at 3% (74.57), likely due to dilution of browning reactants at low substitution levels. However, further increases in additive content to 6% and 9% significantly reduced lightness values (68.99 and 67.55, respectively), possibly due to intensified Maillard reactions and pigment accumulation from the seed additives (Otondi et al., 2020). Redness (a*) values showed a distinct fluctuation with increasing substitution levels.

Table 2. Color values of crackers

Cracker type	***L****	***a****	***b****	**Hue**	**SI**
Sesame	77.71±3.00a	0.48±1.26c	29.21±5.32a	89.41±2.21a	29.23±5.35a
Black cumin	63.85±6.52d	0.75±0.93b	19.07±11.27d	88.13±1.80b	19.08±11.30d
Blue poppy	69.74±4.09c	1.25±0.91a	22.66±9.12c	86.77±2.31c	22.71±9.13c
Flaxseed	72.67±3.37b	1.15±0.86a	24.82±7.74b	87.47±1.67bc	24.85±7.75b
Additive ratio (%)					
0	72.86±0.24a	2.22±0.28a	37.79±0.42a	86.63±0.41c	37.85±0.42a
3	74.57±5.52a	-0.21±0.35d	20.46±3.52b	90.55±0.89a	20.47±3.52b
6	68.99±7.01b	0.49±0.72c	18.88±5.40bc	88.20±2.19b	18.64±5.39c
9	67.55±8.48b	1.11±0.58b	18.62±6.49c	86.38±1.69c	18.92±6.49bc

[1]Means with the same letter within a column are not significantly different ($p > 0.05$). Hue: Hue angle, SI: Saturation index.

The highest a* value was observed in the control (2.22), which significantly decreased at 3% substitution (-0.21), indicating a shift toward green tones. However, a* values increased again at 6%

and 9% (0.49 and 1.11, respectively), suggesting potential pigment release or interaction effects at higher levels of incorporation (Singh & Gaur, 2024). The b* values and SI followed a similar trend, decreasing significantly as the additive ratio increased from 0% to 9%, with the highest yellowness and color saturation found in the control (b*: 37.79, SI: 37.85) and the lowest in the 6% and 9% substituted samples. Hue angle values ranged between 86.38 and 90.55, with the 3% addition yielding the highest hue angle, implying a slight shift toward a greener/yellower hue. These observations indicate that both the seed type and additive ratio significantly affect the visual appearance of crackers, which may influence consumer perception and marketability. Therefore, optimizing seed type and its level of incorporation is essential to balance functional enrichment with acceptable color characteristics.

Physical properties of crackers

The physical characteristics of crackers, such as diameter, thickness, spread ratio, and hardness, provide critical insights into dough behavior during baking and the final product's textural and visual qualities. Table 3 presents the measured physical parameters of crackers enriched with different types and ratios of oilseeds. Significant variations ($p < 0.05$) were observed among seed types in terms of diameter, thickness, spread ratio, and hardness. Crackers containing blue poppy exhibited the largest diameter (45.44 mm), while black cumin samples had the smallest (44.47 mm). However, all samples were within a narrow range, indicating a limited influence of seed type on diameter. In terms of thickness, most crackers showed similar values (around 7 mm), except for the black cumin sample, which had a significantly lower thickness (6.42 mm). Consequently, the spread ratio (diameter/thickness) was highest in black cumin crackers (7.02), suggesting greater lateral expansion during baking. This may be attributed to the oil content and particle size of black cumin, which could reduce dough viscosity and increase spread ability (Mamat & Hill, 2014).

Hardness, representing the force required to break the sample, varied significantly with seed type. The hardest sample was observed in black cumin (1115.75 g), followed by sesame (1076.58 g), while the blue poppy sample was the softest (976.03 g). These differences are likely due to the fat and fiber composition of each seed type, which influences water absorption and dough matrix development during baking (Otondi et al., 2020; Metwal et al., 2011). Higher oil absorption in black cumin may have led to reduced moisture retention, producing a firmer texture.

Cracker samples prepared with increasing additive levels (0–9%) also showed distinct changes in physical properties. The control sample (0%) exhibited the largest diameter (46.30 mm) and thickness (7.59 mm), resulting in the lowest spread ratio (6.13). This suggests that the incorporation of seed powders disrupts the dough matrix and affects its baking behavior, possibly by altering gluten network strength or water distribution (Gupta et al., 2014). As the additive ratio increased, both diameter and thickness decreased significantly ($p < 0.05$). At 3–9% levels, diameter values remained around 44.4–44.8 mm, while thickness dropped to ~6.7 mm, resulting in higher spread ratios (6.66–6.75). This increase in spread ratio with additive incorporation may be attributed to reduced dough cohesion and increased fat migration from the seeds during baking (Altaf et al., 2021).

The most prominent difference was observed in hardness values. The control sample was the softest (864.34 g), while the 9% substituted crackers were the hardest (1304.76 g). The progressive increase in hardness with higher seed addition may be due to higher dietary fiber and protein content, which can reinforce the dough structure, reduce expansion, and limit moisture retention, thereby yielding a firmer texture (Metwal et al., 2011; Naqash et al., 2017). These results collectively indicate that both the type and concentration of oilseed additives significantly influence the physical attributes of crackers, particularly hardness and spread ratio, key determinants of consumer texture perception.

Table 3. Physical properties of crackers

Cracker type	Diameter (mm)	Thickness (mm)	Spread ratio (W/T)	Hardness (g)
Sesame	45.05±1.13a	7.06±0.74a	6.44±0.66b	1076.58±192.75ab
Black cumin	44.47±1.47b	6.42±0.85b	7.02±0.77a	1115.75±220.77a
Blue poppy	45.44±0.87a	7.19±0.65a	6.37±0.56b	976.03±133.34c
Flaxseed	45.10±1.22a	7.05±0.51a	6.42±0.44b	1035.75±171.06b
Additive ratio (%)				
0	46.30±0.39a	7.59±0.51a	6.13±0.48b	864.34±20.38d
3	44.44±1.15b	6.75±0.81b	6.66±0.70a	942.70±40.28c
6	44.44±0.96b	6.66±0.56b	6.72±0.64a	1092.31±106.77b
9	44.88±1.15b	6.71±0.69b	6.75±0.66a	1304.76±90.84a

[1]Means with the same letter within a column are not significantly different ($p > 0.05$).

Sensory Evaluation of Cracker Samples

The sensory evaluation of crackers formulated with different oilseed types—namely sesame, black cumin, poppy, and flaxseed—was conducted using a 7-point hedonic scale to assess attributes including color, appearance, odor, taste, crispness, and overall acceptability (Table 4, Figure 2). The enrichment with oilseeds significantly influenced the sensory characteristics of the final products, in line with previous studies indicating that the type and concentration of added functional ingredients can modify consumer perception and preference (Desai et al., 2010; Gętek et al., 2020).

Crackers enriched with sesame exhibited improved taste, crispness, and overall acceptability, particularly at substitution levels of 3% and 6%. However, a decline in these attributes was noted at the 9% level, suggesting a sensory saturation threshold beyond which product quality may be adversely affected. The positive impact of sesame at moderate levels may be attributed to its pleasant nutty flavor and natural oil content, which contribute to desirable textural and aromatic properties (Yilmaz and Doga, 2012).

In black cumin-supplemented crackers, the highest scores for odor and crispness were recorded at the 6% inclusion level. Nevertheless, sensory acceptance decreased at the 9% substitution rate, particularly for taste and overall liking. This could be associated with the intense and pungent aroma of black cumin, driven by its volatile compounds such as thymoquinone and carvacrol, which at higher concentrations may overwhelm the sensory balance of baked products (Amin and Husain, 2021; Singh et al., 2018).

Poppy seed-enriched crackers demonstrated consistent and balanced sensory profiles across all substitution levels. Notably, the 6% inclusion level yielded the highest scores across all parameters evaluated, indicating an optimal level that enhances sensory perception without compromising palatability. Poppy seeds are known for their mild nutty flavor and appealing texture, making them suitable for incorporation into cereal-based snacks (Koç et al., 2015).

For flaxseed-containing crackers, the 3% and 6% incorporation levels were generally well-received, enhancing taste and crispness. However, at the 9% level, a slight decline in sensory scores was observed in some attributes, potentially due to the mucilaginous nature of flaxseed components or the earthy flavor profile becoming more pronounced (Kajla et al., 2015). The partial decline in liking at higher doses is consistent with reports indicating that flaxseed's sensory contribution is dose-dependent and may influence consumer acceptability when used in excess (Wiesenborn et al., 2000).

Table 4. Sensory evaluation scores of crackers enriched with different oilseeds and substitution levels

Oilseed type	**Color/Appearance**	**Odor**	**Taste**	**Crispness**	**Overall Acceptability**
Sesame	5.88±0.23b	5.68±0.24ab	5.83±0.11a	5.80±0.12a	5.83±0.13ab
Black cumin	5.75±0.10c	5.63±0.31bc	5.58±0.25b	5.65±0.15b	5.65±0.09c
Blue poppy	5.98±0.09a	5.71±0.12a	5.83±0.08a	5.83±0.23a	5.88±0.11a
Flaxseed	5.88±0.13b	5.58±0.09c	5.73±0.12a	5.88±0.06a	5.78±.06b
Additive ratio (%)					
0	5.50±0.08d	5.20±0.09d	5.40±0.11c	5.60±0.06b	5.50±0.06d
3	5.83±0.03c	5.80±0.03b	5.93±0.00a	6.03±0.17a	5.96±0.11b
6	6.00±0.09b	5.98±0.00a	6.00±0.09a	6.95±0.21a	6.05±0.06a

9	6.15±0.11a	5.60±0.11c	5.63±0.24b	5.58±0.13b	6.65±0.05c

[1]Means with the same letter within a column are not significantly different ($p > 0.05$).

Overall, the results underscore that low to moderate substitution levels of oilseeds (particularly in the 3–6% range) are effective in enhancing the sensory quality of crackers without inducing undesirable sensory effects. Excessive enrichment may lead to overpowering flavors or textural imbalances that diminish consumer satisfaction. These findings are in agreement with prior research suggesting that optimal incorporation levels of functional ingredients are critical in balancing health benefits and sensory acceptance in bakery products (Metwal et al., 2011; Nawaz et al., 2021).

CONCLUSIONS

The enrichment of crackers with functional oilseeds such as sesame, black cumin, blue poppy, and flaxseed has shown potential for enhancing nutritional and sensory quality. Moderate incorporation levels (3–6%) improved texture and taste while maintaining structural integrity and visual appeal. However, higher substitution levels (9%) negatively affected hardness and sensory attributes. Overall, the study indicates that oilseed incorporation at optimal levels can contribute to the development of health-promoting bakery products with enhanced consumer acceptance. These findings provide a scientific basis for the formulation of functional crackers with targeted health benefits and desirable quality characteristics.

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CARBON FOOTPRINT OF AGRICULTURAL ACTIVITIES AND THE EMISSION REDUCTION POTENTIAL OF WIND ENERGY IN ALIAĞA DISTRICT

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ABSTRACT

This study quantifies the greenhouse gas emissions of agricultural activities in Aliağa (İzmir,Türkiye) using a cradle-to-gate Life Cycle Assessment and evaluates the mitigation potential of onshore wind energy via scenario analysis. Under Tier 1 methods of the 2006 IPCC Guidelines for agricultural soils, the district's total footprint was estimated at 6,410.38 t CO_2e, with 81.9% arising from fertilizer-driven N_2O (direct and indirect) and 18.1% from fuel use; the area-normalized intensity is 0.56 t CO_2e/ha, consistent with regional/global ranges. The Çandarlı-Yeni şakran sites produce 6-7 m/s wind speeds, enabling four wind-integration scenarios sized 1-5 MW.The most ambitious case (5 MW, mixed irrigation and mechanization, 1.75 GWh/y allocated to farm loads) yields 1,206 t CO_2e/y abatement (16.5% of the baseline) under diesel displacement (0.7-0.9 kg CO_2/kWh); the same allocation under grid displacement (0.4-0.5 kg CO_2/kWh) delivers 790 t CO_2e/y. Combined measures-N management (dose/timing, nitrification inhibitors, organic inputs) and on-farm wind power-can plausibly achieve 20% total reduction.

Keywords: Life Cycle Assessment; agricultural GHGs; nitrous oxide; wind energy; irrigation

INTRODUCTION

Climate change has made the reduction of greenhouse gas (GHG) emissions an urgent global priority. According to IPCC (2006), agricultural activities account for approximately 10-12% of global GHG emissions, primarily driven by nitrous oxide (N_2O) emissions from fertilizer use, carbon dioxide (CO_2) emissions from fuel combustion, and indirect emissions associated with irrigation.

In Türkiye, the agricultural sector contributes 13.4% of the country's total GHG emissions (TÜİK, 2025). Greenhouse gases sources include fertilizer application, mechanization, and energy-intensive irrigation systems. Electric energy use has been reported at 450-700 kWh per hectare for crop production and processes for crop production produced about 20% of total greenhouse emissions (Özkan et al., 2004). For cereal production in Türkiye, fertilizer-driven N_2O emissions may reach 100-120 kg CO_{2e} per hectare, while in the Aegean Region this level can be higher due to clay-loam soils (Çetin & Vardar, 2019). In agricultural areas similar to Aliağa, clay-loam soils with 1.5-2.5% organic matter tend to have higher nitrogen retention, which encourages fertilizer use and further elevates emissions (Kiper, 2018). Mitigation practices such as precision fertilization, drip irrigation, and organic amendments are suggested to reduce emissions by 20-30% (FAO, 2020). Reducing these greenhouse emissions is essential for Türkiye to meet its Paris Agreement commitments and 2053 Net Zero target.

Life Cycle Assessment (LCA) is a standard method for quantifying the carbon footprint of agricultural systems. The international literature indicates that LCA can effectively inform agricultural mitigation strategies; for example, FAO (2020) reports 15-25% emission reductions through LCA-based optimization.

Wind energy emerges as a renewable and sustainable option for reducing agricultural emissions. Globally, wind accounts for roughly 7% of electricity generation, and its use in energy-intensive on-farm operations can reduce fossil fuel consumption by 30-50% (IEA, 2023). Horizontal-axis wind turbines offer high conversion efficiency; a 1 MW turbine can generate around 3,000 MWh annually, yielding an estimated 1,350 t CO_2e reduction (IRENA, 2023). Integrating turbines into agricultural landscapes can maintain production while supplying energy, as turbine footprints typically occupy only 1-2% of land without compromising yields (IEA, 2023).

According to the Ministry of Energy and Natural Resources (2023), national installed wind capacity exceeds 12 GW, with the Aegean Region contributing about 25% of this capacity. İzmir offers attractive wind regimes of 6-8 m/s. In Aliağa, annual average wind speeds of 6-7 m/s indicate strong siting potential (MGM, 2025). In İzmir, applying wind-powered systems to irrigation can reduce energy consumption by 40% and emissions by 15-20% (Kiper, 2018). For example, powering vegetable irrigation pumps in Aliağa with wind energy could lower indirect CO_2 emissions by up to 20%.

In the Netherlands, farm-integrated turbines have supplied energy for irrigation pumps and greenhouse heating, cutting emissions by 25-30% (IRENA, 2023). In Germany's Baden-Württemberg, small turbines used for farm mechanization and electric equipment reduced emissions by 15-20% (IEA, 2023). In Türkiye, the Çeşme wind power plant (WPP) operates near agricultural zones in İzmir, providing power that indirectly supports nearby agricultural processes (Ministry of Energy and Natural Resources, 2023).

Aliağa is a strategic district where agriculture and industry. Aliağa, located in İzmir Province, is a major agricultural hub with 11,370 hectares of farmland producing cereals, fruits, and vegetables. However, industrial activities have led to land loss, soil degradation, and air pollution, necessitating innovative solutions to reduce agricultural emissions.

Aliağa's wind resource strengthens its suitability for agricultural decarbonization. In the district's northwestern sections (Çandarlı, Yenişakran), 6-8 m/s winds are common, offering multiple turbine siting options (MGM, 2025). Kiper (2018) suggests piloting wind integration into irrigation and mechanization in Aliağa. For instance, a 1 MW turbine in Çandarlı could fulfill the irrigation energy demand of approximately 500 ha of vegetable cultivation (350,000 kWh/year) and deliver 157.5 t CO_2e annual emission reductions (IRENA, 2023). However, Çetin & Vardar (2019) and Kiper (2018) analyze agricultural emissions in İzmir, they do not focus on mitigation through wind energy. International literature (IEA, 2023; IRENA, 2023) highlights wind's potential in agricultural decarbonization, but specific applications are still limited in Türkiye.

This study analyzes the carbon footprint of agricultural activities in Aliağa using Life Cycle Assessment (LCA) and evaluates the environmental impacts of agricultural processes in Aliağa by modeling emissions from fertilizers (N_2O), on-farm fuel use (CO_2), and irrigation (indirect CO_2). Pesticide-related and waste-related emissions are excluded due to data gaps. In addition, this study evaluates the potential of wind turbines to mitigate emissions after determination of greenhouse emissions.

MATERIAL AND METHOD

The study (i) quantifies the cradle-to-gate greenhouse gas (GHG) footprint of Aliağa's agricultural production and (ii) evaluates wind-to-farm electrification scenarios for irrigation and mechanization.

The İzmir Provincial Directorate of Agriculture and Forestry (2025) reports that the study area (Aliağa Region) has 11,370 ha of agricultural land distributed as cereals (40%, 4,548 ha), fruits (35%, 3,979.5 ha), vegetables (20%, 2,274 ha), and other crops (5%, 568.5 ha). Wheat (10,291 t) and olive (6,689 t) dominate local agricultural output (BEPA, 2025). Soil is clay-loam with 1.5-2.5% organic matter. The Mediterranean climate features 17°C average temperature, 600 mm rainfall, and 65% humidity.

Life Cycle Assesment (LCA) follows ISO 14040 (2006) using cradle-to-gate approach. Two functional units are used: 1 ha-year and (where available) per-ton of product. System boundaries include upstream supply and on-farm application stages; post-harvest transport/processing are excluded.

Datas for assumptions and calculations;

Primary datas;

Crop Production**:** The 2024 production data for Aliağa were obtained from BEPA (2025) and the İzmir Provincial Directorate of Agriculture and Forestry are given Table 1. Sample datas: wheat (10,291 tons, 4,000 ha), olive (6,689 tons, 3,500 ha), tomato (4,500 tons, 1,000 ha).

Irrigation and Mechanization: Data on irrigation systems (60% drip, 30% sprinkler, 10% surface) and mechanization (tractors, harvesting machines) were collected from the Aliağa Chamber of Agriculture.

Secondary datas;

Fertilizer for crops;

Fertilizer use for production of crops and generally range of fertilizer dose is approximately 100-150 kg/ha according to crops such as creals 100 kg/ha, vegetables 150 kg/ha, fruit 80 kg/ha. Fertilizer generally includes 50% nitrogen-based fertilizer (ürea), 30% phosphorus-based fertilizer (P_2O_5) and %20 potassium-based fertilizer (K_2O). (TÜİK, 2025; Çetin and Vardar, 2019; Kiper, 2018).

CO_2e emissions factors for production of fertilizer;

3,5 kg CO_2e /kg N; 0,7 kg CO_2e /kg P_2O_5; 0,5 kg CO_2e /kg K_2O;

Direct and indirect N_2O emissions;

Calculation of direct and indirect N_2O emissions;

$N_2O\text{-}N_{direct}=Napplied\times EF1$;

$N_2O_{direct}=N_2O\text{-}N_{direct}\times 44/28$;

$CO_2e=N_2O_{direct}\times GWP_{N_2O}$

Direct N_2O emissions are calculated using the EF factor; EF1 = 0.01 kg N_2O-N per kg of applied N.

Indirect N_2O emissions from volatilization and leaching follow IPCC Tier guidelines. N_2O emissions are converted to CO_2e using the Global Warming Potential (GWP) for N_2O: AR5=265 kg CO_2e per kg N_2O.

<u>CO_2e production from Fuel use;</u>

Calculation of CO_{2e} emissions;

kg CO_2e (for fuel usage) = Cultivated area (ha)×diesel consumption for product (L/ha) × diesel EF CO_2e/L

Fuel used for mechanization such as truck etc. at 30 L/ha (cereals), 50 L/ha (vegetables), 40 L/ha (fruit), diesel EF=2.68 kg CO_2/L (Özkan etc., 2004; Çanakçı etc., 2010).

<u>Irrigation Energy;</u>

Irrigation energy includes electric and diesel pumps used for crop irrigation. Typically, electric pumps consume 450-700 kWh per hectare per year, while diesel pumps consume 10-15 liters per hectare per year (FAO, 2020; İzmir Provincial Directorate of Agriculture and Forestry, 2025).

For electric pumps;

CO_2e= kWh×EFgrid

Grid electricity EF (Türkiye); 0,45 kg CO_2/kWh

For diesel pumps;

CO_2e=Ldiesel× Diesel EF

Diesel EF=2.68 kg CO_2/L

<u>Wind capacity;</u>

The Aliağa district lies in a high-wind coastal belt of the North Aegean; Global Wind Atlas and MGM (Turkish State Meteorological Service) indicate 6-8 m/s velocity with power densities of 400-500 W/m².

RESULTS AND DISCUSSION

The İzmir Provincial Directorate of Agriculture and Forestry (2025) reports that the study area (Aliağa Region) has 11,370 ha of agricultural land distributed as cereals (40%, 4,548 ha), fruits (35%, 3,979.5 ha), vegetables (20%, 2,274 ha), and other crops (5%, 568.5 ha). Total greenhouse emissions according to crops in Aliağa Region are given Table 1.

In Aliağa, total emissions amount to 6,410.38 t CO_2e, with 81.9% (5,250.60 t CO_2e) originating from fertilizers and 18.1% (1,159.78 t CO_2e) from fuel use. Fertilizer emissions include N_2O from soil and production of nitrogen, phosphorus and potassium-based fertilizers. Nitrogen based fertilizer emissions include both direct and indirect N_2O. Aliağa's emissions per hectare (0.56 tons CO_2e/ha)

are consistent with those of the Aegean Region (0.5-1.2 tons CO_2e/ha) and global averages (0.6-0.9 tons CO_2e/ha). The intensity is 0.56 t CO_2e/ha, a range consistent with regional and global agricultural intensities for similar systems.

Table 1. Total greenhouse emissions according to crops in Aliağa Region

Product	Production (tons)	Cultivated Area (ha)	Fertilizer Emissions (tons CO_2e)	Fuel Emissions (tons CO_2e)	Total Emissions (tons CO_2e)
Barley	5,376	1,792	666.23	96.05	762.28
Broad Bean (Animal Feed)	150	50	18.58	2.68	21.26
Wheat	10,291	3,430	1,274.67	183.85	1,458.52
Bean	29	14.5	5.39	0.78	6.17
Fig (Grain)	3,825	1,530	568.21	81.94	650.15
Italian Ryegrass	225	75	27.87	4.02	31.89
Corn (Grain)	2,478	413	153.39	22.14	175.53
Corn (Silage) - 1st Planting	16,650	2,775	1,030.80	148.74	1,179.54
Corn (Silage) - 2nd Planting	2,400	400	148.65	21.44	170.09
Chickpea	23	11.5	4.27	0.62	4.89
Cotton	1,215	405	150.43	21.71	172.14
Potato	121	24.2	10.93	1.62	12.55
Alfalfa (Green Forage)	10,000	1,666.67	619.27	89.33	708.60
Oat	239	79.67	29.60	4.27	33.88
Pistachio	97	48.5	18.02	2.60	20.62
Pear	185	37	13.75	1.98	15.73
Quince	52	10.4	3.86	0.56	4.42
Almond	65	32.5	12.07	1.74	13.82
Walnut	6	3	1.11	0.16	1.28
Mulberry	55	11	4.09	0.59	4.68
Apple (Other)	12	2.4	0.89	0.13	1.02
Plum	200	40	14.87	2.14	17.01
Oleaster	5	1	0.37	0.05	0.43
Fig	80	16	5.95	0.86	6.80
Apricot	51	10.2	3.79	0.55	4.34
Mandarin (Other)	20	4	1.49	0.21	1.70
Pomegranate	155	31	11.52	1.66	13.18
Peach	178	35.6	13.23	1.91	15.14
Grape	1,306	261.2	97.04	14.00	111.04
Olive	6,689	2,675.6	994.02	143.34	1,137.36
Broad Bean (Fresh)	90	18	8.13	1.21	9.33
Pumpkin	50	10	4.52	0.67	5.19
Okra	20	4	1.81	0.27	2.08
Pepper (Stuffing)	180	36	16.26	2.41	18.67

Pepper (Hot)	100	20	9.04	1.34	10.38
Cowpea (Fresh)	48	9.6	4.34	0.64	4.98
Tomato (Table, Paste)	1,419	283.8	128.15	19.00	147.15
Bean (Fresh)	35	7	3.16	0.47	3.63
Cucumber (Table, Pickling)	200	40	18.07	2.68	20.75
Squash (Snack and Seed)	75	15	6.78	1.01	7.78
Cauliflower	40	8	3.61	0.54	4.15
Watermelon	450	90	40.66	6.03	46.69
Melon	250	50	22.59	3.35	25.94
Cabbage	50	10	4.52	0.67	5.19
Eggplant	170	34	15.36	2.28	17.64
Leek	100	20	9.04	1.34	10.38

In terms of product group contributions, cereals lead with 43.8%, followed by fruits at 21.3%, vegetables at 5.5%, and others at 29.5%; this distribution reflects both land use shares and nitrogen input profiles. According to calculations, wheat (1,458.52 tons CO_2e), olive (1,137.36 tons CO_2e), and silage maize (1,179.54 tons CO_2e) show highest greenhouse emissions due to large cultivation areas and fertilizer use. Tomatoes (147.15 tons CO_2e) and watermelons (46.69 tons CO_2e) also have high emissions due to intensive inputs. Oleaster (0.43 tons CO_2e) and apple (1.02 tons CO_2e) have the lowest emissions with minimal cultivation areas.

A significant majority 81.9% of total greenhouse gas emissions, amounting to 5,250.60 tons of CO_2 equivalent (tons CO_2e), stem from fertilizer use. In contrast, fuel combustion accounts for the remaining 18.1%, corresponding to 1,159.78 tons CO_2e. Emissions related to fertilizers include both upstream processes, such as the industrial production of nitrogen-based fertilizers, and downstream impacts like nitrous oxide (N_2O) emissions released from soils. Additionally, phosphorus and potassium fertilizers contribute to the total emissions, with estimated emission factors of 0.7 kg CO_2e per kilogram of phosphorus and 0.5 kg CO_2e per kilogram of potassium applied. Emission source and CO_2 gas emissions are given Table 2.

Table 2. Emission source and CO_2 gas emissions (ton CO_2e)

Emission Source	Emissions (tons CO_2e)	Percentage (%)
Nitrogen Fertilizer	4,202.98	65.6
Phosphorus Fertilizer	707.00	11.0
Potassium Fertilizer	340.62	5.3
Fuel (Diesel)	1,159.78	18.1

Aliağa's wind resource strengthens its suitability for agricultural decarbonization. In the district's northwestern sections (Çandarlı, Yenişakran), 6-8 m/s winds are common, offering multiple turbine siting options. Wind energy integration into irrigation and mechanization in Aliağa is possible and effective way to decrease emissions in agricultural area. In the emission reduction calculations, only the portion of wind energy allocated specifically to farm operations was considered. For estimating the impact of diesel displacement, a practical emissions factor ranging from 0.7 to 0.9 kg CO_2 per kilowatt-hour (kWh) was used. This range is based on the specific fuel consumption of diesel engines and the carbon content of diesel fuel. In the case of grid electricity displacement, an emissions

factor of 0.4 to 0.5 kg CO_2/kWh was applied, in line with recent data from the International Energy Agency (IEA) for Türkiye.

The Aliağa district benefits from consistent wind speeds ranging between 6 and 7 meters per second, which are ideal for the operation of modern onshore wind turbines. This renewable energy potential presents a compelling opportunity to substitute conventional energy sources used in agriculture, particularly for irrigation systems and mechanized farm operations. Wind turbines installed in Çandarlı, where wind speeds reach 7 m/s, and in Yenişakran, with 6 m/s, have the capacity to reduce agricultural greenhouse gas emissions by 1.1% to 16.5%, depending on the scale and application. This study evaluates four wind energy deployment scenarios based on their capacity and corresponding emission reduction potential. Scenario results are given Table 3 according to replacement and decreasement of emissions.

Table 3. Wind scenarios and annual abatement (ton CO_2e/y)

Scenario	Site/Capacity	Electricity allocated (kWh/y)	Diesel displacement (0.8 kg/kWh)	Grid displacement (0.45 kg/kWh)
S1	Çandarlı/1 MW	350,000	280	158
S2	Çandarlı/3 MW	1.170,000-1.300,000	938	525-585
S3	Yenişakran/2 MW	190 000-350 000	150-280	158
S4	Çandarlı+Yenişakran/5MW	1,750,000	1,400	788

According to Table 3, a 1 MW wind turbine installation at Çandarlı allocates 350,000 kWh of electricity annually, resulting in a reduction of 280 tons of CO_2e through diesel displacement and 158 tons of CO_2e via grid electricity substitution in Scenario S1. At scenario S2, with a larger 3 MW capacity at the same site, provides between 1.17 and 1.30 million kWh per year, leading to a substantial reduction of 938 tons CO_2e from diesel and 525-585 tons CO_2e from grid displacement. Scenario S3, located in Yenişakran with a 2 MW capacity, allocates between 190,000 and 350,000 kWh annually, achieving 150-280 tons CO_2e reduction from diesel and 158 tons CO_2e from grid electricity. Finally, Scenario S4 combines both sites with a total capacity of 5 MW, delivering 1.75 million kWh per year and yielding the highest overall emission reductions: 1,400 tons CO_2e from diesel and 788 tons CO_2e from grid displacement. These findings underscore the critical role of wind energy in mitigating greenhouse gas emissions, particularly when replacing high-carbon sources such as diesel fuel. For instance, a 5 MW wind turbine could achieve an annual emission reduction of approximately 1,400 tons of CO_2 equivalent, representing the upper bound of this mitigation potential.

CONCLUSIONS

In Aliağa, total greenhouse emissions amount to 6,410.38 t CO_2e, with 81.9% (5,250.60 t CO_2e) originating from fertilizers and 18.1% (1,159.78 t CO_2e) from fuel use. Fertilizer emissions include N_2O from soil and production of nitrogen, phosphorus and potassium-based fertilizers. Nitrogen based fertilizer emissions include both direct and indirect N_2O. Aliağa's emissions per hectare (0.56 tons CO_2e/ha) are consistent with those of the Aegean Region (0.5-1.2 tons CO_2e/ha) and global averages (0.6-0.9 tons CO_2e/ha). The intensity is 0.56 t CO_2e/ha, a range consistent with regional and global agricultural intensities for similar systems.

The Aliağa district benefits from consistent wind velocity ranging between 6 and 7 meters per second, which are ideal for the operation of modern onshore wind turbines. This renewable energy potential presents a compelling opportunity to substitute conventional energy sources used in

agriculture, particularly for irrigation systems and mechanized farm operations. Wind turbines planned to install in Çandarlı, where wind speeds reach 7 m/s, and in Yenişakran, with 6 m/s, have the capacity to reduce agricultural greenhouse gas emissions by 1.1% to 16.5%, depending on the scale and application. Wind energy's role in emission reduction was analyzed through four different scenarios. The most effective scenario, involving a 5 MW wind turbine, achieved a reduction of 1,400 tons CO_2e (16.5%) through combined use in irrigation and mechanization, demonstrating the effectiveness of renewable energy in mitigating agricultural emissions.

To further reduce the carbon footprint of agriculture in Aliağa, the study recommends several strategies: optimizing nitrogen fertilizer use, integrating wind energy, adopting organic fertilizers, shifting to low-emission crop varieties such as oleaster and apple, validating field-level data, and implementing farmer education programs. Through the adoption of precision farming techniques and renewable energy systems, Aliağa holds strong potential to become a model region for sustainable agriculture in Türkiye.

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THE CARBON BORDER ADJUSTMENT MECHANISM (CBAM) AND TURKISH AGRICULTURE: A SUSTAINABLE TRANSITION

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ABSTRACT

The European Union introduced the Carbon Border Adjustment Mechanism (CBAM) in 2023 to prevent "carbon leakage" and ensure fair competition under its Emissions Trading System (ETS). While initially covering carbon-intensive sectors like cement and iron-steel, CBAM's inclusion of nitrogenous fertilizers already impacts agricultural production costs. Furthermore, ongoing EU consultations suggest a high probability of CBAM's direct expansion to processed or new product groups, explicitly mentioning agricultural commodities. For Turkey, a major agricultural exporter to the EU, this presents a critical challenge. To manage this pressure, the paper proposes a dual approach. First, implementing data-driven emission reduction strategies such as precision fertilization, nitrification inhibitors biochar application, and renewable energy integration in greenhouses. Second, establishing robust digital Monitoring, Reporting, and Verification (dMRV) infrastructure, leveraging satellite imagery and IoT sensors to streamline compliance. In conclusion, while CBAM does not yet directly cover agricultural products, its indirect effects are already felt, and direct inclusion is increasingly likely. Proactive adoption of low-emission technologies, institutionalizing digital traceability, and aligning national policies with CBAM are imperative for Turkey to maintain competitiveness and achieve climate goals, transforming the "carbon wall" into a catalyst for sustainable agricultural transition.

Keywords: Agriculture, Carbon Border Adjustment Mechanism, Emissions Trading System, Greenhouse Gas Emissions,

INTRODUCTION

The European Union's Carbon Border Adjustment Mechanism is the world's first seminal regulatory instrument to offset carbon leakage to boost global climate ambition by imposing a levy on the carbon value of imported goods (Lin & Zhao, 2023). Formally enforced in May 2023, the CBAM is aimed at making the carbon price of domestic EU manufacturers falling under the Emissions Trading System equivalent to that of foreign rivals to promote global decarbonization (Perdana et al., 2023). First targeting industries of specific carbon intensity such as cement, iron, steel, aluminum, fertilizers, and electricity, the CBAM is the flagship of the European Green Deal to cut greenhouse gases by 55% by 2030 from 1990 levels, and by 2050 to be carbon neutral (Perdana & Vielle, 2022). The regulatory instrument has marked the onset of its transition period from October 1, 2023, for all incoming importers to report the innate carbon emissions for the first report period ending on January 31, 2024 (Fermeglia, 2023). From 2034, implementation in full swing including imposing of financial levies is to kick in, hence becoming the crucial economic impetus for producers to adopt less carbon-based technologies as part of the mitigation of global climate change (Wang et al., 2024). Although originally

only large industries were to be under the realm of work to be done, the fact that nitrogenous fertilizers are under the jurisdiction of the CBAM already directly impacts the production costs of agriculture, particularly for large agro-exporters of the EU such as Turkey (Kalpakchiev et al., 2023). Its implication already poses serious questions to the viability of Turkish products that are agricultural in nature, therefore necessitating the undertaking of forced reappraisal of hitherto agronomic operations and supply chains' carbon footprint to ensure sustainable access to the markets and yet remain economically viable (Guepie et al., 2023; Zhu et al., 2024). Moreover, ongoing consultations at the EU report a firm possibility that CBAM will be extended directly to processed agri-commodities and other product groups, potentially through a distinct "Agriculture-CBAM" regime (Kalpakchiev et al., 2023). Such extension would broaden the scope of carbon taxing significantly, from today's energy-based industries to the supply chains of agri-commodities, necessitating proper carbon accounting and abatement actions by agri-producers (Perdana & Vielle, 2022; Shuai et al., 2025). The analysis investigates the multi-faceted effects of the CBAM, particularly its potential extension to the agri-sector, for Turkey, the significant trading partner of the EU.

Potential Expansion of CBAM to Agriculture

The current negotiations within the European Union to expand the coverage of the Carbon Border Adjustment Mechanism to agri-food staples is a historic moment in international climate policy, extending its reach from industrial sectors to the complicated record of agri-food production and trade. It is spurred by large greenhouse gas emissions from farms, particularly from synthetic fertilizers and livestock, and would aim to avoid carbon leakage from the agri-food industry (Benedetti et al., 2025). It would need strict methodologies to estimate embedded carbon in agri-products, complicated by regionality of farm activities, local climate variations, and changing supply chains. It would require rigorous understanding of agri-carbon footprints that would consider everything from manure handling to farm practices to energy use at farm businesses, making the compliance space even more challenging for non-EU suppliers (Kolev, 2021). The potential "Agriculture-CBAM" regime could impose another 3-12% cost to non-EU agri-food suppliers, seriously jeopardizing access to markets and competitiveness for countries like Turkey (Ministère de l'Agriculture et de la Souveraineté alimentaire, 2024). It is another call to urgency for Turkish agri-food producers to bring innovative, low-carbon farm practices and to estimate and report their own emissions with precision to stay ahead of the curve in the EU market. Use of Digital Monitoring, Reporting, and Verification (dMRV) technologies that incorporate satellites and IoT sensors will be critical for compliance streamlining and could reduce verification costs by up to 40%. Such advances would facilitate more reliable carbon accounting that would enable producers to gain competitive advantages by demonstrating verifiable reduction of their environmental footprint. Such strategic investment in dMRV infrastructure and the development of a "digital carbon passport" would allow Turkey to credibly demonstrate the lowered carbon intensity of its agricultural products and therefore minimize potential trade barriers and gain wider market access in the EU. Moreover, the development of a national ETS for Turkish agriculture, including a voluntary pilot program by 2026-2028, will provide a key template for standardizing the data and creating common methodologies for carbon accounting, in harmony with international best practice. Such forward-looking approach will not only allow Turkish agriculture to be prepared for potential CBAM expansion but also facilitate the development of a more sustainable and resilient food system and contribute to addressing climate change at the international level. Such strategic coordination at the national and international levels, particularly through harmonized mechanisms of carbon accounting and reporting, would be important to ensure that Turkey would be capable of maintaining and consolidating its role as principal agricultural supplier to the European Union, while at the same time propelling its own domestic decarbonization agenda.

Implications for Non-EU Exporters

Non-EU export nations, especially those with significant agri-food trade to the EU, must rigorously reassess their supply chain and production processes as the Carbon Border Adjustment Mechanism (CBAM) potentially expands. This requires major investments in low-carbon technologies and the maintenance of tough, verifiable carbon emission records to ensure compliance and avoid punitive duties (Benedetti et al., 2025). This process necessitates that countries strengthen their carbon accounting capabilities, invest in green technologies, and adapt quickly to international policies. This builds resilience against policy risks and price volatility (Ren et al., 2025). Countries like Turkey, which have large trade exposures to the EU and carbon-intensive agri-food facilities, are particularly vulnerable to the externalities enforced by CBAM, risking a loss of competitiveness and trade imbalances (Çelik et al., 2025). Therefore, it is an urgent necessity for Turkey to invest strategically in decarbonization projects and to create national regimes that align with or mirror EU carbon pricing mechanisms to offset the negative economic impacts of CBAM (Dy & Yang, 2025). Strategically aligning national and international climate policies, particularly through common carbon reporting and accounting mechanisms, will be indispensable for Turkey to maintain and strengthen its role as a key agri-supplier to the European Union while simultaneously pursuing its own domestic decarbonization goals. Such measures aim to keep Turkish agri-products competitive by minimizing the financial costs of CBAM, thereby guaranteeing market access and trade relationships with the EU (Dy & Yang, 2025). Furthermore, developing a digital carbon passport platform will become essential for transparently revealing the embedded carbon of agri-products, building trust and facilitating smoother trade flows in an increasingly carbon-regulated global trade landscape.

TURKEY'S ROLE AS A MAJOR AGRICULTURAL EXPORTER TO THE EU

Turkey, which exports a lot of high-value agricultural goods to the European Union, is especially vulnerable to the economic effects of the Carbon Border Adjustment Mechanism (CBAM) (Raihan et al., 2023). This risk could grow if CBAM is expanded to include more agricultural products beyond just fertilizers, which would affect an even larger share of Turkish exports. The challenge is made worse by Turkey’s heavy trade reliance on the EU and its use of carbon-intensive farming methods (Çelik et al., 2025). Although Turkey’s exports to the EU may cut carbon emissions by 2.9%, it could also lead to a 0.8% drop in export value (Çelik et al., 2025). This shows why it’s urgent for Turkey to improve its farming policies and practices to limit negative effects and stay competitive in the EU market. As the EU’s sixth-largest trade partner, with €93 billion in exports, Turkey is at high risk from CBAM due to its industries’ high carbon emissions (Çelik et al., 2025). This makes it necessary for Turkey to update its industrial and farming policies to match the EU’s climate goals and protect its exports (Perdana et al., 2023). In the long run, CBAM could be a key driver for Turkey to speed up its shift to greener farming by adopting more sustainable technologies and methods (Çelik et al., 2025). Doing this will not only reduce short-term losses from CBAM but also help Turkey keep access to EU markets and make its agriculture more sustainable globally (Wang et al., 2024).

STRATEGIES FOR EMISSION REDUCTION AND COMPLIANCE IN TURKISH AGRICULTURE

To minimize the economic shock of the Carbon Border Adjustment Mechanism (CBAM) to its agriculture sector, Turkey needs a twin-track approach: adopting advanced low-emission technologies and establishing a credible digital and policy infrastructure to support them. This integrated structure will enhance transparency, accuracy, and compliance with international carbon accounting protocols

(Khalil et al., 2024; Çelik et al., 2025). The policy will enable Turkish industry to estimate its carbon footprint accurately, gain competitive footing within the EU market, and be in line with international efforts to minimize agricultural emissions (Raihan et al., 2023; Schaper et al., 2025).

TECHNOLOGICAL AND AGRONOMIC PRACTICES

A proactive strategy focused on advanced agricultural practices is essential for mitigating the sector's carbon footprint, enhancing resource efficiency, and securing its competitive position in international markets (Baccour et al., 2021; Piras et al., 2023).

Precision Agriculture and Fertilization: Implementing precision agriculture is a critical step to reducing nitrous oxide (N_2O) emissions and conserving water. The use of IoT sensors and AI analytics allows for variable-rate fertilization and localized irrigation, supplying precise doses of fertilizers and significantly reducing over-application (Ogwu & Kosoe, 2025). The use of nitrification inhibitors further reinforces this by blocking the microbial activities that lead to N_2O release from fertilized cropland soils, thereby enhancing nitrogen use efficiency (Pino et al., 2023; Fuerst et al., 2025).

Biochar Application for Carbon Sequestration: Biochar, a long-lasting form of charcoal produced from biomass, is a powerful climate change mitigation technology (Duku et al., 2011). It sequesters carbon in soils for centuries while concurrently improving soil fertility, water-holding capability, and microbial activity (Khan et al., 2024). This dual advantage makes biochar a key component for sustainable intensification and CBAM compliance (Ansari et al., 2023).

Renewable Energy Integration: Greenhouses, which are energy-intensive, can have their carbon footprint significantly reduced by integrating renewable energy sources like solar photovoltaics and geothermal setups (Piras et al., 2023). This strategic shift not only decreases the carbon intensity of agricultural commodities but also improves the long-term energy security of Turkish agro-industries (Mirzamohammadi et al., 2020). State policies and incentives for practices like agrivoltaics can accelerate this transition by optimizing the use of arable land for both energy and food production (Hsu & Yang, 2024).

However, the dissemination of these technologies presents significant implementation challenges, particularly for small and medium-sized farmers, such as high initial investment costs and the need for technical knowledge. Therefore, the success of technological transformation depends not only on financial incentives but also on strong collaboration and widespread training programs among farmer associations, universities, and technology providers.

POLICY AND INFRASTRUCTURE STRATEGIES

Technological adoption must be underpinned by a robust national framework that includes a credible digital Monitoring, Reporting, and Verification (dMRV) system and policies aligned with EU standards.

Digital MRV and the Carbon Passport: Establishing an effective dMRV system is essential to transparently quantify and report greenhouse gas levels. This system would use remote sensing, blockchain technology, and IoT sensors to provide real-time, tamper-proof data on emissions and sequestration rates (Khalil et al., 2024; Łękawska-Andrinopoulou, L., et al. 2024 Benedetti et al., 2025;). The core of this infrastructure would be a "digital carbon passport" platform to transparently

demonstrate the embedded carbon of agricultural products, thereby establishing credibility for smoother trade under CBAM (Su et al., 2024; Çelik et al., 2025).

Integration with TARBİL: Incorporating this digital passport system into existing national agricultural platforms like TARBİL is crucial to leverage current data infrastructure for the auto-collection and verification of carbon footprint data. This enhances traceability and transparency throughout the supply chain.

Facilitating CBAM Declarations: A verified digital passport system will ease the generation of CBAM statements by programmatically retrieving carbon intensity data, significantly reducing administrative costs for exporters and helping them achieve a sustainable competitive edge (Benedetti et al., 2025; Dy & Yang, 2025).

Aligning National ETS with EU Standards: To mitigate the financial impact of CBAM, Turkey must align its national policies with the EU. This involves expanding its domestic Emissions Trading System (ETS) to include a voluntary pilot phase for agricultural emissions, mirroring mechanisms in the EU (Peng et al., 2024; Ceschia & Soussana, 2021). This strategic alignment will reduce the financial burden on Turkish exporters and incentivize domestic decarbonization efforts (Benedetti et al., 2025). This coordinated national response, supported by bilateral agreements on carbon credit recognition, will be crucial for ensuring a smooth transition (Çelik et al., 2025).

Accessing Green Finance: A transparent and traceable carbon data system will facilitate Turkish agricultural producers' access to green financing and climate-friendly capital (Adewusi et al., 2023). This data openness, enabling the automatic creation of digital product passports, is increasingly important for market access and sustainability demonstration (Zhang & Seuring, 2024). To achieve this goal, it is critical to implement concrete financial incentive mechanisms such as "tax reductions for precision agricultural equipment," "grant programs for renewable energy installations," and "green loan opportunities for farmers engaged in low-carbon production."

This comprehensive approach, combining proactive engagement with the EU and strategic domestic policy alignment, can transform CBAM from a trade challenge into a catalyst for innovation and sustainable growth in Turkish agriculture (Zhu et al., 2024).

CONCLUSIONS

This analysis shows how the European Union's Carbon Border Adjustment Mechanism (CBAM) could seriously impact Turkish agriculture. The inclusion of nitrogen-based fertilizers under CBAM is already raising production costs, and future expansions to other processed agricultural goods could make things even more challenging.

Turkey is especially exposed to these new regulations. Still, this also presents an important opportunity. Turkey can use this moment to move toward a more sustainable, low-carbon farming model. Doing so would help protect access to EU markets and make Turkish agricultural exports more competitive globally as environmental concerns grow. To succeed, Turkey needs strong policies that include financial support, training for farmers, and environmental regulations. It's also essential to have reliable systems in place to measure and report emissions. With its new Emissions Trading System (ETS) and digital tools, Turkey can show clear progress in reducing agricultural emissions. By taking action, Turkey can turn the challenge of CBAM into an opportunity for long-term change.

This shift would help make Turkish agriculture more sustainable, protect jobs and exports, and support climate goals at home and abroad.

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IDENTIFICATION OF GENE REGIONS ASSOCIATED WITH IMPORTANT DISEASES OBSERVED IN TOMATO PLANTS USING MOLECULAR MARKERS

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ABSTRACT

Tomatoes are the most widely grown vegetable in the world. Viral diseases are among the main causes of limited tomato production both globally and in our country. Intensive cultivation raises the risk of viral and fungal diseases, such as Tomato Yellow Leaf Curl Virus (TYLCV), Tomato Spotted Wilt Virus (TSWV), and root and root collar rot (Fusarium oxysporum f. sp. radicis-lycopersici, or FORL), which can cause substantial economic losses in tomato-growing areas. Identifying disease-associated gene regions in tomato plants is a crucial and groundbreaking step in this research area. These identifications, accomplished through molecular markers, aim to create a strong foundation for discovering disease-resistant tomato varieties and contributing to our country and globally. The slow pace and limitations of traditional breeding methods have highlighted the importance of molecular markers. Marker-assisted selection (MAS) allows for the detection of gene regions linked to disease resistance and the integration of these genes into the plant's genome for early selection. Molecular markers, especially SSR, SCAR, and CAPS types, are effectively used in tomato breeding programs and have largely replaced conventional testing. Recent research has led to the development and use of numerous molecular markers to identify resistance genes, like Ty-1 and Ty-3 against TYLCV, Sw-5 against TSWV, I-1 and I-2 against Fusarium, and Mi against nematodes. These markers help in screening local genotypes and wild tomato species, facilitating the evaluation of genetic resources. According to the review for this thesis, incorporating molecular markers into breeding programs offers significant benefits, saving time, effort, and costs. It also provides faster, more reliable results compared to traditional methods. Additionally, developing plant lines with multiple resistance genes is essential to protect the production capacity of tomatoes in the face of climate change and mounting disease pressure. In conclusion, the widespread use of the MAS method greatly contributes to developing new, resistant tomato varieties and aligns with sustainable agriculture goals.

Keywords: Tomato, disease, virus, molecular marker

INTRODUCTION

Tomatoes (*Lycopersicon esculentum* L.) belong to the Solanaceae family and the Solanum genus. They are native to South America. It was first discovered in Mexico. The cultivated species was first brought to Europe by the Spanish in the 16th century and from there introduced to South and East Asia, Africa, and the Middle East, eventually spreading worldwide. (Hassan, 2020). According to data from the Food and Agriculture Organization of the United Nations (FAO), Turkey ranks third in the world in tomato production with approximately 13 million tons as of 2022, following China and India (FAO, 2025). According to TÜİK data, Turkey's vegetable production in 2023 is approximately 31.8 million tons. Tomatoes, the most produced vegetable in Turkey, account for 40.88% (13 million

300 thousand tons) of the total vegetable production in 2023 in terms of production volume. When Turkey's tomato cultivation areas are ranked by province in 2023, Bursa is in first place with approximately 19,000 hectares. Antalya follows with approximately 17,000 hectares and Konya with 14,000 hectares in 2023. When looking at tomato production by province, Antalya ranks first with a 19.6% share. Antalya is followed by Bursa with an 11.5% share and Manisa with an 8.2% share (TÜİK, 2025).

In addition to being one of the most widely cultivated vegetables worldwide, tomatoes are used as raw material in human nutrition and various industrial sectors (Kızmaz, 2024). In addition to their taste, tomatoes are rich in vitamins A and C, as well as minerals such as calcium, potassium, and phosphorus, making them suitable for both direct human consumption and as a raw material for industrial products such as ketchup, sauces, and canned goods (Sainju and Dris, 2006; Ayisha et al., 2017). This is due to its widespread cultivation worldwide, its adaptability to ecological conditions enabling its cultivation in different geographical regions, the availability of various forms of use, both fresh and processed, and its prominence as a healthy product in meeting people's fresh vegetable needs. Since the world population is projected to increase by 50% by 2050, an increase in tomato production is expected, as with all products (Aktas et al., 2021).

Tomatoes (*Solanum lycopersicum* L.) are an economically important vegetable crop worldwide, but their production is seriously threatened by diseases and pests. (Yang et al., 2025). Many plant species and varieties have resistance to one or more diseases and pests. Hybrid varieties used in vegetable production, in particular, have multiple resistances. (Pınar et al., 2013). To date, 15 resistance genes have been transferred to tomatoes cultivated from various sources (Barone, 2004). Tomato (*Solanum lycopersicum* L.) production faces significant yield losses due to biotic stresses. The development of genetically resistant varieties offers a sustainable alternative to chemical controls, which are often costly and ineffective. (Hussain et al., 2025). Since the early 20th century, disease and pest resistance has been transferred to cultivated varieties using classical breeding methods. However, resistance to a single factor is insufficient for tomatoes, which are intensively cultivated, have a high number of diseases and pests, and are an important vegetable crop. Under current conditions, at least three or more disease and pest resistances are required depending on the production area and time. As the number of required resistances increases, the breeding period lengthens and may even become impossible. (Pınar et al., 2013).

As with other crops, diseases in tomato cultivation cause significant economic losses. Among these, viral diseases are considered the most important pathogens limiting tomato production, with 312 viruses and viroids (22 families and 39 genera) associated with tomato plants identified to date. Important viruses include species belonging to the Begomovirus, Tospovirus, Cucumovirus, Potyvirus, and Tobamovirus genera (Kızmaz, 2024).

Tomato yellow leaf curl virus (TYLCV) is responsible for tomato yellow leaf curl disease (TYLCD) and causes a significant decline in tomato *(Solanum lycopersicum* L.) yields worldwide. The use of resistant varieties is advocated as a sustainable management strategy. Significant progress has been made in genetically characterizing tomato resistance genes (R genes). Tomato breeding for TYLCV resistance has primarily relied on the introgression of the race-specific resistance gene Ty-3, which originates from wild tomato species. The improvement or development of a cultivated variety can be achieved using marker-assisted selection (MAS). Therefore, the precise and easy use of gene-targeted markers is of great importance for selection in breeding programs (Adedze et al. 2018).

Viral Diseases and Related Resistance Genes

Tomato Yellow Leaf Curl Virus (TYLCV): TYLCV is a DNA virus transmitted by whiteflies (Bemisia tabaci) that causes serious yield losses in tomato production. Six different resistance loci, Ty1, Ty2, Ty3, Ty4, Ty5, and Ty6, have been identified against this disease. Most of these genes have been transferred from wild species such as *S. chilense* and *S. habrochaites*. It has been determined that the Ty1 and Ty3 genes are allelic, and the Pelota (Pelo) gene has been identified for Ty5, leading to the development of a functional dCAPS marker (Lapidot et al., 2015).

Tomato Spotted Wilt Virus (TSWV): The most commonly used resistance gene against this disease, which belongs to the Tospovirus group, is Sw-5. This gene, which originates from S. *peruvianum*, interacts with Avr genes to induce a hypersensitive response. SNP and SCAR markers have been identified for the Sw-5b allele (Van Zijl et al. 1985; Stevens et al. 1991).

Tomato Mosaic Virus (ToMV): The Tm-1 and Tm-22 genes, which provide resistance to this disease, were obtained from *S. habrochaites* and *S. peruvianum*, respectively. The Tm-22 allele is a more resistant and widely used form (Pelham, 1966; Young and Tanksley 1989).

Fungus-Caused Diseases

Verticillium wilt: The Ve1 and Ve2 genes have been identified as conferring resistance to this disease caused by *Verticillium dahliae*. The Ve1 gene encodes a receptor protein with an LRR structure and is characterized by a single base deletion that determines its function (Jung et al., 2015).

Fusarium wilt: The I1, I2, I3, and I7 genes have been identified as conferring resistance against *Fusarium oxysporum*. The I-2 and I-3 genes have an NB-LRR structure and play an important role in genetic resistance (Lim et al. 2006; Gonzalez-Cendales et al. 2015).

Late Blight: Two resistance genes, Ph2 and Ph3, have been identified against the pathogen *Phytophthora infestans.* The Ph3 gene is a functional resistance locus of high importance, detectable using SCAR markers (Black et al. 1996; Kim and Mutschler 2006; Zhang et al. 2013).

Leaf Mold: Resistance genes such as Cf-2, Cf-4, Cf-5, and Cf-9 have been identified against the pathogen *Cladosporium fulvum.* Cf genes typically encode LRR proteins, which interact with the fungus's Avr proteins to induce a hypersensitive response (Jones et al. 1994; Dixon et al. 1996; Thomas et al. 1997; Dixon et al. 1998; Takken et al. 1999).

Nematode-Caused Diseases

Root-knot nematodes (*Meloidogyne* spp.): The Mi-1 gene is the most commonly used resistance gene against this pathogen. The Mi-1.2 allele is a functional form that provides resistance against both nematodes and certain harmful insects (Milligan et al., 1998).

Bacterial Diseases

Bacterial Spot: Various resistance genes such as Rx-1, Rx-3, Rx-4, Xv3, Xv4, and Bs4 have been identified against this disease caused by *Xanthomonas* spp. These genes activate the plant's immune system by recognizing Avr proteins from different strains (Schornack et al., 2004).

Bacterial Speck: The Pto gene has been identified as resistant to *Pseudomonas syringae* pv. *tomato.* Pto triggers the defense response in plants by recognizing the bacterial effectors AvrPto and AvrPtoB. It works in conjunction with the PRF gene to facilitate signal transduction (Kim et al., 2002; Abramovitch et al., 2006).

THE USE OF MARKER-ASSISTED SELECTION IN PLANT BREEDING

Molecular markers have been used extensively in many plants since the 1980s. In particular, markers linked to disease resistance genes have been developed and used in breeding programs for tomatoes (Pınar et al., 2013).

The Marker-Assisted Selection (MAS) Paradigm in Plant Breeding Molecular marker-assisted selection (MAS) is an approach developed to address issues associated with traditional plant breeding, utilizing multiple DNA markers to identify genomic regions responsible for the expression of desired traits in plants. With the development of DNA markers in the late 1970s, breeding programs shifted direction, and the use of markers linked to the desired gene provided a significant advantage (Brumlop et al., 2011). Additionally, it has become an economically important technique that saves time in identifying plants with desired traits (Kanlıtepe et al., 2010).

Molecular markers are not affected by environmental conditions and can be detected at all stages of plant growth, thereby enabling significant progress in identifying genotypes that cannot be detected due to environmental effects (Francia et al., 2005). On the other hand, since many genotypes with undesirable traits will be separated using MAS, it is possible to create a more effective breeding program (Karakaş and Bayrıl, 2024). To date, more than 40 genes (single genes and QTLs) have been mapped. These mapped traits are utilized in breeding programs using the MAS method. Since 1990, the MAS method has been used in breeding programs alongside classical breeding methods. The reasons for using MAS include shortening the breeding period through early selection, facilitating the transfer of resistance through backcrossing, determining the linkage between genes, facilitating the identification of traits that are difficult to screen, and eliminating the need for testing for resistance to certain diseases and pests under quarantine, thereby enabling work with a smaller number of plants and providing advantages in terms of labor and cost (Pınar et al., 2013).

Molecular markers are any gene region or DNA fragment associated with a gene region in the genome. Molecular markers are known as DNA markers because they constitute the DNA-based type of genetic markers. DNA markers are markers that reveal DNA sequence differences between different genotypes in various ways. The use of nucleic acid-based genetic markers in genome analysis is an area of need for breeders. These markers can be used to distinguish and identify crop varieties that are morphologically very similar to each other (Yorgancılar et al., 2015).

LITERATURE REVIEW

Altundas et al., (2024) determined that 12 of the 17 pink tomato hybrids developed as a result of their study were resistant to all of the TSWV, TYLCV, Fol-1/2/3, ToMV, M, and Vd pathogens.

The 12 pink hybrid tomatoes resistant to all the diseases examined were treated with Fe-DTPA at concentrations of 0, 150, 300, and 600 µmol L^{-1} under greenhouse conditions. When the plants reach full maturity, leaf and fruit samples will be collected, and Fe concentrations will be determined using inductively coupled plasma/optical emission spectrophotometry (ICP-OES). The Fe concentrations in the green parts and fruits of the plants will be compared to calculate Fe uptake and translocation efficiency. This will enable the development of tomatoes with the highest Fe concentration, Fe uptake, and translocation efficiency.

The study by Basım and Kandil, (2024) focuses on the cultivation of cluster tomato varieties resistant or tolerant to *Fusarium oxysporum* f.sp. *radicis-lycopersici* (FORL) and Tomato Yellow Leaf Curl Virus (TYLCV) using molecular DNA markers. The breeding program involves isolating genomic DNA from 69 cluster tomato varieties and then using PCR with C2-25 and Ty3P6-25 primers to determine which varieties are resistant or tolerant to FORL and TYLCV, respectively. The results of the tests demonstrated that DNA molecular markers can reliably determine the presence of resistance or tolerance to FORL and TYLCV in cluster tomatoes. Molecular markers have shown the potential to efficiently screen thousands of tomato plants in a shorter time, enabling the selection of higher-quality, resistant, or tolerant varieties.

El Mohtar et al., (2007) developed a marker for the I-2 gene, which confers resistance to *F. oxysporum* f. sp. *lycopersici* race 2. The I-2 gene responsible for resistance to this race was detected in 39 out of 40 tomato genotypes known to be resistant to the same race, while only one tomato genotype did not show the band associated with the I-2 gene. The validity of this method has been reported to be maintained in studies conducted in three different countries using molecular and classical testing.

This study, conducted by Facundo-Angel et al. (2024), was carried out to identify resistance genes against pathogens commonly found in tomato (*Solanum lycopersicum* L.) cultivation. In the study, the presence of genes conferring resistance to important pathogens such as *Meloidogyne* sp., *Fusarium oxysporum*, *Stemphyllium* sp., *Phytophthora infestans*, TYLCV, and TSWV was evaluated in 20 tomato genotypes using 10 different SCAR-type molecular markers. As a result of the study, resistance genes were identified in 17 advanced lines and 3 commercial hybrids. This indicates that the aforementioned markers can be effectively used in selection processes and offer a faster, more economical, and reliable alternative compared to conventional breeding methods. The findings support the applicability of molecular marker-assisted selection (MAS) in tomato breeding programs.

In their study, Hussain E. et al., (2025) used SSR, SCAR, and CAPS markers to assess resistance against six major diseases, including *Fusarium* wilt, late blight, bacterial wilt, root-knot nematode, *Tomato Mosaic Virus* (ToMV), and *Tomato Yellow Leaf Curl Virus* (TYLCV). The study analyzed 964 tomato samples, including both wild germplasm (371) and varieties (593). The results emphasize that wild germplasm has significantly higher resistance levels than cultivated tomatoes, making it an invaluable resource for breeding programs.

Kahraman (2017), in his doctoral thesis titled “Development of Tomato Strains Resistant to *Tomato Spotted Wilt Virus* and Root-Knot Nematodes through Molecular Marker Selection in Tomatoes,” aimed to pyramid the Sw-5 gene, which confers resistance to the *Tomato Spotted Wilt Virus*, and the Mi genes, which confer resistance to soil-borne root-knot nematode species. As a result of the study, resistant advanced breeding lines were developed. Thirty advanced breeding lines resistant to the Tomato Spotted Wilt Virus at the F4 stage and 100 advanced breeding lines

homozygous resistant to TSWV and nematodes at the F4 stage were developed. These lines can be used as references in future molecular and biological testing studies and as parent lines in variety development studies.

Mortazavi et al., (2025) in this study, genetic resistance to root-knot nematode (RKN), which causes significant yield losses in tomato (*Solanum lycopersicum* L.) cultivation, was investigated. A total of 212 tomato genotypes collected from different geographical regions (Turkey, France, Taiwan, USA, Germany, Kyrgyzstan, etc.) were molecularly screened using the Mi23 primer pair (Mi23-F/Mi23-R) to determine the presence of the Mi-1.2 resistance gene. According to the results, a 380 bp band was observed in only six genotypes, and three of these genotypes (Romitel-RHT 3, AVTO0102-CLN2366B, and AVTO0101-CLN2413D) were homozygous, while three (Aysbars-F1, Italian Field [To-23], and Red Pole [To-25]) were determined to be heterozygous resistant. In the remaining 206 genotypes, the resistance gene was not detected, and these genotypes were found to be susceptible to the nematode. These findings demonstrate how effective and critical molecular markers are as tools in identifying resistance genes and developing disease-resistant tomato varieties. The study provides important genetic material for increasing nematode resistance in breeding programs.

Oğuz, (2010) investigated the resistance of tomato genetic resources and some wild genotypes to the pathogen morphologically and molecularly in Turkey. The control was LA 3667, which carries the TSWV resistance gene Sw-5, Formula F_1, and *S. peruvianum* genotypes carrying the TSWV resistance gene Sw-5 as a control. The study concluded that all local genotypes were susceptible to the pathogen.

In the research article titled “Use of Molecular Markers in the Development of Disease- and Pest-Resistant Lines and Varieties in Tomatoes” published by Pınar et al. (2013), multiple markers were tested for a single gene to assess nematode resistance. -23 SCAR markers were used to test for nematode resistance, the Ve2 2720F SCAR marker for *Verticillium* wilt, the At2-F3R3 SCAR marker for the I-1 gene conferring resistance to Fusarium wilt, the TAO901 CAPS marker for the I-2 gene, the JB-1 CAPS marker for the Ty-1 gene, the P6-25 SCAR marker for the Ty-3a gene, and the Sw-5.2 SCAR marker for the Sw-5 gene. It has been demonstrated that these molecular markers could serve as an alternative to classical testing in the development of disease- and pest-resistant varieties and lines.

Pınar et al., (2013) screened 450 tomato lines for resistance to F. oxysporum f. sp. lycopersici using SCAR and CAPS markers developed based on the I-1 and I-2 genes. Eighty-eight tomato lines produced the resistance allele band for the I-1 gene, and 74 lines produced the resistance allele band for the I-2 gene. As a result, it was reported that molecular markers (I-1 and I-2) determining resistance to *F. oxysporum* f. sp. *lycopersici* could be used in breeding programs to develop Fusarium-resistant varieties, thereby enabling the evaluation of more material.

In this study conducted by Yang et al. (2025), 401 tomato samples were analyzed using gene-related molecular markers to identify disease resistance genes in tomato (*Solanum lycopersicum* L.) germplasm. The study evaluated the presence of 21 genes conferring resistance to 10 different diseases, with the frequency of these genes varying between 0.2% and 66.1% across the samples. The I-1, I-2, Sm, Tm-2a, and Ph-3 genes were detected at the highest frequency, while the frequencies of the Ty-2, Ty-4, Mi-HT, I-3, and Tm-2 genes were found to be quite low. 86.3% of the tomatoes examined carried at least one disease resistance gene, with gene frequency and distribution being higher in modern processing and fresh consumption lines and lower in Latin American local varieties

and old varieties. In terms of marker reliability, CAUTy4 and 18IY13 had the highest accuracy rate (100%) for the Ty-4 gene, while the reliability of markers used for the Ty-2 gene was quite low (44.4%). Field observations confirmed the accuracy of marker-assisted gene detection to a large extent. The study demonstrated that disease resistance genes can be reliably identified using markers and that this genetic information can be effectively utilized in tomato breeding programs.

In Zengin, (2016) doctoral thesis, marker-assisted selection breeding in tomatoes was used to develop lines containing multiple resistance genes against Tomato Yellow Leaf Curl Virus (TYLCV), Tomato Spotted Wilt Virus (TSWV), nematodes, and Root and Crown Rot (FORL). As a result, the Institute's four different types of sensitive tomato lines (BATEM1, BATEM2, BATEM3, and BATEM4) were crossed with a resistant F1 tomato variety (Tayfun F1), resulting in the creation of 95 genotypes at the F3 stage through selection based on both disease resistance and agronomic characteristics. These lines, which have good agronomic and morphological characteristics and different combinations of resistance to the aforementioned diseases and nematodes, have been added to the BATEM tomato gene pool.

RESULTS

In this study, gene regions providing resistance to the main biotic stress factors limiting tomato (Solanum lycopersicum L.) production were evaluated using molecular markers, and the effectiveness of marker-assisted selection (MAS) methods in breeding programs was demonstrated. SSR, SCAR, and CAPS-type markers used to determine resistance to important pathogens such as TYLCV, TSWV, FORL, and root-knot nematodes successfully identified multiple resistance genes in different tomato lines.Research indicates that the molecular marker-assisted selection (MAS) method offers numerous advantages over classical breeding methods in the process of acquiring resistance to tomato diseases. The time-consuming and costly nature of classical breeding methods, as well as the difficulties encountered when transferring multiple resistance genes simultaneously, are significantly reduced through the use of molecular markers. In particular, the ability to perform early selection and the detection and screening of genes before they are phenotypically expressed both accelerate the breeding process and ensure more efficient use of resources. This not only provides significant economic and labor-saving benefits but also guarantees the stable transfer of resistance genes into lines. Resistance genes such as Ty genes (Ty-1, Ty-3) against TYLCV, Sw-5 against TSWV, I-1 and I-2 genes against Fusarium, and Mi-1 against nematodes, which are addressed in the study, are successfully identified using molecular markers, thereby enabling the pyramiding of targeted resistance genes. This pyramiding strategy increases the tomato's resistance to multiple diseases and pests, helping to protect the plant's production potential against challenging conditions such as climate change and increasing disease pressure. Literature reviews and experimental studies have shown that molecular markers play a vital role in the introgression of genetic resources from wild tomato species into cultivated tomatoes. Resistance genes found in wild species can be rapidly and reliably transferred to cultivated varieties using molecular markers, thereby increasing genetic diversity and resistance profiles. This not only enhances the resistance capacity of existing varieties but also provides a critical resource for the development of new resistant varieties. As a result, the integration of molecular markers into tomato breeding programs is considered a significant turning point in the development of disease-resistant varieties. This approach is consistent with sustainable agriculture and food security goals, contributing to the spread of environmentally friendly production techniques by reducing dependence on chemical control. Additionally, resistant varieties developed using the MAS method reduce economic losses for producers while enabling the provision of healthier and higher-quality tomato products to consumers.

In future studies, the development of molecular markers using more precise, high-yield, and cost-effective tools, along with the discovery of new resistance genes, will further advance disease management in tomato production. Furthermore, the development of lines carrying multiple gene resistances will offer long-term solutions against new disease threats shaped by climate change. Therefore, the conservation of genetic resources and the support of molecular breeding techniques through multidisciplinary approaches are of vital importance for the sustainability of tomato production.

CONCLUSIONS

This study highlights the effectiveness of molecular marker-assisted selection (MAS) in improving tomato resistance to major pathogens. Compared to conventional breeding, MAS enables rapid and reliable identification, transfer, and pyramiding of key resistance genes (e.g., *Ty*, *Sw-5*, *I*, *Mi-1*), while facilitating the use of wild genetic resources. This approach accelerates breeding, reduces reliance on chemicals, and contributes to sustainable tomato production and food security under increasing disease and climate pressures.

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MACRO ELEMENTAL RISK ANALYSIS OF WATER IN TWO MAJOR DAM LAKES OF THE MARMARA REGION, TÜRKIYE

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ABSTRACT

Reservoirs play a crucial role in freshwater ecosystems, serving key functions such as irrigation, drinking water supply, and flood control. Despite their importance, these artificial lakes are increasingly threatened by anthropogenic pollution. Macro elements like sodium (Na), magnesium (Mg), potassium (K), and calcium (Ca) are essential for both aquatic life and human health, and their elevated levels may indicate ecological stress or contamination. Atikhisar and Alibey Dam Lakes, located in the Marmara Region of Türkiye, are among the region's most vital reservoirs, supplying water to the provinces of Çanakkale and İstanbul. In this study, surface water samples were collected during the dry season of 2022 from both the inlet and outlet zones of each reservoir (a total of four sites). The concentrations of major macro elements —sodium (Na), magnesium (Mg), potassium (K), and calcium (Ca)—were measured, and potential macro elemental risks were assessed. Results indicated that Alibey Dam exhibited higher average concentrations of these elements compared to Atikhisar Dam. Overall, the elemental abundance in both reservoirs followed the trend: Na (81.066 µg/L) > Mg (31.434 µg/L) > Ca (21.867 µg/L) > K (6.353 µg/L).

Keywords: Atikhisar Dam Lake, Alibey Dam Lake, Macro elements, Water quality

INTRODUCTION

Macro elements such as sodium (Na), magnesium (Mg), potassium (K), and calcium (Ca) are essential constituents of natural waters and play vital roles in maintaining aquatic ecosystem function and water quality (Köse et al., 2013; Çiçek et al., 2013; Tokatlı and Helvacıoğlu, 2020; Ali et al., 2025). They are crucial for biological processes in both aquatic organisms and humans, including osmoregulation, enzyme activity, and bone development. However, elevated concentrations of these elements—especially under the influence of anthropogenic inputs such as agricultural runoff, industrial discharges, and urbanization—may lead to ecological imbalances and potential health risks. For instance, excessive sodium or magnesium levels can affect water taste, soil structure, and even plant and aquatic organism health. Therefore, regular monitoring of macro element concentrations in freshwater bodies is essential for early detection of pollution, sustainable water management, and the protection of ecological and human health (Arslan et al., 2012; Tokatlı et al., 2020; Din et al., 2023; Tokatlı et al., 2024; Ustaoğlu et al., 2025).

Atikhisar Dam, located on Sarıçay Stream in the province of Çanakkale, was constructed between 1971 and 1975 primarily for irrigation and flood control. This earth-fill type dam has a body volume of approximately 1.99 million cubic meters and rises about 43 meters from the riverbed. Under normal water conditions, the reservoir has a surface area of 3.30 km² and a storage capacity of 40 hm³, supporting irrigation over nearly 5200 hectares of agricultural land (Anonymous, 2021a; Tokatlı et al., 2023). Similarly, Alibey Dam, situated on Alibey Stream in İstanbul, was built between 1975 and

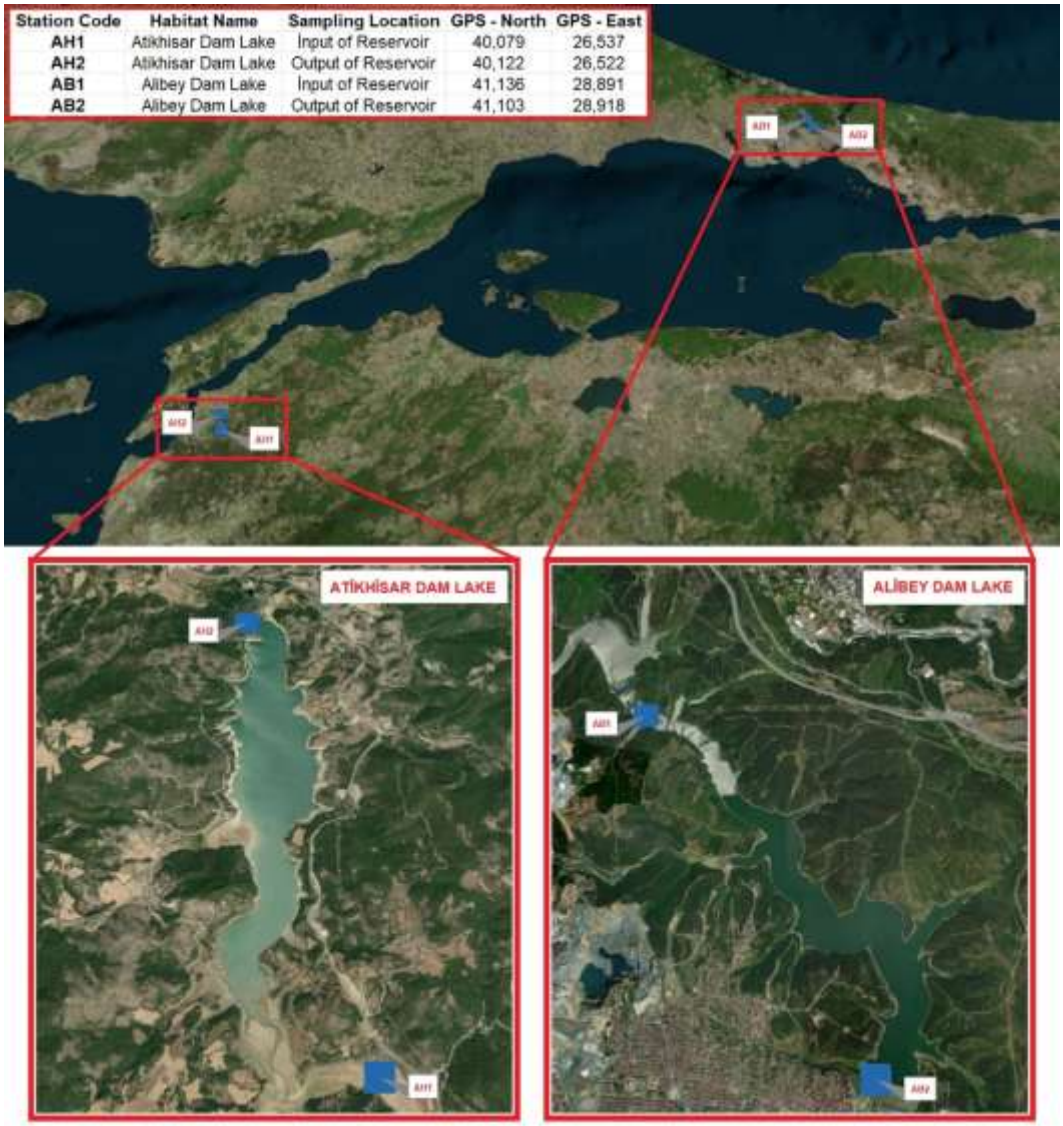
1983 to supply drinking, utility, and industrial water. Also designed as an earth-fill dam, it has a body volume of about 1.93 million cubic meters and a height of 30 meters. The reservoir holds up to 66.80 hm^3 of water and spans a surface area of 4.66 km^2 at normal water levels, providing approximately 39 hm^3 of potable water annually for the city (Anonymous, 2021b; Varol and Tokatlı, 2024).

This study aims to assess the macro elemental composition of two strategically important reservoirs in the Marmara Region—Atikhisar and Alibey Dam Lakes—which serve as key sources of irrigation and drinking water. By focusing on the concentrations of sodium (Na), magnesium (Mg), potassium (K), and calcium (Ca), the research seeks to highlight the current status of these essential yet potentially harmful elements when present in excess. Considering the ecological significance of reservoirs and the increasing pressure from anthropogenic influences, the study underscores the necessity of regular monitoring to ensure sustainable water resource management and the protection of ecosystem and public health.

MATERIALS AND METHODS

Water samples were systematically collected from both the inlet and outlet zones of Atikhisar and Alibey Dam Lakes, comprising a total of four sampling stations, during the dry season at the end of summer in 2022. These specific locations were selected to represent potential spatial variations in water quality between the entry and exit points of each reservoir system. The geographical layout of the study area, along with the precise coordinates of the sampling stations, is illustrated in Figure 1 to provide a clear spatial reference for the sampling design and to enhance the reproducibility of the study.

For macro-elemental analysis, the pH of each one-liter water sample was adjusted to approximately 2.0 by the addition of 2 mL of concentrated nitric acid (HNO_3) to prevent precipitation and ensure element stabilization. Subsequently, the samples were filtered using cellulose nitrate membrane filters with a pore size of 0.45 µm to remove particulate matter. The filtrates were then diluted to a final volume of 50 mL with ultrapure water to standardize the analytical conditions. Concentrations of calcium (Ca), magnesium (Mg), sodium (Na), and potassium (K) were quantified using an inductively coupled plasma mass spectrometer (ICP–MS, Agilent 7700xx) at the internationally accredited laboratory of Trakya University (certified under TS EN ISO/IEC 17025). All measurements were conducted in triplicate, and the mean values were reported to ensure analytical precision and reproducibility (EPA, 2001).



Station Code	Habitat Name	Sampling Location	GPS - North	GPS - East
AH1	Atikhisar Dam Lake	İnput of Reservoir	40,079	26,537
AH2	Atikhisar Dam Lake	Output of Reservoir	40,122	26,522
AB1	Alibey Dam Lake	İnput of Reservoir	41,136	28,891
AB2	Alibey Dam Lake	Output of Reservoir	41,103	28,918

Figure 1. Study area and selected sampling locations

RESULTS AND DISCUSSION

The determined macro-elemental concentrations (Ca, Mg, Na, and K) at the designated sampling locations on both Atikhisar and Alibey Dam Lakes are presented in Figure 2. These values reflect the spatial distribution of the selected elements and provide insight into the ionic composition of the water bodies during the sampling period.

Notable spatial variations were observed between the two reservoirs. Sodium (Na) levels were markedly higher in Alibey Dam Lake, reaching a maximum of 128,219 µg/L at station AB1, whereas the lowest value (25,690 µg/L) was recorded at AH2 in Atikhisar Dam Lake. Similarly, calcium (Ca) concentrations were substantially higher at AH1 (33,837 µg/L), indicating possible geological or anthropogenic influences. Mean concentrations across all stations were calculated as 81,066 µg/L for Na, 31,434 µg/L for Mg, 6,353 µg/L for K, and 21,867 µg/L for Ca.

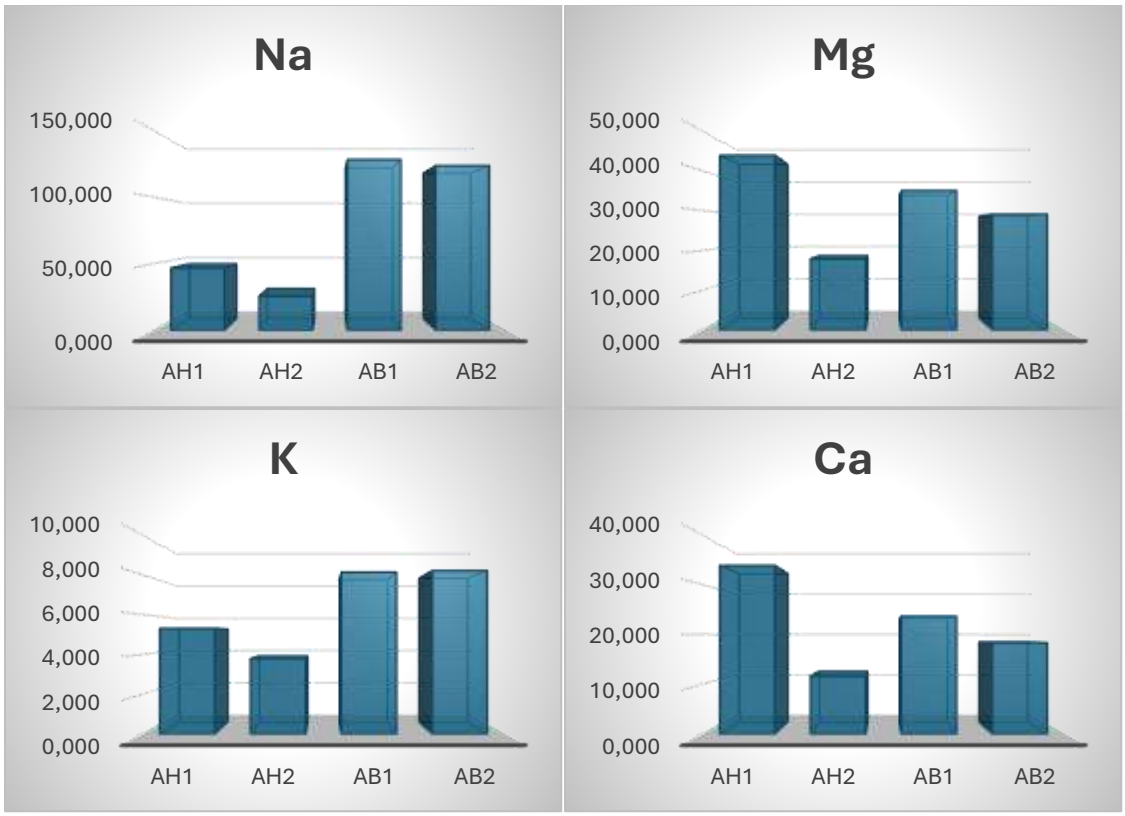


Figure 2. Spatial distributions of macro elements

These data suggest distinct hydrochemical characteristics between the two dam lakes, particularly with respect to the distribution of sodium and calcium. The markedly higher sodium concentrations observed in Alibey Dam Lake, compared to Atikhisar Dam Lake, may be indicative of differences in the geological composition of the catchment areas, such as the presence of sodium-rich sedimentary formations or evaporitic deposits (Hem, 1985; Gibbs, 1970). Moreover, anthropogenic factors such as agricultural runoff, domestic wastewater discharge, and land use practices—particularly irrigation with sodium-enriched water—can further contribute to elevated sodium levels in surface waters (Zhou et al., 2016). On the other hand, the relatively higher calcium content in Atikhisar Dam Lake may be attributed to carbonate-rich lithologies in the surrounding catchment or enhanced dissolution processes of calcareous materials, which are common in Mediterranean hydrological regimes (Meybeck, 1987). Additionally, inflow dynamics, including flow rate, residence time, and seasonal variability, can significantly influence the dilution or concentration of dissolved ions in reservoirs (Lerman et al., 2007). Overall, the observed elemental distribution reflects the combined influence of natural and anthropogenic processes, underscoring the importance of basin-specific evaluations in understanding freshwater chemistry.

CONCLUSIONS

In this study, surface water samples were collected from the inlet and outlet zones of Atikhisar and Alibey Dam Lakes to determine the concentrations of key macro elements (Na, Mg, K, and Ca) during the dry season of 2022. The elemental composition of these two important reservoirs, which supply water to the provinces of Çanakkale and İstanbul, was evaluated in order to assess potential ecological and water quality risks. The results revealed that Alibey Dam Lake exhibited notably higher average concentrations of all analysed elements compared to Atikhisar, suggesting greater exposure to anthropogenic inputs or distinct geochemical characteristics. Among the studied elements, sodium was found in the highest concentrations, followed by magnesium, calcium, and potassium. These findings underscore the need for regular monitoring of elemental loads in freshwater reservoirs, as elevated macroelement levels can be early indicators of ecological stress and potential threats to both aquatic ecosystems and human water use.

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PHENOLIC PROFILE OF DIFFERENT PARTS OF INULA GRAVEOLENS DETERMINED BY HPLC-DAD

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ABSTRACT

Turkey's diverse geography and climatic conditions contribute to a rich flora, particularly in medicinal and aromatic plants that serve as important sources for pharmaceutical, food, and cosmetic industries. Inula graveolens (Asteraceae) is an annual plant characterized by hairy stems and yellow flowers, widely distributed in the Mediterranean region of Turkey. Traditionally, it has been used in folk medicine for respiratory infections, cough, bronchitis, and urinary tract disorders, yet detailed studies on its chemical composition remain limited. This study aimed to determine the phenolic compound profiles of flower, aerial, and root extracts of *I. graveolens* using HPLC-DAD analysis. Plant materials were collected, dried, and separately extracted with ethyl acetate, methanol, and water. The resulting extracts were filtered through 0.20 um membranes and analyzed against 26 phenolic acid standards. In flower extracts, luteolin (11.43 mg/g) and coumarin (8.20 mg/g) were identified as major compounds in ethyl acetate; coumarin (21.65 mg/g) in methanol; and coumarin (11.43 mg/g) in aqueous extracts. In aerial parts, luteolin (14.78 mg/g), quercetin (11.25 mg/g), and trans-cinnamic acid (7.33 mg/g) were major compounds in ethyl acetate; coumarin (18.73 mg/g) and pyrocatechol (7.38 mg/g) in methanol; and coumarin (10.85 mg/g) and pyrocatechol (6.59 mg/g) in aqueous extracts. In roots, luteolin (6.91 mg/g) was predominant in ethyl acetate; rosmarinic acid (5.20 mg/g) and coumarin (4.63 mg/g) in methanol; and pyrocatechol (3.47 mg/g) and coumarin (2.69 mg/g) in aqueous extracts. The findings indicate that *Inula graveolens* is rich in phenolic compounds, particularly luteolin and coumarin, across different plant parts, with concentrations varying according to the solvent used. The high levels of these phenolics suggest potential antioxidant and pharmacological properties. This comprehensive phenolic profile provides valuable insights into the chemical composition of *I. graveolens* and serves as a basis for future studies focusing on its bioactive potential and possible applications in pharmaceutical and nutraceutical formulations.

Keywords: *Inula graveolens*, phenolic compounds, HPLC-DAD, luteolin, coumarin

1. INTRODUCTION

Phenolic compounds are secondary metabolites produced by plants, consisting of a benzene ring with one or more hydroxyl groups and functional substituents. All plants synthesize various phenolic compounds in different types and concentrations as part of their defense mechanisms (Atak & Uslu, 2018). Although the quantity differs among plants, phenolics represent the most abundant class of secondary metabolites (Dai & Mumper, 2010). Plants tend to produce these compounds under stress conditions. In addition to their role in defense, phenolic compounds contribute to the taste and color of fruits and vegetables (Atak et al., 2017). Due to their wide range of biological activities, including antioxidant, antimicrobial, anticancer, and anti-inflammatory properties, phenolic compounds are of great pharmacological significance (Atak & Uslu, 2018).

Turkey is rich in plant and fungal diversity due to its geographical location and climatic variability. The country has a remarkable potential in medicinal and aromatic plants, as confirmed by several studies. In recent years, there has been a significant increase in scientific studies investigating the pharmaceutical, food, and cosmetic applications of these plants.

The genus *Inula*, a member of the Asteraceae family, includes over one hundred species. These plants are mainly distributed in Africa, Asia (with 20 species reported in China), and Europe, particularly in the Mediterranean region. Inula graveolens, an aromatic species of medicinal importance, is widely distributed in the Mediterranean region of Turkey. It grows in a broad range of habitats, from dry, rocky, mountainous areas to moist, shaded lowlands. The species is usually perennial but may also occur as annual plants (Özhan, 2012; Lack, 2007). It is characterized by sticky, hairy stems and yellow flowers, and is valued pharmacologically due to its essential oils and aromatic compounds (Afifia et al., 2015).

Historically, some *Inula* species have been used in folk medicine for treating respiratory diseases such as upper respiratory infections, cough, common cold, and bronchitis. In addition, they have been reported to support the immune system, improve acne, and treat various skin inflammations. Literature also documents their use against urinary tract infections, cystitis, nephritis, and uremia (Kılıç, 2014; Akbulut et al., 2021).

Previous studies reported that extracts of *I. viscosa*, *I. helenium* ssp. *turcoracemosa*, and *I. montbretiana* possess significant antioxidant and antimicrobial activities, attributed to their phenolic composition. Major phenolic compounds identified included chlorogenic acid, caffeic acid, rutin, myricetin, quercetin, luteolin, and kaempferol, with chlorogenic acid, luteolin, and caffeic acid being predominant depending on the species (Gökbulut et al., 2013). Further studies confirmed antiproliferative activity of *I. viscosa* extracts, with methanol extracts showing higher activity than aqueous ones, correlating with their richer phenolic and flavonoid content (Özkan et al., 2019).

For *I. graveolens*, chlorogenic acid (2167 ppb/kg), quinic acid (845 ppb/kg), and hyperoside (146 ppb/kg) were previously reported as major compounds. Mammadov et al. (2023) reported total phenolic and flavonoid contents of methanol extracts as 5.36 mg GAE/g and 3.49 mg QE/g, respectively. Kılıç (2014) identified 1,8-cineole (22.4%), borneol (20.4%), and α-cadinol (11.8%) as major volatile compounds in *I. graveolens*. Afshar et al. (2015) studied the total phenolic, flavonoid, and flavonol contents of *I. graveolens* and their antioxidant activities.

Despite these findings, comprehensive studies on the phenolic profile of I. graveolens remain scarce. Therefore, this study aimed to determine the detailed phenolic composition of flower, aerial, and root extracts of I. graveolens using HPLC-DAD analysis.

2. MATERIALS AND METHOD

2.1. Plant Material

Inula graveolens (L.) Desf. was collected during its flowering stage in late November 2022 from Kızılağaç, Ula district, Muğla Province, Turkey. Approximately 2 kg of plant material was harvested. A voucher specimen has been deposited at the Plant Chemistry Laboratory, Department of Chemistry, Muğla Sıtkı Koçman University.

2.2. Extraction

The plant material was separated into roots, aerial parts, and flowers, which were then dried in a cool, shaded, and well-ventilated place. The dried samples were extracted sequentially with solvents of increasing polarity: ethyl acetate, methanol, and hot water. After maceration, solvents were evaporated under reduced pressure using a rotary evaporator. Aqueous extracts were freeze-dried under vacuum to yield the final crude extracts.

2.3. HPLC-DAD Analysis of Phenolic Compounds

Ethyl acetate, methanol, and aqueous extracts obtained from *I. graveolens* were dissolved in water or alcohol, filtered through 0.20 µm membranes, and analyzed by HPLC-DAD (High-Performance Liquid Chromatography with Diode Array Detection) as described by Küçükaydın et al. (2021). Twenty-six phenolic acid standards were prepared in water and used for profiling. The HPLC-DAD analytical conditions are summarized in Table 1.

Table1: HPLC-DAD Analysis Conditions

HPLC-DAD Instrument Parameters			
Column	C_{18} column (5 µm, 250 mm x 4.6 mm i.d)		
Mobile Phase A	%0,5 CH_3COOH containing H_2O		
Mobile Phase B	MEOH		
Column Oven Temperature	40˚C		
Detector	SPD-M20A DAD detector		
Detector Wavelength	280 nm		
Injection Volume	20 µm		
Analysis Time	55 minutes		
Gradient Program	Time (min)	Flow (mL/min)	%Solvent A
	0,01	1,000	90,00
	5,00	1,000	80,00
	15,00	1,000	70,00
	25,00	1,000	50,00
	30,00	1,000	35,00
	40,00	1,000	25,00
	50,00	1,000	10,00
	55,00	1,000	90,00

3. RESULTS AND DISCUSSION

The phenolic composition of ethyl acetate, methanol, and aqueous extracts obtained from the flowers of *I. graveolens* is presented in Table 2 , while the corresponding HPLC chromatograms are shown in Figure 1. Among the 26 phenolic standards tested, 12 compounds were detected in flower extracts. Eleven compounds were identified in the ethyl acetate extract, seven in the methanol extract, and five in the aqueous extract. Luteolin (11.43 mg/g) and coumarin (8.20 mg/g) were determined as the major compounds in the ethyl acetate extract, whereas coumarin was the predominant phenolic in both methanol (21.65 mg/g) and aqueous (11.43 mg/g) extracts.

The phenolic composition of ethyl acetate, methanol, and aqueous extracts from the aerial parts of *I. graveolens* is summarized in Table 3, with representative HPLC chromatograms shown in Figure 2. A total of 12 compounds were detected in aerial part extracts, including 11 in the ethyl acetate extract, seven in the methanol extract, and five in the aqueous extract. Luteolin (14.78 mg/g), quercetin (11.25 mg/g), and trans-cinnamic acid (7.33 mg/g) were identified as the major compounds in the ethyl acetate extract. In contrast, coumarin (18.73 mg/g in methanol; 10.85 mg/g in water) and pyrocatechol (7.38 mg/g in methanol; 6.59 mg/g in water) were the dominant phenolics in the methanol and aqueous extracts, respectively.

The phenolic composition of ethyl acetate, methanol, and aqueous extracts obtained from the roots of *I. graveolens* is provided in Table 4, with HPLC chromatograms shown in Figure 7. Nine compounds were detected in root extracts, including eight in the ethyl acetate extract, six in the methanol extract, and four in the aqueous extract. Luteolin (6.91 mg/g) was the predominant compound in the ethyl acetate extract, whereas rosmarinic acid (5.20 mg/g) and coumarin (4.63 mg/g) were the main constituents in the methanol extract. In aqueous extracts, pyrocatechol (3.47 mg/g) and coumarin (2.69 mg/g) were identified as the major compounds.

Table 2. Phenolic Profiles of Extracts from *I. graveolens* Flowers

Phenolic Compounds	RT (min)	IGCE	IGCM	IGCS
Protocatechuic acid	8.75	2.51	-	-
Pyrocatechol	11.04	2.36	10.26	7.40
Chlorogenic acid	12.35	0.11	-	-
Syringic acid	16.56	0.32	-	-
p-Coumaric acid	20.56	-	0.42	0.20
Coumarin	24.49	8.20	**21.65**	**11.43**
Rutin	25.30	0.68	3.20	0.39
Rosmarinic acid	26.77	0.71	3.38	-
Quercetin	30.83	5.11	0.18	-
trans-Cinnamic acid	31.33	5.68	0.45	0.12
Luteolin	31.70	**11.43**	-	-
Kaempferol	33.21	0.25	-	-

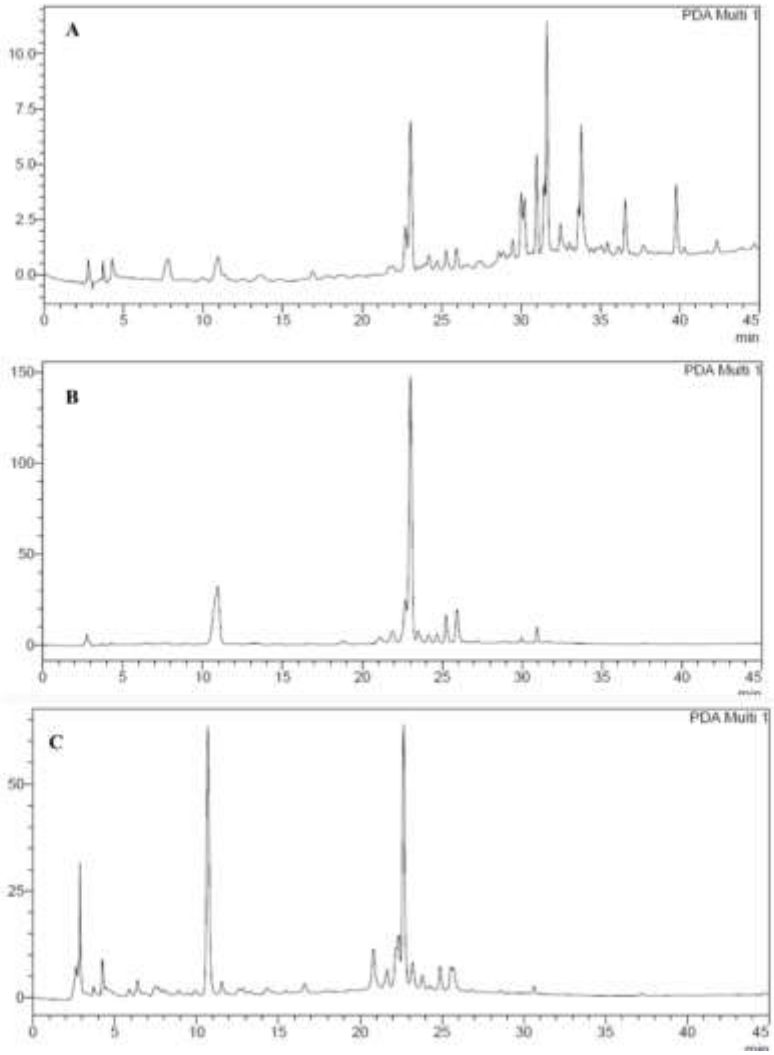

Fig. 1. HPLC chromatograms of *I. graveolens* flower extracts (A: Ethyl acetate, B: Methanol, C: Water)

Table 3. Phenolic Profiles of Extracts from the Above Ground Parts of *I. graveolens*

Phenolic Compounds	RT (min)	IGTE	IGTM	IGTS
Protocatechuic acid	8.75	0.95	-	-
Pyrocatechol	11.04	0.87	7.38	6.59
Chlorogenic acid	12.35	0.07	-	-
Syringic acid	16.56	0.17	-	-
p-Coumaric acid	20.56	-	0.28	0.22
Coumarin	24.49	5.90	**18.73**	**10.85**
Rutin	25.30	0.55	2.56	0.30
Rosmarinic acid	26.77	0.63	2.71	-
Quercetin	30.83	**11.25**	0.11	-
trans-Cinnamic acid	31.33	7.33	0.63	0.10

Luteolin	31.70	**14.78**	-	-
Kaempferol	33.21	0.38	-	-

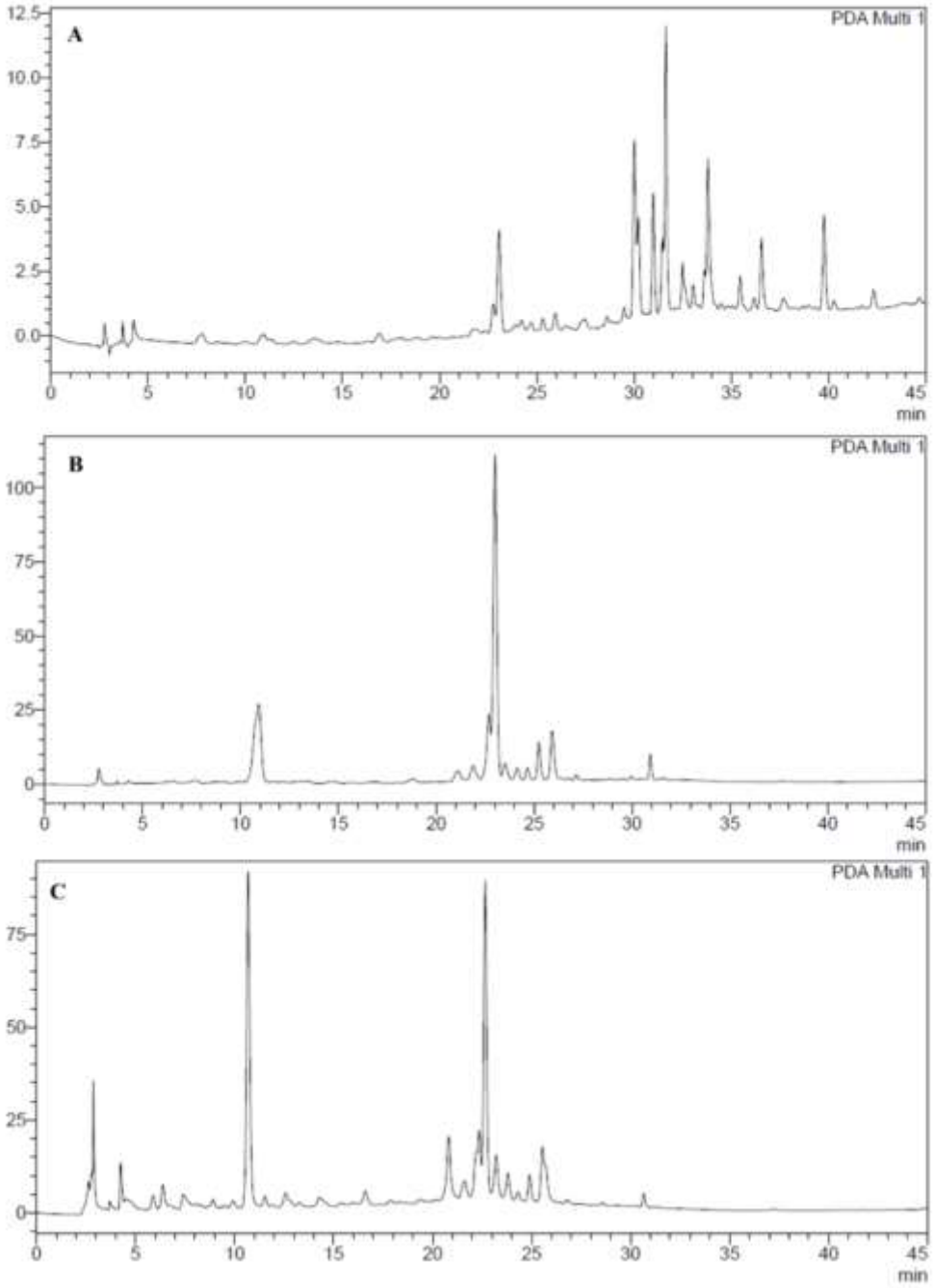

Fig. 2. HPLC chromatograms of *I. graveolens* aerial part extracts (A: Ethyl acetate, B: Methanol, C: Water)

Table 4. Phenolic Profiles of Extracts from I. graveolens Root Parts

Phenolic Compounds	RT (min)	IGKE	IGKM	IGKS
Protocatechuic acid	8.75	0.10	-	-
Pyrocatechol	11.04	0.22	3.15	**3.47**
Syringic acid	16.56	1.15	-	-
p-Coumaric acid	20.56	-	3.86	0.85
Coumarin	24.49	0.51	4.63	2.69
Rutin	25.30	0.35	0.65	0.22
Rosmarinic acid	26.77	0.32	**5.20**	-
trans-Cinnamic acid	31.33	0.96	0.23	-
Luteolin	31.70	**6.91**	-	-

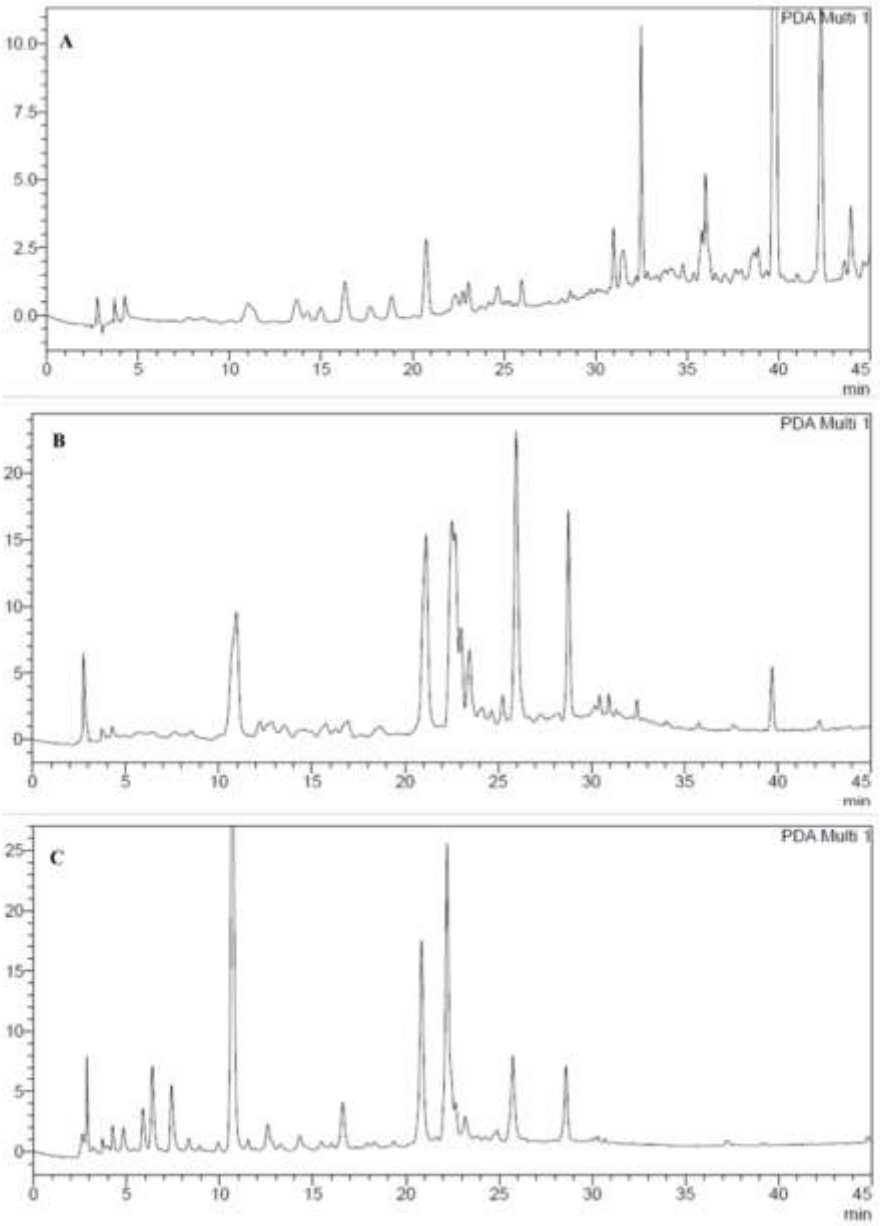

Fig. 3. HPLC chromatograms of *I. graveolens* root extracts (A: Ethyl acetate, B: Methanol, C: Water)

4. CONCLUSION

This study provides a comprehensive analysis of the phenolic composition of Inula graveolens (L.) Desf. using HPLC-DAD. The results demonstrated that different parts of the plant (flowers, aerial parts, and roots) contain diverse phenolic profiles, with luteolin, coumarin, quercetin, rosmarinic acid, and pyrocatechol identified as the major compounds depending on the extraction solvent. These findings emphasize the solvent-dependent variability in phenolic contents and highlight the importance of optimized extraction techniques for phytochemical studies.

The relatively high levels of luteolin and coumarin, along with other bioactive phenolics, suggest that I. graveolens could be a promising natural source of antioxidant and pharmacological agents. This

study contributes valuable insights into the chemical composition of I. graveolens and provides a foundation for future research aimed at evaluating its biological activities and potential applications in pharmaceutical, nutraceutical, and cosmetic formulations.

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IN VITRO A-AMYLASE AND A-GLUCOSIDASE INHIBITORY ACTIVITIES *OF INULA GRAVEOLENS* EXTRACTS

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ABSTRACT

Inula graveolens (L), an aromatic medicinal plant belonging to the Asteracede family, is widely distributed in Europe, Africa, and Asia and is commonly found in the Mediterranean region of Türkiye. This endemic species has long been used in folk medicine for the treatment of respiratory tract infections, coughs, colds, and bronchitis. The present study aimed to evaluate the in vitro antidiabetic potential of flower and aerial part extracts of l. graveolens by assessing their inhibitory effects on a-amylase and a-glucosidase enzymes. Plant materials were separated into flowers and aerial parts, dried, powdered, and sequentially extracted with ethy acetate, methanol, and water. The inhibitory activities of the obtained extracts were determined spectrophotometrically and compared with the standard drug acarbose. At a concentration of 200 (µg /ml, ethyl acetate extracts of aerial parts and flowers exhibited 52.86 ±0.71% and 45.27 ± 0.23% inhibition against a-amylase, respectively, while acarbose showed 80.56 0.52% inhibition at the same concentration. Similarly, the highest inhibition against a-glucosidase was recorded for ethyl acetate extracts of flowers and aerial parts, with 70.20 ± 0.82% and 75.35 ±0.86% inhibition, respectively. These values were higher than that of acarbose (67.30 ±0.45%), indicating superior activity. Methanol extracts also demonstrated notable activity, with inhibition rates of 61.63 ±0.45% and 58.30 ±0.62% for flowers and aerial parts, respectively. These findings highlight that ethyl acetate extracts, particularly from the flower and aerial parts, exhibit remarkable a-glucosidase inhibitory activity, surpassing even the standard drug acarbose. The extracts also showed considerable a-amylase inhibition, following a similar trend. Overall, *I graveolens* demonstrates promising potential as a natural source of antidiabetic agents, warranting further bio-guided isolation studies and mechanistic investigations.

Keywords: *Inula graveolens,* Asteraceae, α-amylase, α-glucosidase, enzyme inhibition,antidiyabetic activity

1. INTRODUCTION

The Asteraceae (Composite) family comprises more than 1500 genera and approximately 23,000 species worldwide (Judd et al., 2007). In Türkiye, this family is represented by about 140 genera and nearly 1186 species. The genus *Inula*, a member of the Asteraceae family, is widely distributed in Europe, Africa, and Asia (Lack, 2007) and is especially common in the Mediterranean region of

Türkiye (Ertem, 2023). The genus includes 33 different species, eight of which are endemic to Türkiye, corresponding to an endemism rate of 28.5% (Güner et al., 2012).

Inula graveolens typically grows along roadsides, riverbanks, and arid areas, and is widespread in the Mediterranean region of Türkiye. It is an annual plant that can reach up to 80 cm in height, characterized by sticky, hairy stems and yellow flowers, containing aromatic compounds and essential oils (Afifia et al., 2015). Traditionally, *Inula* species have been used for treating upper respiratory tract infections, coughs, colds, and bronchitis. They are also reported to support the immune system, alleviate acne, and reduce skin inflammations (Oshadi, 2005). Furthermore, their use in treating urinary tract infections, cystitis, nephritis, and uremia has been documented (Kılıç, 2014; Akbulut et al., 2021).

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia resulting from insulin deficiency or resistance, and its prevalence is increasing globally (Kaveeshwar & Cornwall, 2014; Atkinson et al., 2014; Huang et al., 2016; Yan et al., 2019; Chinsembu, 2019). According to the World Health Organization (WHO, 2020), the number of diabetic patients increased from 108 million in 1980 to 422 million in 2014, with 1.6 million diabetes-related deaths reported in 2016. The International Diabetes Federation (IDF, 2019) estimated that the number of diabetic adults was 463 million in 2019 and is projected to reach 700 million by 2045. Type 1 diabetes is most common in childhood and results from autoimmune destruction of pancreatic β-cells, while type 2 diabetes, representing 90–95% of cases, is characterized by insulin resistance and β-cell dysfunction (Fan, 2017). Current treatments aim at controlling hyperglycemia. Insulin injections are mandatory for type 1 diabetes, while type 2 diabetes is managed through lifestyle modifications and oral antidiabetic agents (Aziz, 2012). Natural plant polyphenols have attracted attention due to their antioxidant properties and ability to inhibit digestive enzymes such as α-amylase and α-glucosidase, thereby delaying intestinal glucose absorption and contributing to glycemic control. Indeed, commonly used antidiabetic drugs such as acarbose and voglibose act via this mechanism (Xu, 2024).

Although several studies have explored the antiproliferative, antioxidant, and enzyme inhibitory activities of different Inula species such as *I. viscosa*, *I. britannica*, and *I. discoidea* (Özkan et al., 2019; Khan et al., 2010; Ceylan et al., 2021), little information is available regarding the antidiabetic potential of *I. graveolens*. Therefore, the present study aimed to evaluate the in vitro antidiabetic activity of hexane, ethyl acetate, methanol, and aqueous extracts prepared from the leaves, roots, and flowers of *I. graveolens* by assessing their inhibitory effects on α-amylase and α-glucosidase enzymes

2. MATERIALS AND METHODS

2.1.Plant Material

Inula graveolens (L.) Desf. was collected during its flowering stage in late November 2022 from Kızılağaç, Ula district, Muğla Province, Türkiye. Approximately 2 kg of plant material was harvested. A voucher specimen has been deposited at the Plant Chemistry Laboratory, Department of Chemistry, Muğla Sıtkı Koçman University.

2.2 Extraction

The plant material was separated into roots, aerial parts, and flowers, dried under shade in a cool and ventilated environment, and extracted sequentially with solvents of increasing polarity: hexane, ethyl acetate, methanol, and hot water. After maceration, organic solvents were evaporated under reduced pressure using a rotary evaporator, while aqueous extracts were freeze-dried under vacuum.

2.3. Determination of Antidiabetic Activity

Hexane, ethyl acetate, methanol, and aqueous extracts were dissolved in dimethyl sulfoxide (DMSO) or distilled water, depending on their solubility, and subjected to in vitro α-amylase and α-glucosidase inhibitory assays as described below

2.3.1.α-Amylase Inhibition Assay

The α-amylase inhibitory activity of the extracts was determined following the method of Quan et al. (2019), with slight modifications. The reaction mixture consisted of 25 µL of extract solution at different concentrations and 50 µL of α-amylase solution (0.1 U/mL in 20 mM phosphate buffer, pH 6.9, containing 6 mM NaCl). After pre-incubation at 37 °C for 10 min, 50 µL of 0.05% starch solution was added and incubated for another 10 min. The reaction was stopped by adding 25 µL of 0.1 M HCl and 100 µL of iodine solution. Absorbance was measured at 565 nm.

2.3.2. α-Glucosidase Inhibition Assay

The α-glucosidase inhibitory activity was determined according to the method of Kim et al. (2000), with modifications. The assay mixture contained 50 µL of 0.01 M phosphate buffer (pH 6.9), 25 µL of 0.5 mM p-nitrophenyl-α-D-glucopyranoside (PNPG), 10 µL of extract solution, and 25 µL of α-glucosidase (0.1 U/mL in 0.01 M phosphate buffer, pH 6.0). After incubation at 37 °C for 20 min, the reaction was terminated by adding 90 µL of 0.1 M sodium carbonate. The release of p-nitrophenol was measured at 400 nm..

3.RESULTS AND DISCUSSION

The α-amylase inhibitory activities of the extracts are presented in Table 1. As shown in the table, the ethyl acetate extract of the aerial parts of *I. graveolens* exhibited the highest activity, with an inhibition value of 52.86 ± 0.71% at a concentration of 200 µg/mL, followed by the ethyl acetate extract of flowers with 45.27 ± 0.23% inhibition. Compared to the reference compound acarbose, the extracts displayed lower α-amylase inhibitory activity overall. Nevertheless, the inhibition values of both aerial and flower ethyl acetate extracts at 200 µg/mL were comparable to that of acarbose at 25 µg/mL, suggesting that these two extracts may possess promising activity.

The α-glucosidase inhibitory activities of the extracts are shown in Table 2. Among the tested samples, the ethyl acetate extracts of flowers and aerial parts exhibited the highest inhibition against α-glucosidase, with activities comparable to the standard drug acarbose. At a concentration of 200 µg/mL, the aerial and flower ethyl acetate extracts demonstrated inhibition rates of 75.35 ± 0.86% and 70.20 ± 0.82%, respectively, while acarbose showed 78.26 ± 0.35% inhibition at the same concentration. Following these, methanol extracts of flowers and aerial parts exhibited considerable activity, with inhibition rates of 61.63 ± 0.45% and 58.30 ± 0.62%, respectively. In line with the α-amylase inhibition results, the root extracts showed the lowest inhibitory activity against α-glucosidase.

Table 1. α-Amylase inhibitory activities of *Inula graveolens* extracts (% inhibition)

Extracts/Standard	25 µg/mL	50 µg/mL	100 µg/mL	200 µg/mL
IGCE	-	19.22±0.30	31.51±0.16	45.27±0.23
IGCM	-	-	17.80±0.43	30.56±0.75
IGCS	-	-	-	12.96±0.11
IGTE	-	22.28±0.14	36.04±0.27	52.86±0.71
IGTM	-	-	18.59±0.16	32.90±0.25
IGTS	-	-	-	13.50±0.60
IGKE	-		-	28.78±0.45
IGKM	-	-	-	21.63±0.15
IGKS	-	-	-	10.50±0.12
Acarbose	46.30±0.37	60.51±0.28	76.38±0.43	87.52±0.77

Table 2. α-Glucosidase inhibitory activities of *Inula graveolens* extracts (% inhibition).

Extracts/Standard	50 µg/mL	100 µg/mL	200 µg/mL	400 µg/mL
IGCE	25.36±0.85	41.05±0.70	56.20±0.60	70.20±0.82
IGCM	18.36±0.42	29.11±0.53	45.27±0.73	61.63±0.45
IGCS	-	12.05±0.22	19.21±0.42	31.70±0.51
IGTE	27.80±0.57	45.20±0.31	59.03±0.75	75.35±0.86
IGTM	15.43±0.28	25.74±0.57	42.09±0.35	58.30±0.62
IGTS	-	13.19±0.86	22.36±0.28	35.69±0.70
IGKE	-	17.48±0.65	28.01±0.70	43.21±0.98
IGKM	-	-	15.27±0.30	26.33±0.92
IGKS	-	-	11.07±0.15	19.05±0.54

Acarbose	32.55±0.26	47.20±0.45	62.40±0.50	78.26±0.35

4.CONCLUSION

This study demonstrated that ethyl acetate extracts of the flowers and aerial parts of *Inula graveolens* exhibited the strongest α-glucosidase inhibitory activity, surpassing even the standard drug acarbose at certain concentrations. Methanol extracts also showed considerable inhibitory effects, while root extracts were the least active. Parallel trends were observed in α-amylase inhibition. Overall, these findings indicate that *I. graveolens* may serve as a promising natural source of antidiabetic agents. Further bioassay-guided studies and mechanistic investigations are required to isolate active compounds and evaluate their pharmacological potential.

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KNOWLEDGE ABOUT THE GENETICAL, MORPHOLOGICAL AND PHENOLOGICAL DIVERSITY OF THREE LINDEN SPECIES (*TILIA CORDATA* MILL., *TILIA PLATYPHYLLOS* SCOP. AND *TILIA TOMENTOSA* MOENCH.) IN BOSNIA AND HERZEGOVINA AND POTENTIAL FOR BETTER MANAGEMENT OF LINDEN FORESTS

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ABSTRACT

The paper presents an analysis of the first report on molecular studies of the *Tilia* genus complex in Bosnia and Herzegovina, with brief information on some measured morphological parameters of leaves in lindens in Bosnia and Herzegovina. Based on the literature search process on the genetic diversity of the species *T. cordata* Mill., *T. platyphyllos* Scop. and *T. tomentosa* Moench., it was noted that genetic studies had not been conducted before 2020. As part of the study of the genetic diversity of the three dominant linden species from Bosnia and Herzegovina, a significant level of diversity was discovered. However, it seems that the full results of the research have not been published and that only some partial results have been published, and that on the website of the research sponsor, which is the *German Federal Environmental Foundation*. It is worth noting that the research focuses on the species *T. tomentosa*, which has been identified as the "tree of the future". Resistance to high droughts and lack of moisture in the soil makes the silver-leaved linden very valuable for establishing commercial plantations in the future, but also important for the survival of beneficial insect species and strengthening overall biodiversity. This tree can significantly help improve the climate of cities and municipalities where linden trees are planted, as they provide shade and protection from harmful ultraviolet rays for human health. Since climate change significantly threatens to disrupt the balance of everyday life, wood markets and local economic conditions, further research into the benefits of linden trees is very necessary for the forestry industry and for the general good of the bosnian-herzegovinian community. To this end, it is of great importance to present to the public that there is molecular research on the genus *Tilia* in Bosnia and Herzegovina and that it indicates that linden can be used for various purposes, as well as that it has a prosperous future in planting.

Keywords: *Tilia cordata, Tilia platyphyllos, Tilia tomentosa*, lime forests, Bosnia and Herzegovina, lime morphology, lime genetics

INTRODUCTION

At the beginning of research on the genus *Tilia* in Bosnia and Herzegovina, it was realized that no molecular studies had been conducted until 2020. The main reason for this is that either there was not enough interest in researching lindens, as they were mainly used as trees for establishing alleys and parks, or there were no funds available to conduct any genetic research at

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that level. According to an **Oral statement by Ballian (2016)**, the main reason for the lack of research on lindens is that they are very complicated for molecular analysis.

According to the **Oral Statement of Sejdić (2020)**, the then director of the *Public Enterprise* "Bosanskohercegovačke šume" in Sarajevo, there were several linden research plots in the Konjic Municipality in Bosnia and Herzegovina, where exclusively phenological observations were conducted in the context of fruiting of trees and potential for honey production and beekeeping. According to **Vejzagić & Omerbegović-Arapović (2025)**, linden trees are very important for considering the establishment of mass commercial plantations, because they can help maintain honey production and help the sustainability of insect populations in forests. The authors state that the exceptional benefits of linden have been proven in the production of not only honey, but also wood pulp, medical preparations, but also cosmetics, all of which have their own significant place in the market for products derived from wood pulp, or forestry.

According to **DBU.de (2021)**, *T. cordata* trees are very difficult to distinguish from *T. platyphyllos* trees in the field, making it difficult to confirm which species they are without molecular identification. The report states that *T. platyphyllos* had to adapt morphologically to new climatic conditions, starting as early as 9,000 years ago. Namely, since the climate has changed significantly in the Scandinavian countries in the past, there has been a significant drop in temperatures in the natural habitats of *T. cordata* and *T. platyphyllos*. With the average temperature falling below 15 degrees Celsius, it has become very difficult for *T. platyphyllos* seeds to germinate. Therefore, the fragmentation of the habitats of *T. cordata* and *T. platyphyllos* began due to climatic factors, and/; species such as *T. platyphyllos* and *T. tomentosa* began to seek their last refuges in more southern habitats, heading towards the very coasts of the Balkan Peninsula (*T. tomentosa*).

The results of research on several forest tree species in Bosnia and Herzegovina and the surrounding countries have shown that, depending on the type of climate, altitude, exposure and vegetation type, there is a significant morphological diversity of species (**Ballian et *al.*, 2017**, **Franjić**, **1994**, **Kvesić**, **2019**, **Hajrudinović-Bogunić**, **2023**). This has been written about by prominent forestry experts and authors of the most important forestry university textbooks in Bosnia and Herzegovina and the surrounding area as part of the description of morphological characteristics and habitat conditions (**Mekić**, **1998**).

The aim of this review is to comment on the main results of molecular and scarce morphological research conducted by a researcher on three linden species in Bosnia and Herzegovina, *T. cordata* Mill., *T. platyphyllos* Scop. and *T. tomentosa* Moench., within the framework of the "MOE-Scholarships" scholarship program in Germany. The Report **(DBU.de, 2021)** notes that the research was carried out in two German institutes dealing with *Forest Genetics*. The research is very interesting from the genetics aspect, especially because it provides information that in Bosnia and Herzegovina, in certain locations, there are trees that have started to develop different fruits, and the discovery was published in the *Forestry Journal of the Faculty*

of Forestry, University of Banja Luka, Bosnia and Herzegovina (**Vejzagić**, **2022**). In the following, comments will be presented on all described results about lindens.

MATERIAL AND METHOD

This review article contains information on the morphological and molecular results of the first comprehensive study of the three dominant linden species in Bosnia and Herzegovina: *T. cordata* Mill., *T. platyphyllos* Scop. and *T. tomentosa* Moench. The report with the results of the study was published on the official website of the *German Federal Environmental Foundation* in 2021, and is written in German. A literature review on the scientific review conducted revealed that no previous articles with a complete overview of molecular and morphological results have been published on this study in any journal. Therefore, one of the goals of this review article is to point out the importance of publishing the overall molecular and morphological results in a genetic journal. In order to obtain additional information about what scientific research on lime trees in Bosnia and Herzegovina has been conducted in addition to this, the following key terms were used in a *Google* search: 1) *Tilia in Bosnia and Herzegovina*, 2) *Tilia cordata in Bosnia and Herzegovina*, 3) *Tilia platyphyllos in Bosnia and Herzegovina*, 4) *Tilia tomentosa in Bosnia and Herzegovina*, 5) *lime forests in Bosnia and Herzegovina*, 6) *Bosnian lime morphology* and 7) *Bosnian lime genetics*.

This review article used a meta-analysis of the available: 1. Scientific research report entitled **DBU.de (2021)**: "Morphological, phenological and genetic characteristics of linden stand structures (*Tilia cordata* Mill., *Tilia platyphyllos* Scop. and *Tilia tomentosa*)", as well as 2. The following three peer-reviewed papers deal with morphological and/or phenological research on the three main linden species in Bosnia and Herzegovina, as follows: a) **Tomić et *al*. (2014):** "FENOLOGIJA CVJETANJA TRI VRSTE LIPE (Tilia sp.) NA PODRUČJU GRADA BANJA LUKE" (engl.: *FLOWERING PHENOLOGY OF THREE SPECIES OF LINDEN (Tilia sp.) IN THE AREA OF THE CITY OF BANJA LUKE*), b) **Vejzagić et *al*. (2021):** "Preliminary research of morphological traits of silver linden (*Tilia tomentosa* Moench.) leaves in Bosnia and Herzegovina and Serbia", and c) **Vejzagić, S. (2022):** "On the discovery of the multibracteate silver-leaved linden (*Tilia tomentosa Moench*.) IZ MOSTAR, BOSNIA AND HERZEGOVINA".

In accordance with the available research material, the analysis and discussion of the results is planned in the following three *sub*-chapters: 1) Molecular investigations of the *Tilia* complex in Bosnia and Herzegovina, 2) Morphological analysis of *T. cordata* Mill., *T. platyphyllos* Scop. and *T. tomentosa* Moench. from Bosnia and Herzegovina, i 3) Other relevant investigations within the *Tilia* complex in Bosnia and Herzegovina.

RESULTS AND DISCUSSION

The results of the meta-analysis will be presented in the following three brief sub-chapters, with help of tables (for better clarity). The first *sub*-chapter consists of the main part

of the text, because the given (analysed) report on molecular results in *Tilia* is much more abundant with molecular results. The second *sub*-chapter is similar but shorter, because it consists of the very brief results on a few morphometric parameters and findings of the

morphological analysis of the leaves in T. tomentosa in separate research **(Vejzagić et *al*., 2021)**. The third *sub*-chapter consist almost no results about the phenology findings in *Tilia* in scope of German institutes conducted research **(DBU.de, 2021)**, but it focuses more on the phenological results of **Tomić et *al*. (2014)** and it mentions discovery of a multibracteal linden fruit in Mostar, Bosnia and Herzegovina. The reason why mentioning this discovery was planned in a third and not in second sub-chapter is to avoid mentioning it twice, and to emphasize the importance of conducting phenological research in the future, by seeking for additional data about existence of the multibracteal fruit in other locations.

Molecular investigations of the *Tilia* complex in Bosnia and Herzegovina

This *sub*-section reviews the most important information from the *DBU* report on molecular research of the *Tilia* complex in Bosnia and Herzegovina. A literature review has established that the report published in 2021 is the first of its kind to address the molecular characterization of linden in that country.

According to the **DBU.de (2021)**, a complex study of the three dominant linden species in Bosnia and Herzegovina was carried out due to the great ecological importance of *Tilia cordata* Mill. (abbreviated as *T. cordata*), *Tilia platyphyllos* Scop. (abbreviated as *T. platyphyllos*) and *Tilia tomentosa* Moench. (abbreviated as *T. tomentosa*) and due to the fact that the genetic and morphological variability of these species attracts great interest from the scientific community. The linden is a tree that is becoming increasingly important in the context of climate change, as it is believed to have the ability to adapt to dry habitats. Therefore, the research focused on molecular analysis but also typification from the aspect of the climate type prevailing in the habitats in which the linden trees were sampled, then from the aspect of altitude, soil type, ecological type of vegetation and tree exposure. One of the goals of the research was to identify potential linden hybrids in nature, possibly new varieties, breeds, etc. **(DBU.de, 2021)**.

The study included a total of 317 trees morphologically identified as *T. cordata*, *T. platyphyllos* and *T. tomentosa*, from several locations in Bosnia and Herzegovina: 1) *Kladanj*, 2) *Srebrenik*, 3) *Čajniče*, 4) *Bosanski Petrovac*, 5) *Kakanj*, 6) *Sarajevo*, 7) *Tešanj*, 8) *Bosansko Grahovo*, 9) *Livno*, 10) *Posušje*, 11) *Mostar*, 12) *Tomislavgrad* and 13) *Tomislavgrad Kovač*. The study relied on data from the author **Herman (1971)**, who described where linden trees grow and how to identify them in the field. The report states that, in addition to populations, so-called meta-populations were formed in the research, in order to cover as much of the territory of Bosnia and Herzegovina as possible (a map of locations is provided), and describes that meta-populations are those that include linden trees from different habitats, which are nevertheless similar in terms of ecological and biological growth conditions.

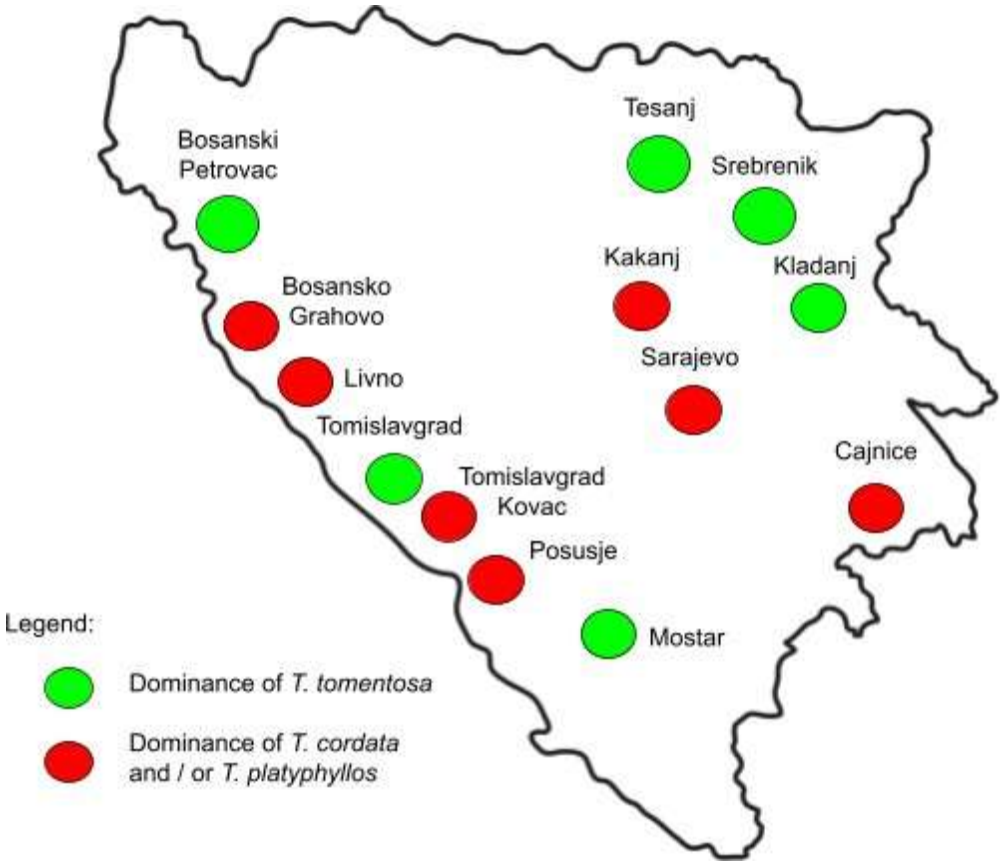

Image 1. Map of the locations of sampled linden trees according to the reviewed report.

The report states that three populations from Germany were taken as *Control* populations, but we decided not to list them, because we want to focus on the molecular results of trees exclusively from Bosnia and Herzegovina. It is important to note that the researcher used *DNA* that he extracted from leaves, twigs (wood) and buds of small-leaved (*T. cordata*), large-leaved (*T. platyphyllos*) and silver-leaved (*T. tomentosa*) linden, which he morphologically identified in the field, and then established their species affiliation using *DNA* analysis.

The importance of the first molecular study for understanding the *Tilia* complex in Bosnia and Herzegovina is best demonstrated by the fact that it is one of the few studies conducted in Europe after the study by **Phuekvilai & Wolff (2013)**, who worked on the characterization of microsatellite loci in *T. platyphyllos* trees and on cross-amplification in related species. They then used a set of 15 microsatellite markers, in which most loci showed a high degree of polymorphism in two populations of *T. platyphyllos*. The results of cross-amplification showed that as many as 12 of the 15 loci were amplified polymorphically in as many as 23 linden species. The research conducted as part of the *MOE*-Scholarship is also useful from the aspect of saving chemicals, because it was found that in the analysis of a large number of *T. cordata*, *T. platyphyllos* and *T. tomentosa* trees from Bosnia and Herzegovina, the *Tc918* marker (locus) only functions in *T. platyphyllos.*

Table 1. Overview of molecular results of genetic analyzes of three linden species (*T. cordata* Mill., *T. platyphyllos* Scop. and *T. tomentosa* Moench.) in Bosnia and Herzegovina **(DBU.de, 2021)**

Research method and focus	Overview of the most important molecular results
The *CTAB* protocol was used to extract *DNA* from	Results from microsatellite analysis showed that the *Tc918* locus was functional only in *T. platyphyllos*. It was therefore omitted
buds, leaves and twigs (wood) of three linden species, which were initially identified as small-leaved (*T. cordata*), large-leaved (*T. platyphyllos*) and silver-leaved (*T. tomentosa*) linden based on macroscopic characteristics in the field. Trees were sampled from 13 (meta)populations from all over Bosnia and Herzegovina, trying to cover as many different habitats as possible, which are characterized by different climatic characteristics, vegetation type, altitude, exposure, slope, as well as the type of soil on which the trees grow. The *DNA* content was measured randomly with a photometer and diluted to approximately 20 *ng*/*µl*. The report states that this solution was subsequently available for *PCR* reactions, using the *Qiagen multiplex* kit. The study was conducted using a set of 12 nuclear microsatellite loci (*Tc5*, *Tc31*, *Tc6*, *Tc7*, *Tc4*, *Tc8*, *Tc915*, *Tc963*, *Tc951*, *Tc918*, *Tc920*, *Tc11*), according to the method of **Phuekvilai & Wolff (2013)**	from the calculations of genetic differences between species and locations. The remaining 11 out of 12 microsatellites were used to characterize populations and identify species and potential hybrids. The application of microsatellite on *T. tomentosa* samples showed that some populations are clearly separated or isolated from others, as is the case with the population from the Srebrenik site. Molecular analyses indicate that the *T. tomentosa* population from Mostar, which grows in a thermophilic habitat, is genetically more similar, or even coincides with a group of *T. tomentosa* populations growing in northwestern Bosnia, in continental and mesophilic conditions. Although it was expected that the mesophilic populations of *T. tomentosa* from northwestern Bosnia would have a stronger genetic influence on the continental populations of *T. tomentosa* from northeastern Bosnia (the Srebrenik and Kladanj sites), this was not the case. The analyses also showed that the existence of potential hybrids resulting from crossing *T. tomentosa*, as well as the occurrence of genetically identical trees, is present. The occurrence of clonal reproduction is part of the question of what are the main reasons for the occurrence of a large number of genetically identical trees. The need for more detailed analyses was indicated by the fact that a certain number of trees identified as *T. cordata* (based on morphological characteristics) turned out to be *T. platyphyllos* (after performing genetic analysis identification), and that a certain number of trees identified as *T. platyphyllos* (also based on morphological characteristics) turned out to be *T. tomentosa*. Deeper analyses are particularly important because trees identified as *T. tomentosa* were also observed, which have morphological characteristics of the fruits of all three dominant linden species in Bosnia and Herzegovina (*T. cordata*, *T.*

and using 6 *cpDNA* microsatellites (*ccmp3*, *ccmp4*, *ccmp5*, *ccmp6*, *ccmp10* and *μkk3*), according to the method of	*platyphyllos* and *T. tomentosa*). The results of the genetic analysis of three linden species from Bosnia and Herzegovina show the existence of clearly separated clusters of *T. cordata*. Certain populations are, however, very similar and form a common cluster. The researcher concludes that there are clear boundaries that prevent populations from networking through gene flow. The researcher compares populations from Bosansko Grahovo and Livno, where it is observed that the peripheral trees of the populations are very close to each other. Comparing the population from Tomislavgrad with the population from Čajniče, it is observed that the two populations are genetically distinct
Weising & Gardner, 1998. The report states that the research fellow conducted the analyses at the Institute for Applied Forest Genetics "AWG" in Bavaria, Germany, and at the Forestry Institute "FVA-BW" in Germany. The researcher focused on determining the tree species involved and the *intra*- and *inter*-population variability of the populations. The analytical programs *Software Structure 2.2* (according to **Pritchard et *al*., 2000**) and Software *Genalex* and *Alrequin* were used for the genetic analyses.	The results of the analysis obtained using the *STRUCTURE* software have allegedly changed the initial picture of the Kladanj population. According to the initial morphological identification, the trees sampled in Kladanj were *T. tomentosa*, however, genetic analysis determined that they were *T. cordata* and *T. platyphyllos* trees. The final considerations of the report on the genetic results obtained on the *Tilia* complex in Bosnia and Herzegovina state that *T. tomentosa* is an important species for understanding the process of fragmentation of lime forests in Southeast Europe and the process of climate change in the context of forest macroclimate. This makes the study of lime significant from the perspective of bioclimatology, climatology, ecology, dendrology and genetics, because it demonstrates how these different scientific disciplines can synergistically explain the overall picture of the evolutionary development of *T. cordata*, *T. platyphyllos* and *T. tomentosa*.

Lobo et *al*. (2018) studied the local adaptation of highly fragmented populations of *T. cordata* and found that populations with a high degree of genetic variation have the ability to respond to selection based on their high degree of heritability. This ultimately indicates the existence of adaptive potential, or plasticity, which allows species to adapt. However, the researchers did not rule out the influence of epigenetics on the creation of local adaptation. It is noted that the research conducted by **Lobo et *al*. (2018)** is interesting for linden research in Bosnia and Herzegovina, since the report puts them in the focus of trees of the future, especially from the aspect of adaptation to climate change.

The **DBU.de (2021)** report indicates that there are populations that are isolated from the rest, and that there are populations that share similarities with geographically distant populations. This is the case with the population from Srebrenik (*T. tomentosa*), which does not have much

similarity with the population from Mostar (*T. tomentosa*), but the population from Mostar is more closely related to the population from Bosanski Petrovac (*T. tomentosa* and other lime species). Research conducted by **Logan et *al*. (2015)** in the United Kingdom indicates the existence of distinct but also shared gene pools. In order to determine the detailed differences in the genetic structure of populations from Bosnia and Herzegovina, it is necessary to publish the results of the overall research project in the future.

Morphological analysis of *T. cordata* Mill., *T. platyphyllos* Scop. and *T. tomentosa* Moench. from Bosnia and Herzegovina

In addition to genetic analysis, the study included morphological analysis of 6340 leaves and 12640 linden fruits.

Table 2. Overview of the results of morphological analyzes of three linden species (*T. cordata* Mill., *T. platyphyllos* Scop. and *T. tomentosa* Moench.) in Bosnia and Herzegovina **(DBU.de, 2021)**

Research method and focus	Overview of the most important morphological results
In morphological analyses of measured parameters of leaves and fruits, a modified standard method for morphological measurements of forest species in the Balkan region was used, created by Prof. Dr. Franjic, from the *Faculty of Forestry, University of Zagreb*. The methodology for measuring linden fruits was considered because in Bosnia and Herzegovina, silver-leaved linden (*T. tomentosa*) fruits with more than one wing (*bracteum*), i.e. fruits with primary, secondary and tertiary rows of bracts, occur. The following parameters were measured in leaves: 1) *leaf length*, 2) *leaf width*, 3) *central nerve length*, 4) *angle of intersection of the first developed right nerve with the leaf base*, 5)	The results of morphological measurements are given very sparsely, and only a few obtained values are stated. The report states that as part of the measurement of morphological parameters of linden in Bosnia and Herzegovina, the longest leaf petiole was measured in *T. tomentosa*, and that in Mostar. The Mostar population of silver-leaved linden is characterized by thermophilic vegetation and the presence of a *sub*-Mediterranean climate. The average petiole length for the entire population is 32.21 mm. As for the shortest leaf petiole measured, it was measured in the linden metapopulation sampled in the area of Tomislavgrad and Čapljina. The average length of the leaf

peduncle length, 6) *number of teeth on each 1 cm of length*, 7) *type of hairiness* and 8) *type of serration*. The following traits were measured in fruits: 1) *peduncle length*, 2) *length of primary, secondary, and tertiary wings*, 3) *length of the base of primary wings, 4) separation position*, 5) *seed width*, 6) *seed length, and number of ridges*.	petiole was measured for the entire metapopulation and is 23.12 mm. At the end of presenting these results, the researcher states that the morphological measurement patterns created based on morphological measurement methods in Croatia and Bosnia and Herzegovina yielded mostly expected results (patterns of morphological forms and characteristic leaf dimensions in individual climatic regions).

From the available data of the **DBU.de (2021)** report, it is observed that only two results for the average leaf petiole length are specifically stated, namely for the population from Mostar and the meta-population *Tomislavgrad-Kovac*. In the population from Mostar, the average leaf petiole length for the entire population was recorded as 32.21 mm, while for the metapopulation that is represented with the average value of 23.12 mm. Accordingly, the average leaf petiole length is as much as 28.22 % shorter in the *Tomislavgrad-Kovac* metapopulation compared to the population from Mostar.

When reviewing the results of the morphological analysis of *T. tomentosa* trees in Bosnia and Herzegovina, conducted by **Vejzagić et *al*., (2021)**, the results of several morphological parameters of leaves are observed compared to the *DBU* report. The study measured the morphological properties of silver-leaved linden leaves from the Sana River Valley (two locations) and from the Neretva River Canyon (two locations). As Control populations, the researchers took two populations from two locations in the Republic of Serbia, but we will not describe them in detail here because we want to focus on domestic / bosnian-herzegovinian populations. The researchers sampled 30 trees per location and 30 leaves per individual tree.

The results obtained by morphological measurements are: 1) The average value of the length of the petiole for all populations, which is 40.3 mm, 2) The average length of the leaf, which is 111.0 mm, 3) The average width of the leaf, which is 90.7 mm, 4) The average distance of the first right developed vein from the base of the leaf, which is 0.3 mm, 5) The average angle of the first right developed vein that closes with with the central vein, which is 47°7’, 6) Average length of the central vein, which is 90.4 mm, 7) Average *length*/*width* ratio of the blade, which is 1.3, 8) Average *width*/*length* ratio of the blade, which is 0.8, 9) Average total length of the central blade with petiole, which is 130.7 mm, 10) Average length midrib *versus* petiole length, which is 2.5, 11) The average length of the petiole / the total length of the central vein and the petiole, which is 0.3, as well as 13) The number of primary teeth *per* 1 centimeter of the length of the leaf edge (the largest number of individuals had two primary teeth *per* 1 cm of the length of the leaf edge).

Analysis of variance for leaf morphological properties indicates the existence of statistically significant differences for all measured leaf properties by population, while Duncan's test showed the separation of all populations into separate groups for the majority of measured properties. Therefore, from the data that can be compared, it is visible that in the **DBU.de (2021)** research the average length of the leaf petiole of all measured trees reached 32.21 mm (population in Mostar), while for all the measured populations in the research of **Vejzagić et *al*. (2021)** this value was 40.30 mm (what is about 25.1% more compared to the average leaf petiole length from the *DBU* report).

Although according to the **DBU.de (2021)** study, the Mostar population consists of *T. tomentosa* trees, it is observed that the trees sampled in the **Vejzagić et *al*. (2021)** study differ significantly in leaf petiole length, so that trees in the Sana River canyon do not reach a length of 40 mm at all, while trees from the Neretva River canyon exceed a petiole length of more than 40 mm. The discussion leads to the logical question of whether climatic oscillations and the time of sampling of plant material significantly influenced the length of the petiole in the year of plant material collection, and whether significant differences in leaf petiole length, observed on herzegovinian (southern) locations, differ on the local level. For sure that should certainly be investigated in future studies of linden in Bosnia and Herzegovina, especially in locations with linden in the Neretva River canyon.

Other relevant investigations within the *Tilia* complex in Bosnia and Herzegovina

Among other significant research conducted on linden trees in Bosnia and Herzegovina, an article published in the *Forestry Journal* of the *Faculty of Forestry* in Banja Luka stands out, where the authors **Tomić et *al*. (2014)** published the results of a phenological study of three linden tree species in the city of Banja Luka: *Tilia cordata* Mill., *Tilia platyphyllos* Scop. and *Tilia argentea* (which is one of the synonyms of *T. tomentosa* Moench. in Bosnia and Herzegovina. The authors researched the phenological flowering phases on 27 sampled trees of three species of linden, namely at the locations *Park Mladen Stojanovic*, *University Campus* and *Starcevica*. The authors defined five phases of flowering that they followed, namely: 1) Appearance of the first flowers, 2) Beginning of full flowering (when about 25 % is open flowers), 3) The time of full flowering (when most of the flowers are open, on more than 50% of the inflorescence), 4) The end of full flowering (when about 75% of the flowers do not have a flower sheath or perianth) and 5) The end of flowering (when all flowers remain without a perianth).

According to the results of the research, the first flowers at the Park *Mladen Stojanovic* site appeared on 27.05. for *T. platyphyllos*, and for *T. cordata* on 10.06. The species *T. argentea* began to bloom later, on 16.06. The first flowers at the *University Campus* site appeared on 28.05. for *T. platyphyllos* and on 10.06. for *T. cordata*. The species *T. argentea* began to bloom the latest, on 15.06. At the Starcevica site, *T. platyphyllos* bloomed first, on 26.05., while *T. cordata* began to bloom on 09.06. The species *T. argentea* was the last to bloom, on 17.06. As

for the last phenological phase that was observed, which is the *End of flowering*, at the Park *Mladen Stojanovic* location, the leaves of *T. platyphyllos* fell first. On the same day, the *End of flowering* of *T. platyphyllos* was spotted at the *University Campus* location, and two days earlier, on 11.06., the *End of flowering* for *T. platyphyllos* was observed at the *Starcevica* location. On 23.06., the *End of flowering* of *T. cordata* was observed at the *University Campus* and *Starcevica* locations, while at the *Park Mladen Stojanovic* location, the *End of flowering* was observed on 24.06. For the species *T. agentea*, the *End of flowering* occurred on a) 27.06. at the *University Campus* location, b) 28.06. at the *Park Mladen Stojanovic* location, and c) 29.06. at the *Starcevica* location. **(Tomić et *al*., 2014)**

The authors state that one *T. cordata* tree at the *Starcevica* site had a flowering period that was 6 days shorter than other trees. The authors assume that the reason for the shorter flowering period of *T. argentea* and *T. cordata*, compared to *T. platyphyllos* (which flowers somewhat longer), is dependent on the value of average daily temperatures. The authors also state an interesting fact, that a greater change in the value of relative air humidity for the flowering period of *T. platyphyllos* was probably observed with a longer duration of its individual flowering phases, and this was the case at all investigated sites. **(Tomić et *al*., 2014)**

The phenological, morphological and genetic characteristics of linden depend on numerous factors, such as growing conditions, climate, weather fluctuations, other plant species in the community, slope, altitude, etc. The authors **Logan et *al*. (2019)** studied the genetic diversity and reproductive characteristics of linden and concluded that leading-edge populations have reduced genetic diversity and reduced reproductive output, which, according to the authors, may limit their potential to successfully rank in expansion under the influence of climate change. **De Jaegere et *al*. (2016)** reviewed the ecological, physiological and silvicultural characteristics of small-leaved (*T. cordata*) linden and came to results that indicate a broader context of high resilience to drought and suitability for mixed forest management in terms of different possible climate change scenarios. Certainly, there will be an idea of thinking about the phenology of flowering and leaves of linden trees in the future in terms of climate change. **Ruņģis & Krivmane (2021)** investigated the genetic diversity and population structure of *T. cordata* in Latvia and found that there is a relatively high level of genetic variation but weak population differentiation, indicating good potential for conservation and sustainable forest management. There are several other significant studies that indicate the importance of genetic diversity in linden and how it can later be used for sustainable forest management in the context of climate change. It is worth noting that the authors **Wolff et *al*. (2021)** studied *T. cordata* and *T. platyphyllos* from the Bavarian Forest National Park and concluded that both linden species are characterized by sufficient genetic diversity for conservation. It is believed that careful management can achieve the planting of *non*-native species in the future, and that guided restoration efforts must be resorted to in order to preserve the balance and ecological differences between species. **Heinrichs et *al*. (2021)** studied the impact of *T. tomentosa* on plant diversity in elevational transects in western Romania and concluded that the dominance of *T. tomentosa* may

have an impact on reducing understorey diversity. Therefore, it was observed that *T. tomentosa* provides potential for transitional habitats under the influence of climate change and supports shade-tolerant species. If this research were to be brought into the context of morphological and phenological studies, it is likely that patterns of phenological parameter measurements could be found in such forests, because the dominance of *T. tomentosa* may also raise the question of whether leaves and flowers are better developed and whether the species generally has the conditions for the development of larger vegetative organs as well as possibly better conditions for leafing, flowering and fructification.

From the numerous listed studies on lindens and those that included lindens in their scope of research, it is noticeable that lindens are very interesting to study, especially from the perspective of genetics. Lindens are greatly underestimated and insufficiently researched, as evidenced by the research of **Zhelev et *al*. (2020)**, who studied the taxonomic diversity and genetic resources of *Tilia* species, and emphasized that it is necessary to pay attention to lindens, to document the existing conservation genetic variation, in order to contribute to raising awareness of the genus *Tilia* in terms of forest management and supporting the resilience of forest tree species under the influence of climate change. That the study of morphological and genetic research on lindens in the context of the future of sustainable forest management in Europe is also important for Bosnia and Herzegovina, wrote **Vejzagić et *al*. (2021)**, but also the **DBU.de (2021)** report, which places *T. tomentosa* and linden in general at the very top of the list of "trees of the future" that could save forest biodiversity, the survival of bees and other insects **(Vejzagić & Omerbegović-Arapović, 2025)**, but also save the market for forest wood assortments at least at the local level (production of furniture, small construction equipment, *etc.*).

Potentials in research are seen in additional research on a new type of fruit in *T. tomentosa* in Mostar **(Vejzagić, 2022)**, which is characterized by rows of primary, secondary, and tertiary bracts on the fruit, which the author believes can help in further dispersal of the fruit and seeds by wind, which could help in the spread of the species to further habitats. Further potentials, for research at the molecular level, are also seen in clarifying the occurrence of clonal trees in natural habitats and studying potential apomixis. The **DBU.de (2021)** report points to these potentials of molecular research, and recommends continuing work in the field of genetic diversity of trees of the genus *Tilia* in Bosnia and Herzegovina.

CONCLUSION

This review article has reached the most significant conclusions about linden trees from Bosnia and Herzegovina, which are:

1. Within the *Tilia* complex in Bosnia and Herzegovina, there are significant genetic differences between (*interpopulation* variability) linden populations but also within (*intrapopulation* variability) the same species, as is the case with populations composed of *Tilia tomentosa* trees.

2. Morphological measurements of trees of three linden species from Bosnia and Herzegovina (*T. cordata* Mill., *T. platyphyllos* Scop. and *T. tomentosa* Meonch.) show that the length of the leaf petiole differs significantly among populations, suggesting a significant influence of local ecological factors and microclimatic conditions.
3. A study of phenological parameters in the city of Banja Luka in Bosnia and Herzegovina showed that phenological differences in flowering between species and locations confirm that average daily temperatures and relative humidity affect the length and onset of flowering, and that they should be given full attention in future phenological studies.
4. The report of the *MOE*-Scholarship Program, **DBU.de (2021)**, and the research of **Vejzagić et *al*. (2021)** consistently point to the importance of molecular analyses in understanding the population structure of linden, since trees with interesting morphological characteristics provide an excellent basis for genetic research.
5. Molecular differences between the Herzegovinian and Krajina populations in Bosnia and Herzegovina, especially Mostar and Bosanski Petrovac, suggest a complex history of the spread and isolation of linden species in the country, which is why it would be interesting to follow the "Gene Flow" of linden in Bosnia and Herzegovina and the countries of the Region, and to name genotypes and haplogroups.
6. The presence of specific fruit shapes in *T. tomentosa* in Mostar represents an interesting potential for future research into the dispersal of silver-leaved lime seeds in nature, and especially for investigating the evolutionary adaptation of this species. This phenomenon should also be investigated in the countries of the Region.
7. Research on clonal trees and the possibility of apomixis may open new questions about the reproductive biology of linden under natural conditions in Bosnia and Herzegovina, and it is necessary to devise a way to finance molecular research on *Tilia* in the country in the future.
8. Phenological studies **(Tomić et *al*., 2014)** indicate that *T. platyphyllos* has the longest flowering period, while *T. cordata* and *T. tomentosa* show shortened phenophases under certain climatic conditions, which shows the special potential of large-leaved linden in the future from the aspect of insect pollination and importance for insect biodiversity.
9. The observed statistically significant morphological differences between populations emphasize the need to preserve local linden genotypes in Bosnia and Herzegovina in future planning of sustainable management of forest ecosystems.
   1. Linden is confirmed as the "tree of the future" for climate-adapted forests in Bosnia and Herzegovina, with important roles in biodiversity conservation, as well as in the context of pollinating insects and in the maintenance of the local timber industry and sustainable planning of forest plantations.

Acknowledgements: This paper is the result of nine years of research on linden in Bosnia and Herzegovina and the Region and is planned as a basis for the theoretical part of the doctoral thesis at the *International Burch University* in Sarajevo. Due to the impossibility of publishing the overall molecular results **(DBU.de report, 2021)**, this paper is the only opportunity to show the

public the richness of the *Tilia* "Gene Pool" in Bosnia and Herzegovina and to introduce the academic community and Bosnian-Herzegovinian society to linden in Bosnia and Herzegovina as a potential solution to climate change at the local (state) level.

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SOURCES OF INSTITUTIONAL SUPPORT FOR SUSTAINABLE MANAGEMENT OF TREES FROM THE GENUS Tilia sp. IN BOSNIA AND HERZEGOVINA

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ABSTRACT

The research of the three dominant linden species in Bosnia and Herzegovina as part of the DBU "MOE" 2021 scholarship program opened the door for a new perspective of these species in the Bosnian-herzegovinian economy, primarily forestry as an industrial branch. Species from the genus *Tilia* in Bosnia and Herzegovina are characterized by a wealth of intra- and interpopulation diversity, and due to their good properties (primarily resistance to long-term drought) they are considered trees of the future. The goal of the wider research project is to assess the possibilities for raising linden nurseries in Bosnia and Herzegovina in the future, so that the country can provide high-quality, highly resistant planting material, which would be used in mass afforestation. Based on the review of transparent information of forestry companies in the country, it was learned that several companies are already growing linden trees in their nurseries. This article discusses how support could be provided to forestry experts, who could possibly promote the idea of more frequent planting of trees from the genus *Tilia* in Bosnia and Herzegovina in the future. Namely, Bosnia and Herzegovina as a country has a relatively complex administrative structure, however, this should not be an obstacle to sustainably managing forests of interesting genetic importance throughout the territory of Bosnia and Herzegovina. Namely, research conducted as part of the MOE scholarship (DBU.de, 2021) (if the report of this research is reviewed) indicates that linden, especially the species *Tilia tomentosa*, should be viewed as a potential tree of the future. Since the facts indicate that linden spreads in almost all habitats in Bosnia and Herzegovina and other countries of the Western Balkans, a logical question arises: "Why not support innovative ideas in order to find sustainable solutions for the wood market on the Balkan Peninsula?". Although it was relatively difficult to obtain detailed information on the popularity of linden in nursery production based on the data available on the websites of forestry companies, it is noticeable that the websites of forestry companies are relatively transparent and aesthetically adequately arranged. Forestry companies in Bosnia and Herzegovina transparently provide contact information, so that it is possible to establish contact and inquire about cultivation, protection and sale, as well as prices of planting material. This article relies on a literature review method, as well as an overview of available relevant information on the work of forestry companies and the species that are represented in nursery production for filling forest crops, planting plantations, but also for planting in alleys, on river banks and in floodplains. The main results of the research indicate that the functioning of forestry enterprises in Bosnia and Herzegovina is sufficient to sustainably manage species of the genus *Tilia*, especially considering that the Governments of both entities are willing to support the Master Plans for Afforestation and that there is an interest in carrying out the necessary afforestation of forest land with adequate tree species, as well as supplementing forest crops with valued commercial tree species.

Keywords: *Tilia*, Plant nursery, Sustainable Management, state organisation, governmental support

INTRODUCTION

In Bosnia and Herzegovina, according to Yugoslav sources (Herman, 1971), which are the most detailed on the subject of linden, there are relatively large areas of linden, which is considered a rare noble tree in many European countries. According to an oral statement by Professor Dr. Mikic (Oral Communication, 2016), there are two large linden sites on the European continent, namely Croatia (with linden dominance on the Bilogora mountain) and Serbia (with large linden complexes on the Fruska Gora mountain). In Bosnia and Herzegovina, there are relatively large linden forest stands in the Cazinska Krajina area, with a distribution center in Bosanski Petrovac and Sanski Most, while relatively large forests of the thermophilic species *Tilia tomentosa* extend in the Neretva River canyon (Herzegovina). Bosnia and Herzegovina is a country with a very complex administrative structure, which is closely correlated with all segments of the functioning of the state, and the administrative structure of the country directly reflects on the management of forest ecosystems. However, the existence of a complex state administration is not and does not have to be a reason for unresponsive forestry, and it is possible to establish an integrated system of management supervision of tree species of special importance, with the aim of preserving important highly resistant trees of the future for future generations.

The results of the Second Large-Scale Forest Inventory in Bosnia and Herzegovina (Lojo et *al*., 2008), whose methodology was developed by prominent forestry experts in the country, indicate significant potential for future forest land reforestation with appropriate tree species, appropriate to the given forest ecosystems and soil conditions (its quality and history). Some of the forest land can be planted with species of the genus *Tilia*, as indicated by research within the MOE scholarships (DBE.de, 2021), within which a report was published indicating the importance of conservation and cultivation of linden trees, as well as the preservation of the genetic pool of existing linden forest stands in Bosnia and Herzegovina. In addition to this research, there is additional research indicating the importance of preserving linden for the future, in relation to protecting habitats from negative climate impacts. Thus, the authors Vejzagić, Ballian and Memisević-Hodzić (2021), in their study of the morphological diversity of

T. tomentosa trees in Bosnia and Herzegovina and Serbia, state that this species is significant in the context of climate change, while Vejzagić (2022) also points to a similar significance, describing the potential connection between climate change and the appearance of multiple bracts in the fruits of silver linden (in the short communication on discovery of multibracteate linden in Mostar).

The institutions of the Federation of Bosnia and Herzegovina and the Republika Srpska can significantly help preserve the linden gene pool, not only in Bosnia and Herzegovina, but also in the wider region consisting of the Western Balkan countries. Thus, the authorities in Bosnia and Herzegovina can support the preservation of both linden forests and individual linden trees, which are important for genetic diversity (protection from illegal logging and any fragmentation of linden habitat). The importance of the Government of the Federation of Bosnia and Herzegovina and the Government of the Republika Srpska is also reflected in the support for the potential future production of seeds and high-quality seedlings resistant to drought and temperature fluctuations, which could help preserve populations of beneficial insects, such as bees, as well as protect the

climate of local cities from overheating. Taking into account the research of Pigott (1992), who described the linden in Europe and presented information on pollen maps of Europe, according to which the linden was the dominant tree in the past, as well as the statements of Finschi et *al*. (2003) who explain what happened in the past with the genus *Tilia* in Europe due to the onset of the Ice Age, and if we add to the context of consideration the fact that forestry companies are obliged to do everything possible in cooperation with the academic community and authorities to ensure sustainable forest management, it is noted that there is a need to investigate the framework of potential support for sustainable linden management in Bosnia and Herzegovina. Since there is no previous knowledge about this problem (scientific articles in Bosnia and Herzegovina), and since forest management elaborates of individual forest companies are extremely large documents, which are difficult to analyze in a short period of time, there was a need to collect basic information about which forestry companies are engaged in linden cultivation in Bosnia and Herzegovina, and whether there are documents that support the idea of linden production and planting in Bosnia and Herzegovina. The research was also guided by the fact that the countries of the Western Balkans chronically suffer from a lack of statistical data (Mueller and Hofmann, 2022), and care was taken to try to collect basic information about the problem under study.

MATERIAL AND METHOD

Research obtained basic information from different forestry companies in Bosnia and Herzegovina, that are existing and functioning at the moment, but also whose management data are transparently available at the web-sites. Since the simple review method of the given information was used, the main or key terms in the website search related to: 1) territory of management, 2) main services and production aims, 3) data about the plant nurseries and 4) data about the linden production. The report data of this review work should provide background information on where the potential for wider use of linden could be explored in the future.

However, since the organization of forestry companies in Bosnia and Herzegovina is complex in accordance with the division of responsibilities into different administration units, this indicates the need to conduct basic research whether the Governments of Federation of Bosnia and Herzegovina and Republika Srpska do have certain framework for the future afforestation, concretely whether the afforestation with linden trees is has already been planned. For the purposes of this exploration, the readings of two documents, “Republika Srpska Afforestation Master Plan” (Govedar and Mataruga, 2021) and “Afforestation Manual” (Visnjic, 2024) (provided on the website of the Association of Forestry Engineers and Technicians of Bosnia and Herzegovina), were analyzed. The aim of the readings was to identify whether linden species had been planned for the afforestation in the Federation of Bosnia and Herzegovina and Republika Srpska.

Some additional data about the forest companies, such as human resources or monitoring capabilities, were added in the results, since they could indicate the potential of individual enterprise level and the institutional support together to keep linden forest resources sustainable. Finally, to be emphasized is that the research did not use data from the forestry enterprise elaborates (which consist potentially more detailed data on the amount of linden trees planned for afforestation, in the frame of Plans of afforestation), but the research focused on the publicly available data from the web source.

Studied were the websites contents obtained from the following forestry companies: 1) Public company “Forestry economic society of Zenica-Doboj Canton” Zavidovici L.l.c., 2) Cantonal

public company “Sarajevo-forests“ L.l.c. Sarajevo, 3) Public company "Forests of Tuzla Canton" J.s.c. Kladanj, 4) Forestry economic society “Central Bosnia forests” L.l.c. Donji Vakuf, 5) Forestry economic society “Herzeg-bosnian forests” L.l.c. Kupres, 6) Forestry economic society “Una – Sana forests” L.l.c. Bosanska Krupa, 7) Public company "Bosnian-herzegovinian forests" e.o. Sarajevo, 8) Public company "Forests of Bosnian Drina" L.l.c. GORAZDE, 9) Forestry “Central Neretva” J.s.c. Mostar, 10) Public company

„Herzegovina-Neretva forests“ L.l.c. Mostar, 11) Forestry "PRENJ" J.S.C. Konjic, 12) "FORESTRY LJUTA" L.l.c. Konjic, and 13) Forestry company "FORESTS OF REPUBLIKA SRPSKA" J.s.c. Sokolac.

After reviewing basic data about the companies, basic information related to the possibilities of improving the sustainability of linden stands and/or their individual trees, through the existing framework of institutional support and company capabilities, was clarified by the information sources from the institutional level (governmental supported documentation).

RESULTS AND DISCUSSION

The functioning of forestry companies in Bosnia and Herzegovina is to a certain extent supported by municipal (individual) and cantonal, but also Republika Srpska institutions, either through the means of those institutions directly, or through funds related to them and their services (offices), such different cooperations between forest companies and foreign organisations, who support afforestation projects in Bosnia and Herzegovina. There are different types of institutional support for the work of forestry companies in Bosnia and Herzegovina, about which a lot of information can be found on the official websites of forestry companies. Companies in general take care to publish information about partnerships and important projects on their websites. In the context of the analysis of potential addresses for seeking support in the future, when companies would seek help to harmonize their operations with the parallel negative consequences of climate change on forestry as an industry, and what also refers to support for the establishment of nursery production of resistant species of trees, from the available information it is known that support can be provided by the institutions in Republika Srpska and Federation of Bosnia and Herzegovina.

Individual forestry companies data

The first company analysed is the P.C. "Forestry economic society of Zenica-Doboj Canton” Zavidovići L.l.c.. According to the transparent content on the website, this company deals with the cultivation and protection of forests, production planning and hunting. This company has been managing Cantonal forests since 2006. There are several business units (*Olovo*, *Vares*, *Visoko*, *Kakanj*, *Zenica*, *Zepce*, *Zavidovici*, *Maglaj*, *Tesanj*, and *Business unit "Natural Monument TAJAN Zavidovici"*), which are united in five wider forestry areas (*Gornjebosansko*, *Olovsko*, *Krivajsko*, *Kakanjsko* and *Natron-Usorsko-Ukrinsko*). This area manages forests and forest land on an area of 183,389.20 hectares, of which 69.12% are forests with natural regeneration, 15.22% are vegetative origin forests, 9.18% are forest cultures and plantations, and 6.48% are bare and unproductive areas. The mined areas cover 21,946.40 hectares. Seed and nursery production is very successful. Seed production of silver fir, Norway spruce, Serbian spruce, green Douglas fir, Scots pine, Austrian pine, European larch, European beech, ash, sycamore, Norway maple, Scots elm, small-leaved linden, sessile oak and chequer is stable. The nurseries owned by this company are: "Olovo", "Ajdinovici", "Zepce", "Zavidovici" and "Tesanj". (SPDZDK.ba, 2025)

In Sarajevo Canton is a center of the company C.P.C. "Sarajevo-forests" L.l.c. Sarajevo, which manages the forests and forest land of Sarajevo Canton. The activities of this company are: 1. Forest cultivation, 2. Forest protection, 3. Forest exploitation, 4. Construction and maintenance of forest transport infrastructure, 5. Bioengineering, 6. Environmental projects, 7. Hunting and hunting tourism, 8. Nursery production, and 9. Various projects and collaborations. The company was founded on November 24, 1999 with the main goal of ensuring the protection of forests and forest lands in the Canton area, and to this day it fulfills the mission and vision of the company. The area of forestry is divided into two sectors, of which the first sector, which is responsible for cultivation, protection and use of forests, includes: 1. Service for cultivation and protection of forests, 2. Service for planning and analysis, 3. Service for preparation and design,

1. Service for transport and maintenance of machinery, 5. Service for maintenance of forest communications and facilities, 6. Service for hunting and tourism, 7. Service for other forest products, 8. *Srednje* Forestry Administration, 9. *Hadzici* Forestry Administration and 10. *Trnovo* Forestry Administration, *while* the other sector has jurisdiction over ecology and the management of protected areas, which is known as Service for Protected Areas. There are two nurseries, in Tarcin and in the settlement of Sedrenik. The two nurseries have a total area of 28,538 m2, and the total capacity of these two nurseries is 700,000 seedlings. (SARAJEVO-SUME.ba, 2025) Forestry company P.C. "Forests of Tuzla Canton" J.s.c. Kladanj is a cantonal forestry company engaged in the following activities: -Forest cultivation, -Forest protection, -Seed and nursery production, -Planning and production, -Hunting, and -Non-timber (secondary) forest products (sale). The cantonal company has over 530 employees, and in addition to the Directorate, there are four business units: Forestry "KONJUH" Kladanj, Forestry "SPRECKO" Zivinice, Forestry "MAJEVICKO" Srebrenik, and Forestry "Vlasenicko" Turalici. The area manages forests and forest land on an area of 44,713 hectares. The nursery production is good developped, and the "Budim potok" nursery is characterized by the successful production of the following species/tree *generas*: silver fir, Norway spruce, blue spruce, Scots pine, Austrian pine, European larch, *Juniperus spp.* and *Thuja spp*. (JPSUMETK.ba, 2025)

Forestry company F.E.S. "Central Bosnia forests" L.l.c. Donji Vakuf deals with the following activities: -Production, -Seed production and nurseries, -Ecology and protection, and

-Forestry. Within this company there are 12 forestry units, which are named after the names of municipalities (*Jajce*, *Dobretici*, *Donji Vakuf*, *Travnik*, *Bugojno*, *Novi Travnik*, *Vitez*, *Gornji Vakuf - Uskoplje*, *Fojnica*, *Busovača*, *Kreševo* and *Kiseljak*). Twelve smaller units are included in four wider forest management areas: Forestry “Gornjevrbasko”, Forestry “Srednjevrbasko”, Forestry “Lasvansko” and Forestry “Fojnicko”. The company has three nurseries: “Bugojno” Nursery, “Busovaca” Nursery and "Slimena" - Travnik Nursery. The nurseries have the following trees in the production: Norway spruce, Scots pine, Austrian pine, Bosnian pine, ash, and as for other species such as silver fir, Douglas fir, European beech, sycamore and oak, the organization of collection and production is ongoing. (SBKSUME.ba, 2025)

Next for the analysis was the F.E.S. "Herzeg-Bosnian forests" L.l.c. Kupres company, which performs the following activities: -Forest cultivation and other activities in forestry,

-Forest exploitation, -Auxiliary services in forestry, -Hunting and service activities related to hunting, -Collection of uncultivated forest fruits and products, except forest assortments,

-Processing of seeds for plant nursery material *etc*. The work within this company is carried out in the following business units: Directorate, Forestry *Bosansko Grahovo*, Forestry *Drvar*, Forestry *Glamoc*, Forestry *Kupres*, Forestry *Tomislavgrad*, Forestry *Livno*, and Work unit *RASADNIK*. The

"Przine" nursery within this company has a very successful production of the following trees: Austrian and Scots pine, Norway spruce, silver fir, *A. platanoides*, *A. pseudoplatanus*, *A. negund*o, *F. excelsior*, *S. aucuparia*, *S. aria*, *C. betulus*, *T. platyphyllos*, *P. avium*, *M. sylvestris*, and *P. pyraster*. (HBSUME.ba, 2025)

Forestry company F.E.S. "Una - Sana forests" L.l.c. Bosanska Krupa is a cantonal company that performs the following activities: -Utilization of forests, -Timber wholesale trade,

-Forestry (citizen) services, -Forest cultivation and protection, -Hunting, (trapping) and care of wild animals including appropriate services, -Forest cultivation and protection, -Production of other wood products, -Wood retail trade, -Cork and wicker production. The company has the following filiales: *Bihac*, *Bosanska Krupa*, *Bosanski Petrovac*, *Buzim*, *Cazin*, *Kljuc*, *Sanski Most*, and *Velika Kladusa*. This company manages 172,687.14 hectares of forests and forest lands. In addition to operations within the units, the activity is also carried out in the "Cazin" nursery. (USSUME.ba, 2025)

In the eastern part of Bosnia and Herzegovina is the center of the forest company P.C. "Forests of Bosnian Drina" L.l.c. GORAŽDE. That cantonal forestry company in the Bosnian-Podrinje Canton for its main activities has: -Planning and production, -Forest cultivation and protection, -Additional activities (SUMEBPK.ba, 2025).

Forestry "Central Neretva" J.s.c. Mostar is a forestry company based in Mostar, in the Herzegovina-Neretva Canton. The main activity of the company is the utilization and cultivation and protection of forests. The company has been operating for about 25 years and has 34 employees. (SUMARSTVOMOSTAR.ba, 2025)

Forestry company P.C. "Herzegovina-Neretva forests" L.l.c. is located in Mostar and it participate in the following activities: -Cultivation and protection of forests, -Use and sale of all products of forests and forest land, -Construction and maintenance of forest communications and facilities, -Protection, maintenance and improvement of the general useful functions of forests,

-Management of protected areas and forests with special purpose, -Planning in forestry, -Seed production, -Nursery production, and -Hunting and fishing and Tourism in forestry. The company has several units, namely: *Capljina*, *Jablanica*, *Konjic*, *Prozor-Rama*, *Mostar* and *Citluk*. (SUMEHNZK.com, 2025)

Forestry "PRENJ" J.s.c. Konjic is a company registered in the municipality of Konjic, and it participates in the following activities: -Forest cultivation, -Forest protection, -Forest utilization, -Construction and maintenance of forest transport communication, and -Nursery production. The nursery "Boracko jezero" has 2.60 ha surface. (SUMARSTVOPRENJ.ba, 2025). "FORESTRY LUTA" L.l.c. Konjic is a company whose activities are: -Production, -Cutting and export of forest wood assortments, -Sales, and -Transport. (SUMARSTVO-LJUTA.ba, 2025)

In Sarajevo there is a center of P.C. "Bosnian-Herzegovinian forests" e.o. Sarajevo, the forestry company that can be singled out as an example of a company that provides support to all the aforementioned forestry companies, and whose professional assistance in reviewing and revising project documentation and forest management fundamentals is a huge professional help and support to all forestry companies' demands (BHSUME.com, 2025). This is thanks to its different departments from which every one provides professional service of writing, reviding and supporting forestry elaboration documentation related to Forest management, Forest exploitation, Forest breeding, Forest protection and all sorts of forestry economic analyses.

Large forestry company F.C. "FORESTS OF REPUBLIKA SRPSKA" a.d. Sokolac is a comprehensive forestry company in the Republika Srpska, whose activities are: -forest utilization, -service activities in forestry, wood wholesale trade, and -Wood retail trade. The company manages 1,000,608.56 hectares of forests and forest lands, of which: - forests with natural regeneration reaches 469,164.64 hectares, - degraded forests reaches 14,528.97 hectares,

- coppice forests reaches 226,336.88 hectares, - forest cultures reaches 48,687.63 hectares, - areas suitable for afforestation and management reaches 154,846.61 hectares, -areas unsuitable for afforestation reaches 68,374.82 hectares, -usurpations reaches 18,669.01 hectares. The volume of total wood mass amounts to 202,426,428 m3, of which deciduous forest wood participates with up to 122,429,391 m3, and conifers make up to 80,997,037 m3. The annual yield amounts to 5148250 m3. (SUMEREPUBLIKESRPSKE.org, 2025)

The business units of this company are: Forestry "Banja Luka", Forestry "Ostrelj-Drinic", Forestry "Panos", Forestry "Birac", Forestry "Gradiska", Forestry "Doboj", Forestry "Zelengora", Forestry "Cemernica", Forestry "Vrbanja", Forestry “Majevica", Forestry "Lisina", Forestry "Botin", Forestry "Jahorina", Forestry "Prijedor", Forestry "Ribnik", Forestry "Sjemec", Forestry "Romanija", Forestry "Drina", Forestry "Maglic", Forestry "Borja", Forestry "Visocnik", Forestry "Vucevica", Forestry "Gorica", Forestry "Treskavica", Forestry "Klekovaca-Potoci", Forestry "Milici", Forestry "Rudo", "Karst Management Center" with headquarter in Trebinje, "Research-development and project center" with headquarter in Banja Luka, "Center for seed and nursery production" with headquarter in Doboj, Forest house "Ognjiste" with headquarter in Pale, and "Directorate of Public Enterprise" with headquarter in Sokolac. The nursery production is well developed and stable, and the company has the following nurseries: "Stanovi" and "Usora" in Doboj, "Stupine" Nursery in Nevesinje, "Bisina" Nursery in Sekovici, "Gornja Puharska" Nursery in Prijedor, Trebinje Nursery (*Cupressus spp.*, *P. pinaster*, *P. halepensis*, *P. nigra*, *P. heldreichii*, *Thuja spp.*, *Cedrus spp.*, *B. sempervirens*, *P. granatum*, *L. nobilis*, *Lavandula officinalis*, palm trees, *F. ornus*, *Euonymus spp.*), and the Nursery "Petkovaca" in Brcko (50 hectares surface). (SUMEREPUBLIKESRPSKE.org, 2025)

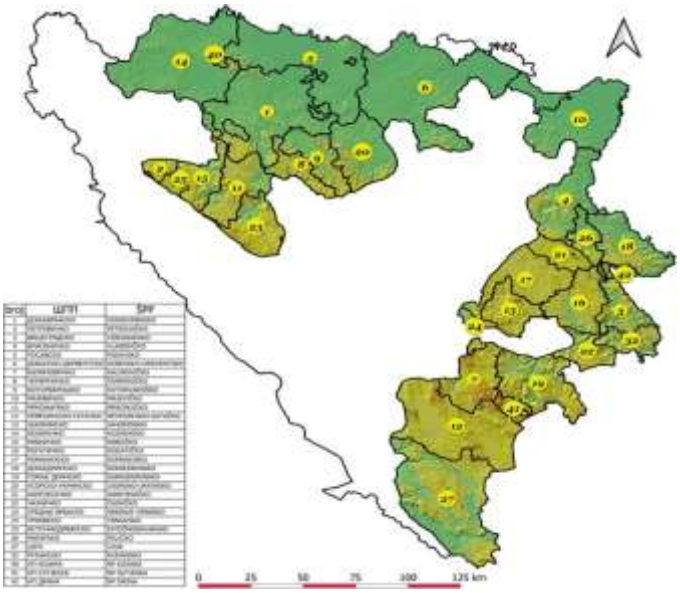

Figure 1. Forests of Republika Srpska, with clear administrative borders among Srpska's Forestry units/area. Source: https://sumerepublikesrpske.org/index.php/strana-1/rg-niz-ci

Institutional support in the form of afforestation approving documents

Forestry companies in the Federation of Bosnia and Herzegovina and Republika Srpska are encouraged to implement afforestation programmes on their territory of management. Special care for the noble species of trees in the regional forest ecosystems should be anyway taken in the drough-affected areas, since they are mostly affected by climate, and so the forestry companies should take measures and plan activities to concretize protection measure, so that the forestry production does not suffer, as warned Kumar (2021).

The assumption that lindens can compete with oaks in Europe in terms of their biodiversity should be an incentive for increasingly frequent further molecular research and the search for hybrids in existing natural linden stands (populations). In order to preserve the linden trees in Bosnia and Herzegovina, and conserve the naturally occurring species with their subspecies and varieties for the future, it is necessary to learn in detail the behavior of linden trees within the species and between species, as well as the behavior of several linden species within one mixed stand. In this direction, it would be possible to go with a survey method, where the possibilities within forestry companies in the Republika Srpska and the Federation of Bosnia and Herzegovina could be investigated. If there are capacities (a sufficient number of employees) for additional monitoring of the behavioral patterns of linden trees in relation to changed environmental conditions, observing insects species that might appear (macroscopic identification on the terrain) and tracking whether the leaves and fruits of linden trees deviate from their typical appearance, there are then fundamentals for an integrated high quality health condition and vitality parameters observations. The typical appearance of lindens growing in the territory of the former Socialist Federal Republic of Yugoslavia was described by Herman (1971), and that literature source should not be neglected in the process of systematic monitoring of behabioral patterns of linden complexes in the context of climate change.

Institutional support to forestry companies in the Republika Srpska and the Federation could, therefore, help further fragmentation of linden forests, and potential planning and financing of nursery production. Each forestry company is located within the territory of Republika Srpska and Federation (nurseries in Brcko District should be investigated in the future), which is characterized by different climatic, hydrological, relief and vegetative characteristics. It is important to note that through the review of transparent information on the activities of forestry companies and the species they grow for afforestation purposes, it was noticed that there is linden cultivation, both in the continental part of Bosnia and Herzegovina) (Zavidovici area) in the west-southern Mediterranean parts (Kupres) of the country. It has been observed that there is an interest in buying linden seedlings, considering that this is a very attractive species for ornamental purposes and park planting, but the question arises whether and to what extent there is interest in planting this species as part of the afforestation program, which needs to be analyzed separately in the future. In a meanwhile the forestry companies exploit lawful support in the institutional documentation, such “Republika Srpska Afforestation Master Plan” (Govedar and Mataruga, 2021) and “Afforestation Manual” (Visnjic, 2024) (provided on the website of the Association of Forestry Engineers and Technicians of Bosnia and Herzegovina).

According to the data of the Federal Forestry Administration (FMPVS.gov.ba, 2025), the support of the Federal Forestry Administration has as part of its scope of work the collection of data and the maintenance of a database on the development of all forests in the Federation of Bosnia and Herzegovina, including their inventory. The federal administration, therefore, helps forestry companies that manage state-owned forests by preparing a draft of the Forestry Program of the Federation of Bosnia and Herzegovina. In addition, this institution determines the monitoring

of the dynamics of the creation of forest management bases, which are documents that include all important information on forest management, (so forest stands as well as individual trees), which grow on public forest land. The provision of expert assistance for the development of forest management bases for forest management areas and forestry economic units in scope of companies *is* one of the most important assistances of this federal institution, but in addition, the development of plans and programs for extended biological reproduction of forests and programs and plans for afforestation of karst and bare forests is ensured. Establishing and maintaining registers and records of facilities for the production and processing of forest seeds and producers of forest trees and shrubs are under the authority of the institution. Monitoring the health status of forests, carrying out the work of the reporting and forecasting service and monitoring the condition and degree of forest damage is a very important function of institution, which can help in monitoring the health and condition of the habitus of the remaining linden trees, which may have the potential for cloning, i.e. more mass clonal nursery production, or which may be interesting from the aspect of seed collection for the necessary production of healthy and resistant linden seedlings. Since these trees may have commercial use in the future, and the production of linden wood for the production of furniture can potentially develop over time, it is important to familiarize all the stake-holders interested in protection of remaining linden trees and their stands with the described function of the Federal Forestry Administration, but also governmental offices in Republika Srpska. Namely, monitoring the economic condition of forestry industry, based on data obtained by cantonal administrations in the Federation of Bosnia and Herzegovina and from the Republika Srpska Governmental administration, as well as monitoring the prices of forest wood assortments and secondary forest products, including informing the public about the vitality condition of forests and the development of forestry branche, enables forestry companies to contact the Forestry Administration of the Federation of Bosnia and Herzegovina and Government of Republika Srpska at any time, in order to propose possible solutions for their local market, the needs of their partners - customers, but also to suggest species for planting in the future. The development of an adequate solution plan in front of each forestry company is the product of its engineers, i.e. experts in forestry and various fields, which means the adequacy of those proposals for consideration and support by the Federal Administration and Srpska's Government. According to the obtained data, it is clear that forestry institutional based legislation is really made by forest experts and the full implementation could serve as an example how complex administration can in final provide a healthy framework for forestry laws implementation, when all legal criteria are met.

The preparation and creation of programs in the field of forestry, which are financed or co-financed from the dedicated fund of the Federation of Bosnia and Herzegovina, is closely correlated with the preparation and implementation of scientific research activities and the transfer of knowledge in the field of forestry. New knowledge useful for forestry must, in a certain way, be forwarded to the Federal Forestry Administration, so that it has insight into the solutions offered by forestry scientists, who may work in federal forestry companies (engineers, Masters and *PhD*s of Forestry). The preparation of expert opinions for the establishment and revision of forest management areas is the domain of the Federal Administration (FMPVS.gov.ba, 2025). Also, in order to protect an area with an existing high-quality stand, it is necessary to propose it to the Federal Forestry Administration. The reason for this is that the preparation and giving of opinions for the declaration of protected forests *and* forests with a special purpose is carried out in accordance with the powers and support of the named Administration. The organization of consultations and the provision of professional assistance to cantonal administrations and cantonal forest management societies is one of the functions of the Federal Administration, on which companies can always rely, therefore, frequent communication of forestry companies is significant and useful compared to sporadic ones.

This kind of communication partially meets the recommendation of Kumar (2021). The same complex task in a form of huge support to Republika Srpska forestry units in giving the governmental office for forestry, which also supports the exchange of new scientific knowledge, ideas and solutions for existing or potential problems and helps developing cooperation between companies and institutions, as well as in terms of inter-units cooperation and strengthening awareness of the importance of protecting forests from the negative consequences of climate change. The Federal Forestry Administration and Government of Republika Srpska are supporting scientific achievements and experts recommendations from faculties for forestry (Faculty of Forestry in Banja Luka, Republika Srpska, and Faculty of Forestry in Sarajevo, Federation of Bosnia and Herzegovina), whose research projects draws the resources from the support of the Federal Ministry of Agriculture, Water Management and forestry companies themself, and Government of Republika Srpska. Strengthening the bond between Ministries, forestry faculties and forestry companies increase the capacity to help the development of wider forestry regions. According to the Association of Forestry Engineers and Technicians (USIT.ba, 2025) of the Federation of Bosnia and Herzegovina (in Sarajevo), the support of the Croatian Forestry Society and the Faculty of Forestry of the University of Sarajevo is also very significant, not only in terms of scientific and professional assistance , but also in joint lobbying to find the best solutions for forestry as a whole and to promote solutions in the society, academic and business community. On the other hand, Faculty of Forestry in Banja Luka is host to Forest institute, which gathers forestry experts from the country and the Region, in aim to promote forest studies results and lobby for implementation of good scientific practices in the forestry of Republika Srpska (SF.UNI.BL.org, 2025).

In Republika Srpska, support is provided to the company F.C. "Forests of Republika Srpska" J.s.c. Sokolac, which is further implemented individually by all its individual business units, in accordance with the needs of those units. Due to climate differences and the presence of different ecological-phytocenological areas in Bosnia and Herzegovina, there are certain differences in the focus of forest restoration support between the Federal Ministry of Agriculture, Water Management and Forestry and the Ministry of Agriculture, Forestry and Water Management of the Republic of Srpska. For example, the percentage representation of conifers and deciduous trees is different in Federation of Bosnia and Herzegovina and Republika Srpska, so there are certain differences in the amount of planting material for forest restoration and differences in the tree species, too. For example, the focus of nursery production within the business units of the “Forests of the Republika Srpska” company for the units located in the southern municipalities is focused on species that are designated as Mediterranean, while in the case of the northern municipalities, focus in production is more on continental species. In the Federation of Bosnia and Herzegovina, for example, the need to restore forests using Mediterranean species is in close correlation with the needs of the southern and west-southern cantons, on whose territory grow the species that require Mediterranean habitat and cultivation conditions. Therefore, there are certain differences related to the flow of information such as what are the needs of forest units regarding their climate and ecological conditions. Because of the complexity of the administration in Bosnia and Herzegovina, there are also certain specifics that play a huge role in the information flow and sometimes even can affect the speed of decision making processes, i.e. whether information from business units through forestry companies must go through the processing procedure first in the cantonal institutions, and then continue its journey to the competent ministry, or (as is the case in the Republika Srpska) that information travels directly, in the sense that it does not undergo processing at some mediate levels, but can continue its journey faster to the Ministry of the Republika Srpska. Ways of supporting forestry companies with all their units, can be found on the

organized and transparent pages of the Ministry in the Republika Srpska. (VLADARS.rs, 2025, FMPVS.gov.ba, 2025)

Document "Republika Srpska Afforestation Master Plan" (Govedar and Mataruga, 2021) treats even *Tilia* species, by giving recommendations on breeding, planting, planting density index and percentage participation in the mixed forest stands. Recommendations for the linden planting are made also for the forest types, taking into consideration the habitat and vegetation characteristics for different forestry areas.

Precise data from this afforestation master plan are even given by individual *Tilia* species, as follows (Table 1).

Table 1. Surface for afforestation with *Tilia* specimens in hectares (according to Mataruga and Govedar, 2021)

Species	Dinarids (*ha*)	Mediterranean region (*ha*)	Pannonian region (*ha*)	Illyrian-Moesian region(*ha*)	Total surface for Republika Srpska(*ha*)
Tilia cordata	605,08	710,47	13,01	147,96	1.476,52
Tilia platyphyllos	441,83	754,06	13,55	6,86	1.216,30
Tilia tomentosa	301,06	1.208,87	177,76	23,16	1.710,85

At this moment it is unknown (when analysing forestry companies website content) how much of the recommended (calculated) plant material from *Tilia* genera have been planted, but it is obvious that detailed calculations exist in accordance to climate and habitat requirements.

Similar recommendations but more general, were published in Bosnia and Herzegovina in 2024 ("Afforestation Manual" by Visnjic, 2024, - provided on the website of the Association of Forestry Engineers and Technicians of Bosnia and Herzegovina), consisting detailed lists of species (non)resistant to drought, frost and temperature turbulences. Light requirements are also taken into considerations, while afforestation soil preparation process and preparation of the planting material was also described in detail. So, it is possible to conclude that governmental supported legislation and documents supporting afforestation programmes are completed by professional and fulfilling forest experts and Forestry professors works.

CONCLUSION

The institution support of the Government of the Federation of Bosnia and Herzegovina and the Government of the Republika Srpska could significantly help in the future, if the forestry companies decide to request the help of institutions for mitigating climate change. In accordance

with the reviewed literature that describes the ecological-phytocenological areas in the country, and transparent informations provided by forestry companies that manage area with small-, large- and silver-leaved linden forests, an analysis was performed in order to preliminarily suggest which forest company and under what conditions could be considered for potential feasibility study on mass linden production. In accordance with the natural habitat characteristics of the plant habitats and respecting the requirements of the species from the genus *Tilia*, which were described for the area of the former Socialist Federal Republic of Yugoslavia by the author Herman, and adding the findings of the linden research presented in the DBU.de report, which indicates a high degree of variability within and among linden populations, the recommendation of conducting wider research on establishing linden nursery for mass afforestation purpose in the future, in order to combat the negative consequences of climate change, could be given to Zavidovici-Teslic region (since natural conditions for growing linden in the nurseries already gave good results, as seen in the transparent information about nursery activity in the given company). It is interesting that the pollen maps of Europe, according to which linden pollen dominates in all habitats of the continent, raise a legitimate question whether linden cultivation in a nursery with various natural conditions in Bosnia and Herzegovina can result in quality linden trees that can adapt to all habitat conditions. Institutional support for the research of this possibility as well as various variants can be sought in the financing of scientific-research projects by forestry funds that belong to the Federal, that is, the Ministry in charge of forestry in the Federation of Bosnia and Herzegovina, and in the Republika Srpska Governmental institutions, that also could support scientific research and needed expertise. Areas such Una-Sana Canton also represents an interesting location for linden nurseries respecting the fact about existence of wider linden forest stand structures there, where the focus is on *T. tomentosa*. Successful production of *T. platyphyllos* in the Kupres, as seen from the website data collection of there active forestry company, gives hope that wider production of this species could be expected also in the future, with upcoming of higher temperatures and more frequent heat waves. Potential for further afforestation in Republika Srpska is giving optimism that the *Tilia* trees can provide better climate conditions for the local cities areas, what could results in the sustainable development of support of local forest management, forestry as industry and perseverance of local climate and biodiversity, as main fighter in mitigating climate change.

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CORRELATION OF BIOMARKERS OF COMBINED STRESS TOLERANCE IN EGGPLANTS AND EVALUATION WITH POLAR HEATMAP

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ABSTRACT

Abiotic stress factors are among the primary causes of yield losses in agricultural production, with drought and salinity being particularly prominent among these stresses. Drought stress, increasingly felt due to global warming, severely restricts plant production, especially in arid and semi-arid regions. Drought often causes soil salinization, while excessive soil salinity also leads to yield losses in many crop plants. The simultaneous occurrence of both stresses leads to even more serious negative effects on plant development and yield. Therefore, understanding the tolerance mechanisms that plants develop under such combined stress conditions is crucial for developing effective agricultural strategies. This study evaluated the relationships between biological markers that play a role in combined stress tolerance in eggplant under combined drought and salinity stress conditions and that provide concrete indicators of the multifaceted stress responses. As a result of evaluations using correlation analysis and a polar heatmap, strong relationships were identified among shoot dry weight (SDW), malondialdehyde (MDA), proline, total anthocyanins, total phenolic compounds, some antioxidant enzyme activities, and the K^+/Na^+ ion ratio. The polar heatmap comprehensively revealed the relationships between biomarkers in stress responses in terms of direction and intensity. These findings demonstrate that these parameters are important biomarkers reflecting the level of tolerance to combined stress in eggplant and provide guidance for selecting genotypes with high stress tolerance or for planning future studies.

Keywords: antioxidant enzymes, biological markers, combined stress, eggplant, ion homeostasis,

INTRODUCTION

Drought and salinity are among the most significant abiotic stresses, negatively impacting plant production and causing serious problems resulting in yield loss. Losses are particularly high in vegetable species with high water content. This situation is even more critical in arid and semiarid regions with limited water and land resources and inadequate water use (Hussain et al., 2024). Researchers estimate that the area affected by drought is increasing and that yield losses will increase further unless measures are taken to mitigate the negative effects (Golldack et al., 2014; Cao et al., 2023). Drought, particularly the effects of climate change, is currently exacerbating the severity of these stresses.

Drought and salinity, two primary stress factors in agricultural production, often occur simultaneously. In arid areas, soil salinity develops much more rapidly than in rainy regions due to the combination of factors such as low rainfall, high evaporation, irrigation with poor-quality water, and inadequate drainage (Zelm et al., 2020). These stresses lead to physiological

and biochemical problems in plants, such as loss of turgor, decreased stomatal conductance, decreased chlorophyll content, ion imbalance, decreased photosynthetic capacity, and lipid peroxidation, protein denaturation, and DNA damage due to increased reactive oxygen species (ROS) (Kumar et al., 2016; Hasanuzzaman et al., 2019; Kıran and Baysal Furtana, 2023). Plants develop various defense mechanisms against these adverse conditions, such as antioxidant enzymes (SOD, CAT, APX, GR), proline, and phenolic compounds (Kıran and Baysal Furtana, 2023; Sharma et al., 2023).

The physio-biochemical responses of plants to combined stress are remarkable, and their responses to these complex stress conditions are generally unpredictable by their responses to single stresses. However, existing studies on the effects of combined stress are quite limited. Eggplant (*Solanum melongena* L.) is an important vegetable species widely grown in tropical and subtropical regions of the world due to its low calorie content and rich vitamin and mineral content. Eggplant is reported to be moderately tolerant to drought and moderately sensitive to salinity (Kıran et al., 2022; Cebeci et al., 2025). While plant responses to drought and salinity have been studied separately, the responses that occur under combined stress conditions have been more clearly defined (Kıran and Baysal Furtana, 2023). In this context, characteristics that can be considered biomarkers, which are distinctive indicators of stress responses, have been identified, and their importance as biomarkers in revealing the effects of combined stress has been emphasized. Indeed, biomarkers play a critical role in determining plant tolerance not only to single stress conditions but also to combined stress conditions.

In this context, for the purpose of this study, examining prominent biomarkers that can be used to assess biological responses to drought and salinity stress in eggplant using correlation and polar heatmap analyses will significantly contribute to both elucidating tolerance mechanisms and providing guiding data for genotype selection.

MATERIALS AND METHODS

The study was carried out as pot experiments in a controlled glass greenhouse (22–26/18–22°C day-night temperature, 50–55% humidity). Seeds of open-pollinated 'Kemer' eggplant variety were used in the study. Seeds were sown in vermiculite-perlite (1:1) mixture, and 30 days later, seedlings with 3–4 true leaves (DAS-30 days after planting) were transplanted (8 seedlings/pot) to 7 L pots containing medium textured soil (N: 0.18%, P: 3.60%, K: 0.86%, pH: 7.75, EC: 1.28 dS m^{-1}). Seedlings were irrigated at field capacity until stress treatments. Field capacity was determined by leaving the pots saturated in water for 48 hours and draining the gravity water. Drought and salt stress treatments were initiated at 37 DAS according to a randomized plot design with four replicates. Control plants were irrigated at field capacity (FC), drought stress (DS) pots were irrigated at 60% FC, salt stress (SS) pots were irrigated with water containing 50 mM NaCl, and combined stress (DS + SS) pots were irrigated at 60% FC with 50 mM NaCl. Tap water (EC: 0.42 dS m^{-1}) was used for control and DS irrigation. The study was terminated at 75 DAS and sampling was performed.

Harvested plants were dried at 65°C until they reached constant weight and weighed to obtain SDW values. K^+ and Na^+ analyses were performed by microwave digestion (Kacar and Inal, 2008). Total phenolic content was determined by the Folin-Ciocalteu method (Singleton and Rossi, 1965), proline by Bates et al. (1973) and anthocyanin were determined according to the method of Mancinelli et al. (1975). Lipid peroxidation (malondialdehyde (MDA) as a lipid peroxidation product) was measured using the methods of Velikova et al. (2000) and Heath and Packer (1968). Superoxide dismutase (SOD), Catalase (CAT), and Glutathione reductase (GR) activities were determined using standard enzyme assays (Beyer and Fridovich, 1987; Çakmak and Marschner, 1992).

Data Analysis

Relationships between biological markers were visualized using a correlation diagram, and hierarchical clustering between data sets was represented by a polar heat map. Both visualizations were prepared using OriginPro 2024 (OriginLab Corporation, USA) software.

RESULTS AND DISCUSSION

In this study, correlation analysis was conducted to examine the relationships among traits considered as biomarkers. The correlation table reveals the multifaceted relationships between some important biochemical and physiological parameters examined in response to drought, salinity, and their combined treatments. Images of plants exposed to drought and salt stress, both individually and in combination, are presented in Figure 1, while a heatmap visualizing the correlation data is presented in Figure 2. Biomarkers were identified using a previous study (Kıran and Baysal Furtana, 2023) by selecting traits showing significant differences against stress conditions using ANOVA **(**analysis of variance**)** and then using PCA **(**principal component analysis**)** to identify parameters effective in distinguishing groups.

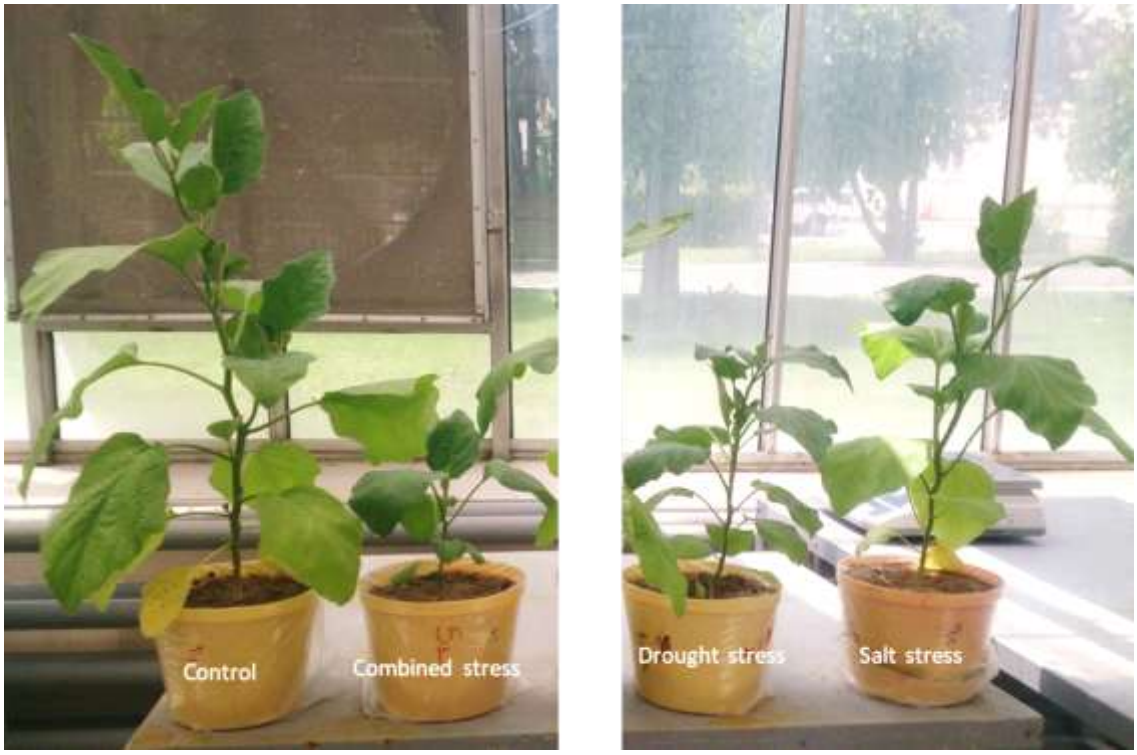

Figure 1. Eggplant plants exposed to single and combined applications of drought and salinity stress

According to correlation analysis, a positive relationship was observed between SDW (shoot dry weight) and anthocyanin (Anthc), while negative and significant correlations were found with proline, total phenolic compounds (Phenol), glutathione reductase enzyme (GR), and MDA. The positive relationship between SDW and anthocyanin levels may reflect the plant's adaptation to stress conditions by synthesizing anthocyanins. Anthocyanins, a potent antioxidant, protect cells from oxidative stress. Under combined stress, high anthocyanin accumulation contributes to the relative maintenance of plant metabolic activities and growth. Therefore, SDW increases under stress may occur. Results confirming this were also reported by Dabravolski et al. (2023).

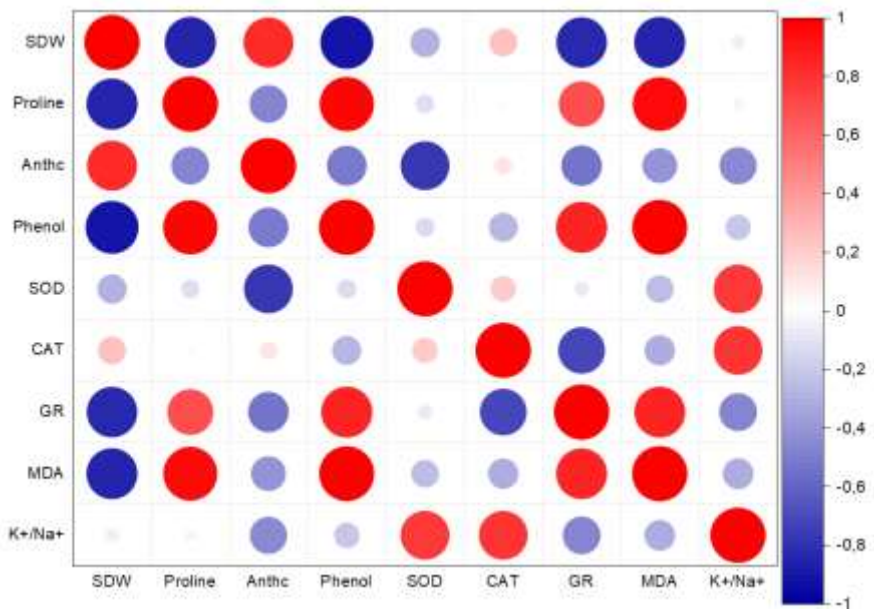

Figure 2. Correlation heatmap of biomarkers evaluated under combined stress ($p<0.05$).

However, proline generally accumulates in plant cells in response to stress, enabling osmoregulation. However, under the combined conditions of drought and salt, which create extreme stress, proline accumulation may indicate that the plant's growth capacity is reduced and that it is diverting necessary metabolites to stress tolerance. Therefore, a negative correlation with SDW is observed. Similarly, phenols also act as antioxidants under stress conditions; however, the severity of stress indicates that the plant is under severe oxidative stress and growth processes are suppressed. This indicates an inverse correlation with SDW.

On the other hand, MDA accumulation, an indicator of membrane lipid peroxidation, indicates that cell damage increases further under severe stress and negatively affects plant growth. This explains the negative correlation with SDW. The GR enzyme, which is effective in neutralizing MDA, is activated to reduce oxidative stress. However, high GR activity indicates that the plant is under intense stress and growth is significantly inhibited. In other words, plants expend energy on the stress response rather than growth to limit cellular damage; hence, a negative correlation with SDW arises. Indeed, Qadir et al. (2022), Soujoud et al. (2024) and Kiran et al. (2025) also reported negative relationships between SDW and proline, phenol, GR, and MDA.

The positive and significant correlation of proline with MDA and phenolic compounds is crucial for stress tolerance. Furthermore, while an increase in MDA levels indicates the severity of stress, proline accumulation also increases to alleviate the plant's stress load and reduce cellular damage (Kiran et al., 2025). In other words, as stress increases, both MDA and proline levels increase. Conversely, phenolic compounds, which are potent antioxidants, accumulate more, strengthening the plant's antioxidant defenses. This suggests that under combined stress, the plant simultaneously activates adaptive responses. Similar findings were also reported by Patanè et al. (2022) and Soujoud et al. (2024).

Phenolic compounds exhibited a significant positive correlation with MDA and GR. In stressed plants, phenolic compounds protect cells from reactive oxygen species (ROS) damage during oxidative stress (Carvalho et al., 2021). The positive correlation of phenolic compounds with MDA suggests that as stress intensity increases, the plant strengthens its antioxidant defenses by increasing phenolic compound synthesis (Zahedi et al., 2023). On the other hand, the GR enzyme plays an important role in combating oxidative stress. The positive correlation

of phenolic compounds with GR suggests that the plant simultaneously activates enzymatic and non-enzymatic antioxidant systems. This suggests that stressed plants activate both metabolic and enzymatic defense mechanisms simultaneously.

Among the antioxidant enzymes, SOD was found to have a significant negative correlation only with anthocyanin, while CAT showed a significant positive correlation only with the K^+/Na^+ ion ratio. SOD and CAT are enzymes that play important roles in antioxidant defense mechanisms against stress in plants. The negative correlation between SOD and anthocyanin in plants under combined stress indicates that the plant detoxifies some of the ROS under stress via anthocyanins, and therefore, SOD activity becomes relatively less critical. Indeed, similar findings were reported by Bigham Soostani et al. (2025) and Ullah et al. (2025).

On the other hand, the positive correlation between CAT and the K^+/Na^+ ratio suggests that the K^+/Na^+ ratio is an indicator of cellular ion balance and salinity tolerance. The positive correlation of CAT enzyme with this ratio suggests that under combined stress, including salinity stress, the plant increases CAT enzyme activity to maintain ion homeostasis and detoxify ROS (Zafar et al., 2024). Furthermore, the positive correlation of GR with proline suggests that proline not only maintains osmotic balance in plants under combined stress but also contributes to the antioxidative defense system together with GR. Furthermore, MDA was found to have a strong and positive correlation with GR. This suggests that as lipid peroxidation increases, oxidative stress in the cell also increases, which, in turn, enhances the conversion of GSSG to GSH, leading to further stimulation of GR (Rao et al., 2024).

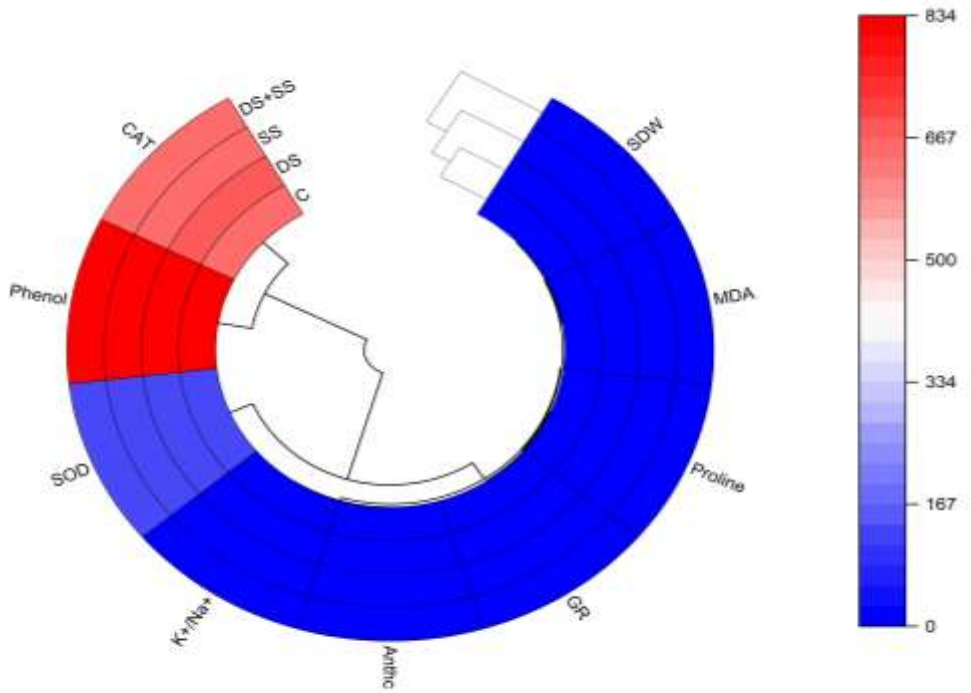

Figure 3. Polar heatmap with dendogram created to display associations between biomarkers evaluated under combined stress

The dendrogram heatmap visualizes similarities and relationships between parameters, revealing which traits change together. SDW, MDA, proline, GR, anthocyanin, and K^+/Na^+ are grouped in the same cluster in blue tones; blue indicates negative correlations or low values between these parameters. The presence of these parameters in the same cluster suggests that

these traits play a role in similar biological and metabolic processes in response to stress conditions. For example, the presence of SDW, MDA, proline, GR, anthocyanin, and K^+/Na^+ levels in the same cluster indicates that the plant simultaneously activates its growth, enzymatic and non-enzymatic antioxidant defense, and osmotic regulatory mechanisms (Sharma et al., 2022). This minimizes cellular damage and protects growth and metabolic functions (Dabravolski et al., 2023).

In contrast, the presence of GR and SOD in a different cluster suggests that this trait has a more independent response. This direct clustering suggests that these enzymatic antioxidant systems work together and in conjunction, while the observation of different shades of blue reflects that the two enzymes have relatively different activity levels in the stress response. This suggests that the capacity of enzymatic antioxidant mechanisms increases in the combined stress environment, where the most severe stress is observed in plants, thus enabling plants to achieve higher tolerance to combined stress. Indeed, it has been previously reported that plants activate their enzymatic antioxidant systems more intensely under stress. (Rao et al., 2024).

Similarly, the presence of phenolic compounds and the CAT enzyme in the same cluster and their positive correlation with high values reveal that non-enzymatic and enzymatic antioxidant mechanisms are activated simultaneously (Bigham Soostani et al., 2025). This finding suggests that plants provide more effective defense against oxidative stress by using antioxidant mechanisms more intensively under combined stress conditions (Sharma et al., 2022).

CONCLUSIONS

Correlation analysis between biological markers and a dendrogram heatmap displaying their relationships enabled a multifaceted assessment of biochemical and physiological responses to combined stress conditions. The polar heatmap and correlation analyses revealed that enzymatic biomarkers, in particular, are the most reliable and important markers for understanding stress responses. However, for practical applications, it has been demonstrated that more easily measurable parameters, such as phenolic compounds, proline, the K^+/Na^+ ratio, and MDA levels, can be used to assess stress tolerance. Therefore, they are recommended as reliable and practical indicators for assessing stress tolerance and selecting tolerant genotypes.

Authors' Contributions

All authors contributed equally to the design, data collection, analysis, and writing of the manuscript.

Conflict of Interests

The authors declare no conflict of interest.

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A RESEARCH ON IN VITRO MICROPROPGATION OF GERANIUM (Pelargonium graveolens)

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ABSTRACT

Geranium (*Pelargonium graveolens*) is a perennial, herbaceous shrub-like aromatic plant species that contains essential oil in its leaves and flowers. The essential oil of the geranium (*Pelargonium graveolens*) plant is widely used in the perfume and cosmetics industries. The demand for high-quality geranium essential oil is increasing day by day. However, in Turkey, geranium plants grown for landscaping purposes are not cultivated for the essential oil industry. As a result of verbal discussions with the essential oil industry in Turkey, it has been concluded that the widespread cultivation of geranium plants is necessary. It is understood that the export potential of lavender essential oil currently imported from abroad is also high. The rose-scented "Bourbon" variety of the lavender plant is preferred in the perfume and cosmetics industries. However, since this variety is a hybrid, it does not produce fertile seeds, meaning seed production is not possible. Therefore, the plant is propagated vegetatively. To promote the widespread cultivation of geranium in Turkey, it is necessary to develop and optimize a rapid, healthy, and mass micro-propagation method. This project was conducted to determine the appropriate nutrient medium combination for *in vitro* propagation of the geranium plant and to identify the suitable rooting medium for *in vitro*-propagated plantlets. To serve the propose MS medium combined with 1 BAP (mg/l) + 0.01 IBA (mg/l), 2 BAP (mg/l) + 0.02 IBA (mg/l) and Vacin &Went medium combined with 0.5 BAP (mg/l) + 0.01 IBA (mg/l), 1 BAP (mg/l) + 0.01 IBA (mg/l), 2 BAP + 0.02 IBA (mg/l). Results revealed that MS medium supplemented with 0.5 BAP+ 0.01 IBA mg/l gave the best result regarding number of shoots, leaves and shoot length and MS0 media was good enough for rooting.

Keywords: Geranium, micropropagation, *in vitro*, shoot and leaf formation, number of leaves, shoot length

INTRODUCTION

Pelargonium spp. belongs to the *Geraniaceae* family and is commonly known as geranium. There are approximately 280 species within the genus Pelargonium, and these species are predominantly native to South Africa (Kellen, 1983). According to research, *Pelargonium zonale* was introduced to Europe in the early 1600s (Craig, 1960; Ewart, 1981). Although there are 280 different species in the *Pelargonium* genus, only a limited number of them are commercially important in terms of flower color, species, leaves, and growth habits. Although these commercially important species differ according to the literature, they are generally divided into four groups: *Pelargonium* x *hortorum*, *Pelargonium* x *domesticum*, *Pelargonium peltatum*, and scented geraniums *Pelargonium* sp.

Pelarganium forms part of many high-grade perfumes, has antifungal, insect repellent and antibacterial activity, so it has widespread use in the field of medicinal and agricultural

chemicals. The oil is non-toxic, non-irritating and generally does not cause sensitization in humans. The oil is a sought-after ingredient in cosmetic products such as soaps, colognes, lotions and creams because of its "rose-like" floral odour. It stands out with its relaxing effects in the field of aromatherapy; its effects on conditions such as stress and anxiety have also been reported. Geranium oil has GRAS (Generally Recognized As Safe) status by the US Food and Drug Administration (FDA) and is used as a flavor additive (Boukhatem et al., 2013). Thanks to its anti-microbial properties, it is considered as a natural preservative agent in food preservation. In addition, leaves and flowers, tea, sweets, jelly, syrups, and tobacco are used as flavoring.

Pelargonium plants are cultivated for their floricultural value as well as their use as a source of essential oil. World production of Pelargonium oil is estimated at 250-300 tons, while the demand is over 800 tons per year. Therefore, both for the production of oil for various industries and for the utilization of *Pelargonium graveolens* for various other purposes, it is clear that there is a great demand for Pelargonium plants, which requires large-scale cultivation. In summary; *Pelargonium graveolens* is a plant of high economic and industrial importance not only for its aroma chemical composition and strong biological activity profile, but also for its economic production potential, global demand and versatile uses. It offers a strategic agricultural and industrial opportunity for potential producers such as Türkiye.

Pelargonium is traditionally propagated by stem cuttings or by seed. Regarding propagation by seed; although seed-propagated varieties of *Pelargonium* have become a popular alternative, they lack some of the agronomically superior and important characteristics found in vegetatively propagated varieties, such as semi-double flowers and lack of fragmentation (Harney, 1982). It is also important to emphasize that *Pelargonium* seeds are very expensive (Stokes Growers Guide, 2000). When the issue come to propagation by cuttings it is worth of stating that a limited number of cuttings can be taken from each mother plant. Propagation by cuttings carries a high risk of infectious diseases, e.g. soil-borne pathogens (Fusarium, Pythium) (Mastalerz, 1971). In addition, there is a seasonal dependence, so that the taking of cuttings is sensitive to environmental factors such as temperature and light; therefore, year-round production is difficult. In summary, propagation by cuttings has significant limitations in practical use. *Pelargonium* propagation from seed can be difficult for several reasons. Due to cross-pollination it can be difficult to know whether the seed is of the true type. The long seed germination time can also be a problem for some geranium species. In addition, propagation from seeds can alter the genetic composition of the plant, which may result in undesirable changes in terms of oil composition or yield. This may make it unsuitable as a source of oil for the perfumery, cosmetic or pharmaceutical industry, where it is mainly grown.

The aforementioned obstacles associated with large-scale propagation of *Pelargonium* can be overcome by using *in vitro* propagation techniques. For example the use of in vitro propagation techniques in the propagation of high-value Pelargonium plants is of great importance for obtaining pathogen-free plants (Laughner, 1993). Compared to the above-mentioned negative aspects of seed and cuttings propagation, in vitro nodal culture can be used to obtain Pelargonium plants that are true to their name and have genetic stability and stability. In this sense, it is important to optimize an efficient and cost-effective, sustainable propagation and acclimatization protocol for *in vitro* micropropagation of Pelargonium plants and this research was carried out for this purpose.

MATERIAL AND METHOD

Plant material

Plant material used in the experiments was introduced from abroad by Güney Agripark Ltd and all work was carried out in the laboratories and greenhouses of Güney Agripark Ltd. The donor plants from which the explants were taken were watered regularly every day and treated with a 1.5% fungicide solution every two weeks until they were used as explant sources, ensuring they were grown in a physiologically balanced and healthy manner.

Culture media and hormonal treatments

Murashige & Skoog (1962) (MS) and Vacin & Went (1949) stock solutions were used as basic media for macro, micro and vitamin compositions. Stock solutions were prepared by dissolving the appropriate amount of macro and micro nutrients and organic supplements using distilled water. Similarly, BAP and IBA growth regulator stock solutions were prepared in a 1/1 ratio (1 mg/1 ml) and dissolved with 1 N NaOH or ethanol and the total volume was adjusted using distilled water. The pH of the medium was adjusted to 5.7 with 0.1 N NaOH or 0.1 N HCl as required and 0.7 g/l agar was added to the medium and boiled to mix the agar with the medium. The concentration of sucrose used in the medium was 3%. Finally, the medium was sterilized by autoclaving at 121°C for 20 minutes under 2 atm. pressure.

In the study, combinations were created using two different culture media and three different hormone concentrations (see Table 1). Each different combination was repeated five times.

Table 1. Media compositions used in the research

MS 0
MS 0.5 mg/l BAP + 0.01 mg/l IBA
MS 1 mg/l BAP + 0.01 IBA
MS 2 mg/l BAP + 0.02 mg/l IBA
Vacin & Went 0
Vacin &Went 0.5 mg/l + BAP 0.01 mg/l IBA
Vacin &Went 1 mg/l BAP + 0.01 mg/l IBA
Vacin &Went 2 mg/l BAP + 0.02 mg/l IBA

Sterilization

Healthy explants taken for sterilization were first washed with tap water for 30 minutes. The fresh shoots of the washed explants were cut at a length of about 10 cm including the internodes and internodes. The washed and cut explants were placed in a sterile cabinet and treated with 70% ethanol for 1 min and then a drop of Tween-20 was added, and sterilization experiments were carried out to determine the appropriate sterilization conditions by keeping them in 20%, 30% NaClO concentrations in 5, 15 and 20 minutes.

Culture conditions

Sterilized nodes were cultured in MS medium and Vacin & Went medium prepared at different hormone concentrations. One node explant was used for each culture. Culture jars were incubated under a cold white fluorescent lamp (4000 lux) at 24 ± 2 °C with 16/8 h light/dark cycles. The media were renewed two weeks after the internodes were planted. Data on the number of internodes that started to proliferate were recorded after four weeks of culture.

RESULTS AND DISCUSSION

The sterilization tests table and results for *Pelargonium graveolens* are given in Table 2. As can be seen from the table, the best result for sterilization of *Pelargonium graveolens* plants was obtained by sterilization with 30% concentration of sodium hypochlorite for 20 min and the rate of contamination-free plants was 90%.

Table 2. Results of different sterilization treatments

NaClO concentrations	5 min.	15 min.	20 min.
% 20	% 50	% 65	% 80
% 30	% 60	% 75	% 90

Sterilization results clearly showed that increasing the NaClO concentrations and duration increase the percentage of no contaminated plants in other words number of clean plants.

Regarding the experiments conducted the find out the best media and hormone concentration and combination, as also stated above, MS (Murashige & Skoog, 1962) and Vacin & Went (1949) media were used as basic media for *in vitro* shoot regeneration experiment. Agar (0.7%) was used as solidifier and sucrose (3%) was used as carbon source and the results obtained are given in the table below.

Table 3. Results obtained for *in vitro* shoot regeneration experiments in different media combinations

Media composition	Number of Leaves	Number of Shoots	Shoot Length
MS 0	3,0	2,0	2,5
MS 0.5 mg/l BAP 0.01 mg/l IBA	10	3,5	4,0
MS 1 mg/l BAP + 0.01 mg/l IBA	6,0	2,0	3,0
MS 2 mg/l BAP + 0.02 mg/l IBA	7,5	2,5	2,0
Vacin & Went 0	3,0	1,2	1,1
Vacin &Went 0.5 mg/l BAP + 0.01 mg/l IBA	6,9	2,3	3,1
Vacin &Went 1 mg/l BAP + 0.01 mg/l IBA	4,8	1,6	2,2
Vacin &Went 2 mg/l BAP + 0.02 mg/l IBA	4,2	1,4	2,0

As can be seen from the table, the best result in terms of the parameters examined was obtained in MS 0.5mg/l + BAP 0.01mg/l IBA medium.

Figure 1. Some pictures from *in vitro* shoot regeneration studies

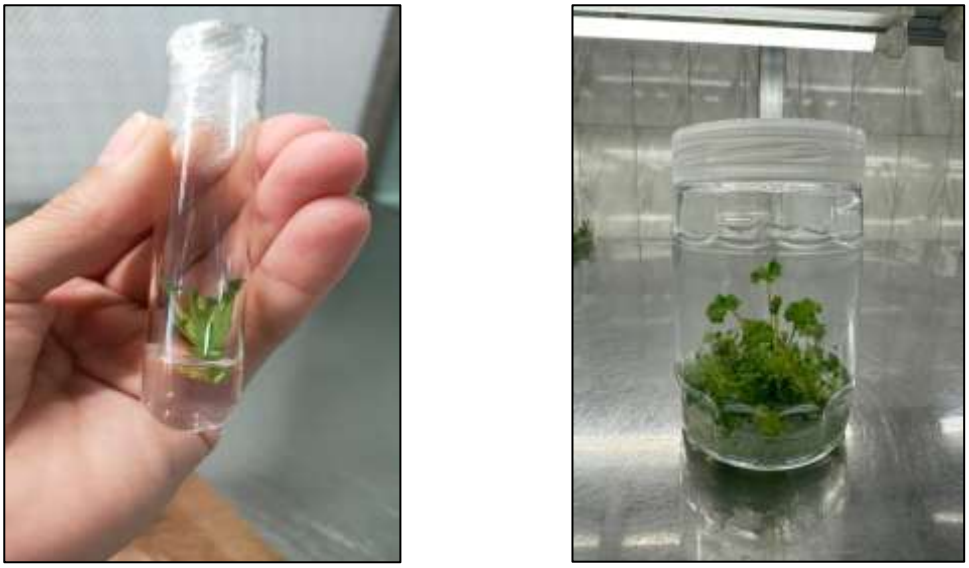

Figure 2. Shoots obtained from the nodes used as explants in *in vitro* culture studies

Results obtained revealed that the best results regarding number of leaves, number of shoots and shoot length obtained MS basic medium was better than Vacin & Went medium if one makes comparison between two basic media (MS and Vacin & Went). As it also clearly seen from the results given in Table 3, low amount of BAP combined with low amount of IBA gave better results than high amount of BAP and IBA in MS basic medium yet again in terms of number of leaves, number of shoots and shoot length. As it is known, the classical rule for shoot propagation: cytokinin dominance stimulates axillary buds, low auxin promotes shoot elongation, whereas high auxin pushes to the root or callus. The chosen dose/ratio, the ratio of 0.5 mg/l BAP to 0.01 mg/l IBA, provides a high but below toxic threshold cytokinin effect for axillary shoot stimulation, while very low IBA allows shoot elongation without forming weak

callus. Therefore, the ratio of 0.5 mg/l BAP to 0.01 mg/l IBA might have resulted in better shoot number, leaf number and shoot length at the same time.

As it is also known MS medium provides a richer supply of total nitrogen (especially NO_3^- + NH_4^+) and macro/micro elements compared to Vacin & Went. This composition is generally more favorable for rapidly dividing meristematic tissues and axillary shoot emergence. High nitrate and balanced ammonium favour an increase in protein synthesis/chlorophyll and thus leaf number + shoot length. When the same BAP/IBA ratios are tested on VW medium, the performance of Vacin & Went medium can therefore be expected to lag behind due to lower osmotic/ionic support and micronutrient differences. In addition, VW is essentially designed for different nutritional needs such as orchid cultures; total nitrogen, ammonium/nitrate balance and sulphate/phosphate levels are different from MS. In this sense, the high basal salinity and microelement profile of MS in shrubby, fast vegetative mass-forming aromatic species such as Geranium usually gives more shoots + longer shoots. Therefore, the proliferation/extension observed in VW at the same hormone ratios may have been weak in comparison.

Subculturing studies were conducted which was conducted to increase number of plants in MS basic medium combined with 0.5 mg/l BAP + 0.01 mg/l IBA as stated medium and hormone combinations gave better results than other combinations. In subculture micro-propagation studies using MS 0.5 mg/l BAP + 0.01 mg/l IBA medium, 6 times more plants were obtained at the end of the 4th subculture as can be seen from down given pictures.

Figure 2. Increased number of plants in jars after subculturing

Regarding *in vitro* rooting studies; MS0 was used in the rooting stage since the plants were observed to root even in the propagation stage. 100% success was achieved. The rooting of the shoots obtained was realised 7 days after rooting in MS0 medium. The average root number of the shoots was 3.75 cm and the average root length was 1.30 cm in the specified environment.

For acclimatization studies, *in vitro* rooted plantlets rooted in hormone-free MS medium were removed from the culture jar and washed under tap water to remove agar from the roots. The washed plantlets were planted in vials containing sterilised sand, soil and peat mixture (2:2:1) and covered in the greenhouse for about two weeks for acclimatisation. Then, the cover was removed and kept in the greenhouse for two weeks.

Figure 3. *In vitro* propagated Pelargonium plants which were acclimatized in the greenhouse by removing the cover

After the cover was removed, the plants were kept in the greenhouse for two weeks and then placed in pots and placed outdoors under normal climatic conditions. Stated acclimatization protocol was found to be very effective and successful for *in vitro* micropropagated *Pelargonium* plants. Planting in a mixture of sterilized sand:soil:peat (2:2:1) during acclimatization seems to have worked very well and this may be due to the substrate properties and the proportions used. Probably the sand (2 parts) improved drainage and aeration and prevented hypoxia in the roots, the soil (2 parts) provided mechanical adhesion and basal nutrient buffer, and the peat (1 part) with its high-water retention capacity may have prevented water stress to the *in vitro* formed leaves accustomed to high humidity.

CONCLUSIONS

At the end of the research, a sustainable micropropagation, rooting and acclimatization method with a high multiplication coefficient for obtaining healthy and mass plant material in Pelargonium plants under *in vitro* conditions has been successfully developed. This study demonstrates that MS medium supplemented with 0.5 mg/l BAP and 0.01 mg/l IBA is optimal for the *in vitro* micropropagation of *Pelargonium graveolens*. The findings can facilitate large-scale propagation of elite cultivars and support Türkiye's efforts to expand its aromatic plant industry and reduce dependence on imported essential oils.

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A RESEARCH ON IN VITRO MICROPROPGATION OF GINGER (Zingiber officinale Roscoe)

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ABSTRACT

Medicinal plants have long been used to treat various diseases. Ginger (*Zingiber officinale* Roscoe), a herbal plant from the *Zingiberaceae* family, is one of these plants. It is used as a spice and medicine in the treatment of cancer; cardiovascular diseases; diabetes; and some other diseases such as colds, nausea, asthma, and coughs. Polyphenols and flavonoids, which are found in high amounts in leaves, stems and rhizomes, play an important role in the pharmacological effect of ginger. These polyphenols and flavonoid compounds are natural sources of antioxidants. Ginger rhizome is a rich source of these antioxidants and is also naturally used in food preservation due to its antimicrobial activity. Due to all these unique properties, the demand for ginger is increasing in the world markets. Due to the rapid increase in soil diseases, the yield of ginger, which is an expensive product, decreases in rhizome production. Tissue culture method provides disease-free plants. In addition, a large number of plants can be obtained in a very short time with tissue culture technique. The present research was aimed to determine the optimum conditions for ginger production by tissue culture. For *in vitro* shoot multiplication, ginger shoots were sterilized, and explants cut approximately 0.5-0.8 cm were cultured in 35 mL jars containing different hormones and dosages of MS medium supplemented with 30 g L^{-1} sucrose and solidified with 2.5 g L^{-1} gelrite. MS medium supplemented with different types of plant growth regulators (IBA and IBA + IAA combinations), and medium MS0 as a control were used for shoot multiplication. MS medium supplemented with 4.5 mg L^{-1} BAP was found to be the best media composition regarding number of shoots, leaves and shoot length and MS0 medium was found to be good enough for rooting.

Keywords: Ginger, micropropagation, *in vitro*, shoot and leaf formation, number of leaves, shoot length

INTRODUCTION

Medicinal and aromatic plants have recently attracted a great deal of attention worldwide and that is why there is a high demand for medicinal and aromatic plants. In response to the increase on some diseases such as covid, cancer; cardiovascular diseases; diabetes, medicinal and aromatic plants are considered as a rich source of important compounds to be used in drug development. Due to that fact some medicinal and aromatic plants in danger of extinction due to this overharvesting (Kumari and Priya, 2020). One of the most important medicinal and aromatic plant is ginger (*Zingiber officinale* Roscoe) which belongs to family *Zingiberaceae*. Ginger is valued as an important spice thanks to its nutritional and medicinal properties (Devina et al., 2016) and that is why it is cultivated in many countries, including India, China, Japan, Indonesia, Australia, Nigeria and the West Indie, generally as a perennial plant. Although different authors reported different numbers about the total number of species and genera it is

assumed that there are 51 genera and 1500 species (Newman, 2001). Ginger has many fibrous roots, leafy pseudostems and an underground root rhizome, which is used for many purposes such as vegetative propagation and storage of foodstuffs (Ravindran and Nirmal Babu, 2005). Ginger is being sold as an herbal supplement in the United States, as reported by Gang and Ma, (2008). It is a known fact that Ginger (*Zingiber officinale* Roscoe) is being used as a spice and in traditional medicine for the treatment of some diseases such as cancer; cardiovascular diseases; diabetes; and several other diseases. It has been also reported to help alleviate severe virus symptoms during the COVID-19 pandemic and reduce the recovery time in these patients. It is reported that polyphenols and flavonoids, which are found in high amounts in leaves, stems and rhizomes, play an important role in the pharmacological effect of ginger since these polyphenols and flavonoid compounds are natural antioxidant sources. Since ginger rhizomes are a rich source of these antioxidants, they are also naturally used in food preservation due to its antimicrobial activity.

Due to all these unique properties, the demand of ginger in the world markets is increasing considerably. In this sense, abundant and high quality starting material is needed in a short time. Ginger is an infertile species not suitable for seed production. Therefore, it cannot be propagated sexually as its rhizomes are used for vegetative reproduction. Since rhizomes are an economically utilized part of the plant, the high use of ginger rhizomes as starting material for growing the plant in the next growing season has a negative impact on its supply in the market. More over quite some of the diseases are easily transmitted and transmitted from rhizomes through vegetative propagation. In addition, the bulkiness of ginger rhizomes as planting material makes their processing costly and laborious (Shaaban et al, 2023). Therefore, *in vitro* propagation can be a good alternative for propagation of ginger (Behera et al., 2010). Long-term vegetative propagation of ginger, which is mostly produced by rhizomes, causes weakening of the rhizomes. Ginger is also one of the most difficult plants to propagate from seed due to poor seed set with flowering (Mosie, 2019). For these reasons, it is necessary to produce a high amount of disease-free plants in a short time for commercial ginger cultivation. On the other hand, using *in vitro* techniques, it is possible to produce millions of disease-free plants with the same genotype in a short time. In this study, it was aimed to develop a protocol for rapid and high-rate *in vitro* propagation of ginger plant using different plant growth regulators. With the tissue culture method, pathogen-free plant production may be possible after optimization of the media, thousands of plants can be obtained in a short time with high reproduction rate, year-round production can be carried out without seasonal restrictions, genetic homogeneity can be achieved in the reproduction material thanks to this reproduction method and as a natural result of all these, yield increase in production can be achieved and quality plant material suitable for the pharmaceutical and cosmetic industry can be obtained.

MATERIAL AND METHOD

Healthy ginger rhizomes were obtained from Gazipaşa, Antalya and all studies were carried out in the laboratories and greenhouses of Güney Agripark Ltd. Ginger rhizomes were kept in a moist, sterilized sand medium at 25 ± 2°C in the dark for 3 weeks to germinate the buds and the rhizome was allowed to germinate. Buds (1-1.5 cm) sprouted from the rhizome were cut from the rhizome and used as explants. The cut buds were washed thoroughly with a few drops of liquid detergent and 2-4 drops of Tween 20 under running tap water for 30 minutes. After washing, rhizome sprouted bud explants were surface sterilized with 70% (v/v) ethanol for 1 min. These explants were then surface sterilized with two different concentrations of commercial bleach (20% and 30%) with two drops of Tween-20 for 5, 15 and 20 min immersion time. Surface sterilization of explants with 70% ethanol for 1 min was considered as control treatment. Finally, surface sterilized explants were rinsed 4-5 times with sterilized distilled water. After sterilization explants cut approximately 0.5-0.8 cm were cultured in 35 mL jars

containing different hormones and dosages of MS medium supplemented with 30 g L^{-1} sucrose and solidified with 2.5 g L^{-1} gelrite. The pH of all media was adjusted to 5.8 using 0.1 N NaOH or HCl solutions before adding gel rite. They were then autoclaved at 121 ºC and 2 atm pressure for 20 min. For all cultures, a light intensity of approximately 4000 lux was maintained in the culture room at 25 ± 2 °C under a 16 h photoperiod.

In micropropagation studies, Murashige & Skoog (1962) (MS) basic medium was used and the following hormones and combinations were tested (see Table 1.)

Table 1. MS basic media and hormone combinations used in propagation studies

MC	MS0
M1	1.5 mg L^{-1}BAP
M2	2.5 mg L^{-1}BAP
M3	3.5 mg L^{-1}BAP
M4	4.5 mg L^{-1}BAP
M5	0.4 mg L^{-1}BAP + 0.2 mg L^{-1} IAA
M6	0.6 mg L^{-1}BAP + 0.2 mg L^{-1} IAA
M7	0.8 mg L^{-1}BAP + 0.2 mg L^{-1} IAA
M8	1.0 mg L^{-1}BAP + 0.2 mg L^{-1} IAA

The media were evaluated according to the following parameters.

- Number of shoots/explant
- Number of leaves/shoot
- Shoot length (cm)

The medium composition which was found to be successful in terms of above stated parameters was used for propagation and was also used to determine the propagation coefficients depending on the number of subcultures.

RESULTS AND DISCUSSION

Regarding sterilization studies, as it can be clearly seen from Table 2, the best result in the sterilization studies of *Zingiber officinale* plants was obtained by sterilization at 30% concentration of sodium hypochlorite for 20 minutes, as can be clearly seen in the table below. Under the specified conditions, the success rate of the plants to remain sterile was measured as 96%.

Table 2. Results obtained from sterilization studies

NaOCl doses	5 minutes	15 minutes	20 minutes
% 20	% 60	% 70	% 80
% 30	% 70	% 80	% 96

NaOCl is a chlorine-based oxidative disinfectant; it kills or removes microorganisms by oxidizing proteins and cell membranes. 20% NaOCl and/or short duration (5-15 min) applications provided lower sterility. The reason for this may be that the lower concentration does not provide sufficient penetration, especially in hard tissues on the rhizome surface. 30% NaOCl is found to be a high enough concentration for sterilization application. When used in combination with Tween-20, it overcomes the waxy layer on the leaf and rhizome surface,

allowing better contact of the disinfectant with microorganisms. The 20-minute duration provided sufficient time to destroy surface contaminants (especially fungal spores and bacterial biofilms). Shorter duration may be ineffective against spores and resistant microorganisms.

The effects of different growth regulators added to the MS medium on shoot proliferation of *Zingiber officinale* is given in the Table 3 below based on hormone dosages and combination.

Table 3. The effects of different growth regulators added to the MS medium

Code	**MS medium**	**Number of shoots/explant**	**Number of leaves/shoot**	**Shoot length (cm)**
MC	MS0	1.00	3.25	2.73
M1	1.5 mg L^{-1} BAP	4.25	10.50	3.18
M2	2.5 mg L^{-1} BAP	6.00	11.00	2.93
M3	3.5 mg L^{-1} BAP	6.25	12.00	3,85
M4	4.5 mg L^{-1} BAP	8.00	15.50	4.10
M5	0.4 mg L^{-1} BAP + 0.2 mg L^{-1}IAA	2.25	7.75	4.03
M6	0.6 mg L^{-1} BAP + 0.2 mg L^{-1}IAA	3.25	9.75	3.33
M7	0.8 mg L^{-1}BAP + 0.2 mg L^{-1}IAA	4.00	8.25	3.38
M8	1.0 mg L^{-1} BAP + 0.2 mg L^{-1}IAA	3.00	6.50	2.28

As clearly seen in Table 3, the best results in terms of the investigated parameters were obtained with the addition of 4.5 mg L^{-1} BAP to the MS medium. This result indicates that cytokinins, especially 6-Benzylaminopurine (BAP), effectively trigger meristematic cell division and stimulation of axillary buds. The activity of BAP is known to result from the capacity of adenine-derived cytokinins to accelerate DNA synthesis, increase protein translation and regulate cellular metabolism. However, high concentrations of BAP may lead to undesirable physiological results such as hyperhydricity, callus formation and unbalanced organogenesis. In this study, 4.5 mg L^{-1} was determined as the optimal level and BAP concentrations above this level may cause adverse effects, although it was not tested in this present study. Regarding IBA and IAA hormone combinations, BAP + IAA combinations did not improve the multiplication coefficient at the dose-composition tested in the study and even caused a decrease in some parameters.

Figure 1. Some pictures from shoot proliferation studies

The total number of plants and growth coefficients obtained as a result of subculturing at 4-week intervals using MS + 4.5 mg L^{-1} BAP medium are given in the Table 4.

Table 4. Results of different subcultures by using MS + 4.5 mg L^{-1} BAP medium on obtained number of plants

Zingiber officinale	Number of Plants (Number)	Multiplication Coefficient
Start	300	
1st Subculture	800	2,6
2.Subculture	3600	4,5
3.Subculture	15120	4,2
4.Subculture	68040	4,5

Regarding rooting of obtained shoots in jars, the plantlets that reached a certain size were directly transferred to MS0 medium for rooting and were rooted without the need for any plant growth regulator (see Figure 2).

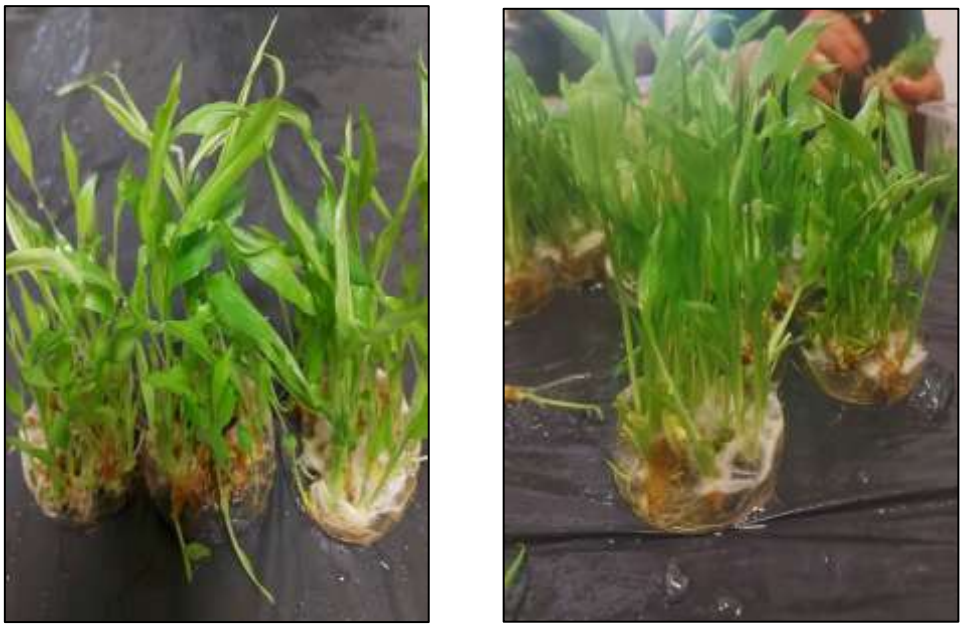

Figure 2. Rooted ginger plantlets in MS0 medium

In vitro shoots that have "reached a certain size" in development naturally synthesise auxins, mainly IAA (indole-3-acetic acid), in their apical/axillary meristems and nodal regions and transport them basipetally (tip to base). This endogenous auxin influx can provide the threshold level necessary for rhizogenesis (root initiation) for MS0 medium without the addition of external auxin.

Regarding acclimatization studies; *in vitro* grown plants were washed thoroughly with tap water to remove agar from the roots. They were then immersed in 0.3% fungicide solution for 15-20 min and then washed with tap water. The plantlets were then transplanted into the vials filled with peat. Care was taken not to damage the roots when the plants were transplanted

into the vials, they were moistened regularly at periodic intervals and acclimatized successfully under *ex vitro* conditions (see Figure 3 and 4).

Figure 3. Ginger plantlets which were removed from jars and washed thoroughly with tap water to remove agar from the roots

Figure 4. The plantlets which were transplanted into the vials filled with peat.

In vitro propagated ginger plants, which were regularly watered and acclimatized in peat-filled vials, were transferred to greenhouse conditions after 2 months, completed their growth and development in greenhouse conditions and the first rhizomes were successfully obtained after 8 months. The developed acclimatization protocol was successful because it managed the three critical risks of acclimatization (excessive water loss, root/crown rot, substrate incompatibility) with the correct sequence and appropriate protective steps. In this sense, agar removal → protective fungicide → peat medium → controlled humidity → greenhouse after 2 months → rhizome in 8 months. It is possible to say that the acclimatization method developed has a scientifically consistent and technically feasible sequence; in particular the principles of complete removal of agar, avoidance of root injury and gradual stressing explain the reported successful acclimatization and rhizome output at 8 months.

CONCLUSIONS

This research successfully established an efficient micropropagation protocol for *Zingiber officinale* using MS medium supplemented with 4.5 mg L^{-1} BAP was found to be the best media composition regarding number of shoots, leaves and shoot length and MS0 medium was found to be good enough for rooting. This composition significantly enhanced the number

of shoots, shoot length, and leaf development, offering a viable solution for mass propagation of disease-free and genetically uniform ginger plants. The study contributes to the commercial cultivation of ginger and provides a platform for further biotechnological interventions such as in vitro conservation, genetic transformation, and secondary metabolite production. Further studies may explore the rooting phase and acclimatization of *in vitro* plantlets under greenhouse conditions.

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AI AGAINST HONEY ADULTERATION: EFFECTIVE AND RELIABLE HONEY BOTANICAL ORIGIN AUTHENTICATION

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ABSTRACT

Honey is a highly valued, nutritious natural product well-known healing benefits. Due to their unique features and limited availability, monofloral honeys (from one predominant floral source) are highly demanded and prized. Unfortunately, this economic incentive brings along fraudulent activities, such as misrepresenting a common honey as a rarer monofloral type or adulteration with sugar syrups and low-grade honey. Therefore, the authentication of botanical origin and accurate detection of adulteration are critical for consumer trust and regulatory compliance. Although there are several approaches, such as physicochemical profiling, organoleptic evaluation, and spectral analysis, melissopalynology (microscopic pollen analysis) is the gold standard for verifying floral origin. Nevertheless, traditional methods of examining pollen in honey are slow, prone to human error, and subjective. In recent years, deep learning (DL) methods (particularly convolutional neural networks, CNNs) have achieved significant performance at microscopic pollen authentication. Along with study domains such as allergology, archeology, and forensics, honey authenticity analysis also benefits from these techniques. This study aims to evaluate the current status and future trends of these techniques to identify new opportunities to improve effective and reliable honey botanical origin authentication.

Keywords: Monofloral honey classification, Pollen classification, Melissopalynology, Microscopy, Deep learning, Explainable AI

INTRODUCTION

Honey is defined as a natural sweet substance produced by honey bees (Apis mellifera). Botanical Origin refers to the specific plant or flower sources from which the bees collected nectar. Monofloral/unifloral honey types are predominantly from one nectar source, often requiring a pollen proportion of over 45% for most species, and command a higher market value due to their unique organoleptic and therapeutic properties (Haidamus et al., 2019). Honey obtained from various types of flowers is referred to as multifloral/polyfloral, which has pollen grains from several plants, none of which are predominant, and is generally less expensive (Geană et al., 2020; Tsagkaris et al., 2021).

Monofloral honeys are highly valued not only for their unique sensory characteristics but also for their distinct healing effects such as antioxidant, antibacterial, anti-inflammatory, liver-protective, gastroprotective, and anticancer properties, which are directly influenced by their botanical origins (N. Li et al., 2024; Mărgăoan et al., 2021). Medicinal effects of monofloral honeys have been reported in several studies, highlighting their potential in functional foods and complementary medicine (Cianciosi et al., 2020). Antiproliferative effect of Manuka honey on murine melanoma, colorectal and human breast cancer cells; weight, adiposity, and triglyceride level reduction effects of Acacia honey are some of the reported results of the

previous studies in the domain (Mărgăoan et al., 2021). Due to these benefits, monofloral honey is a high-value commodity and is the third most adulterated food products globally (N. Li et al., 2024).

Dilution with sugar syrups or other cheaper substances (Banach et al., 2025; X. H. Zhang et al., 2023), mislabeling multiforal honeys as monofloral types, or blending with low-grade honey are the most commonly applied adulteration techniques to increase the yield and profit margins. These fraudulent practices not only compromise consumer trust but also pose significant challenges for regulatory authorities aiming to ensure food safety and authenticity (Ahmed, 2024; Da Silva et al., 2016).

Honey adulteration primarily poses numerous risks in terms of consumer health by compromising food safety standards. The consumption of honey adulterated with sucrose may increase the risk of diabetes, and undeclared components may be toxic or cause allergies (Fakhlaei et al., 2020; Samat et al., 2018). Consumers purchase honey based on its declared botanical and geographical origin, expecting specific taste, nutritional value, and medicinal properties. False labeling and adulteration deceive consumers, undermining their trust in the product and the market.

Honey authentication refers to the processes, including verification of true botanical and geographical origin and ensuring that it is free from any adulteration with sugar syrups and low-grade honey (Brar et al., 2023). Sensory (taste and smell) evaluation and visual (color depth, luster, and viscosity) inspection; analysis of physicochemical parameters such as acidity pH, electrical conductivity, specific quality indicator contents (moisture, brix, proline, 5-hydroxymethylfurfural (HMF), melissopalynological analysis (manual identification and quantification of pollen grains in microscopic images of honey samples) are widely used conventional methods within the scope of honey authentication (X. H. Zhang et al., 2023).

The botanical source significantly influences honey's chemical composition, flavor, aroma, nutritional value, health benefits, and market price. Within the scope of adulteration detection, honey botanical origin authentication is the critical process to verify the declared plant source of honey to ensure its quality, authenticity, and market value. Melissopalynology is the gold standard traditional method for authenticating the botanical origin of honey. While newer methods exist, melissopalynology remains the preferred analysis method that fulfills official standards and norms. For instance, this manual process is still cited as the most accurate method and the only one which fulfills official standards and norms in certain countries, such as Germany (Viertel and König, 2022). Despite this significance, due to its manual nature, melissopalynology is labor-intensive and slow. Besides, it requires considerable expertise and is prone to human error, which restricts its scalability for large-scale quality control.

In the last decade, artificial intelligence (AI), particularly deep learning (DL), based methods, emerged as powerful tools to enhance botanical origin authentication processes. By automating pattern analysis and image recognition, rapid and highly accurate AI-based solutions, often exceeding 90% and sometimes reaching near 100%, have been reported (Viertel and König, 2022).

Despite significant advancements, there are remaining challenges, such as substential risk of misclassifications for certain edge cases (e.g., morphologically similar pollen grains) (Banach et al., 2025). Ensuring that the accuracy and generalization capability of a model in botanical origin authentication is at the highest possible level is the indispensable requirement for effectiveness and reliability. Starting from here, in this study we will review the recent notable research studies of AI-based pollen grain analysis with a critical review of their methodologies and findings.

PALYNOLOGY AND MELISSOPALYNOLOGY

The study and analysis of pollen grains, known as palynology, is a broad field that plays an important role in various disciplines, including allergology, archaeology, and forensics, as well as honey production. Daily measurement and estimation of atmospheric pollen concentrations are critical processes to track allergenic pollen levels, enabling patients and healthcare providers to plan outdoor activities and manage treatments. Identification of fossil pollen preserved in sedimentary archives is a powerful proxy for reconstructing past vegetation and climate. Pollen specimens are valuable in forensic analysis, aiding in identifying individuals or locations due to their unique characteristics.

As the automated and rapid pollen identification solutions are required by all these domains, the focus of AI-based pollen identification studies is not limited to honey botanical origin identification. The common aspect of all these studies is using image datasets of several pollen types to train the models, to be used for the recognition of a certain pollen type. These images may be collected from various sources, including pollen-related books and websites, museum specimens, environmental samples, and directly from plants or honey samples.

Training of deep learning models needs pollen datasets with significant diversity and scale. Therefore, a small number of research groups constructed new datasets of the pollen types of their regions and even made them available (open access) to address this need. Table 1 presents the datasets commonly used by the studies in this domain.

Table 1. Pollen Datasets

Reference	Dataset	Source	Size and Types
(Gonçalves et al., 2016)	Polen23E	Honey (Brazil)	23 type of 805 images
(Astolfi et al., 2020)	Pollen73S	Flowers (Brazilian Savannah)	73 type of 2523 images
(Battiato et al., 2020)	Pollen13K	Airbone samples	5 types of 13.000 images
(Valiente et al., 2025)	Pollen24SP	Honey (Spain)	24 types of 32,285 images
(Tsiknakis et al., 2021)	Cretan Pollen Dataset (CPD)	Flowers (Crete)	20 type of 4.034 images
(Holt, 2020)	New Zealand Pollen Dataset (NZPD)	Mostly honey (New Zealand and the Pacific)	46 types of 19.500 images
(Rostami et al., 2025)	Great Basin Desert (Nevada) Pollen Dataset(GBDPD)	Herbarium collection, (UNRMNH)	6 types of 1353 images
(C. Li et al., 2023)	Urticaceae Family Dataset	Flowers and herbarium collection (NBC, Leiden)	5 types 6472 images

CPD: Cretan Pollen Dataset, NZPD: New Zealand Pollen Dataset, GBDPD: Great Basin Desert Pollen Dataset, UNRMNH: Reno Museum of Natural History, NBC: Naturalis Biodiversity Center

MACHINE LEARNING (ML) IN POLEN GRAIN ANALYSIS

Early studies utilizing artificial intelligence in pollen grain analysis used ML algorithms considering several physicochemical properties (Kibar, 2024; Mateo et al., 2021), mineral profiles (Al-Awadhi Mokhtar and Deshmukh, 2023), and spectral characteristics(Ahmed, 2024; Zhao et al., 2020) as features for the training of the models. Inspired by mellisopalynology,

some of those ML-based studies used extracted features from microscopic pollen images (Gonçalves et al., 2016; Marcos et al., 2015; Travieso et al., 2011).

In Gonçalves et al. (2016), a pollen dataset (POLEN23E), which was also constructed within the scope of the study, is used. This annotated image dataset for the Brazilian Savannah consisted of 805 images of 23 pollen types (Figure 1). The achieved overall accuracy performance using features such as shape, color, and texture was around ~64% accuracy, with higher than 90% accuracy for some pollen types.

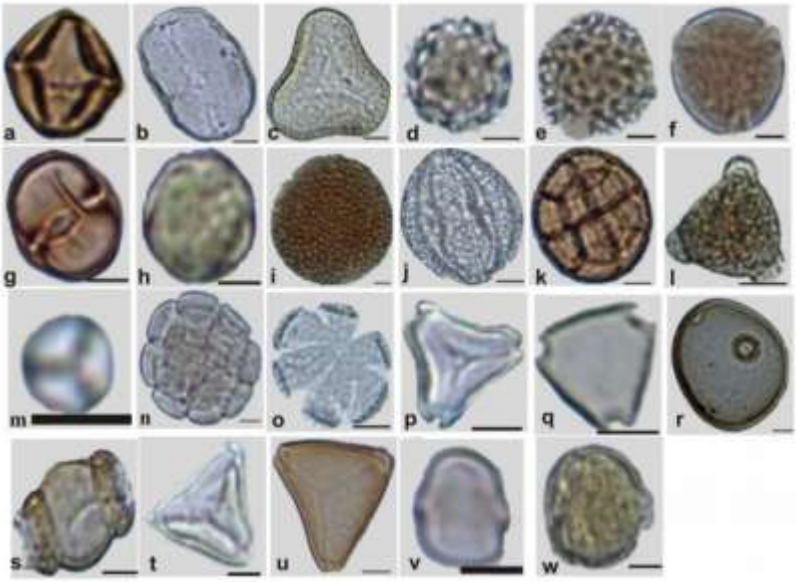

Figure 1**:** Sample images for each of the 23 pollen types a)Anacardiaceae: Schinus terebinthifolius; b-c) Arecaceae: b- Arecaceae; c- Syagrus. d-e) Asteraceae: d) Chromolaena laevigata; e) Tridax procumbens. f) Bignoniaceae: Fridericia florida. g) Burseraceae: Protium heptaphyllum. h) Combretaceae: Combretum discolor. i-j)Euphorbiaceae: i) Croton urucurana; j) Mabea fistulifera. k-n) Fabaceae: k) Anadenanthera colubrina; l) Dipteryx alata; m) Mimosa somnians; n)Senegalia plumosa; o) Lamiaceae: Hyptis. p-q) Myrtaceae: p) Eucalyptus; q) Myrcia. r) Poaceae: Urochloa decumbens. s) Rubiaceae: Faramea t-u) Sapindaceae: t) Matayba guianensis; u) Serjania laruotteana. v) Urticaceae: Cecropia pachystachya. w) Vochysiaceae: Qualea multiflora (Gonçalves et al., 2016)

Although accuracy performances exceeding 90% were achieved in similar studies (Marcos et al., 2015; Travieso et al., 2011) using morphological features such as texture and contour, ML-based methods have some drawbacks. Feature extraction work is labor-intensive and time-consuming. Besides, this fetaure extraction work needs to be repeated for the new pollen types to be included in the scope of the updated models. To overcome these drawbacks, domain studies in the last five years have focused on deep learning methods, which find the determinative features and patterns in the dataset on their own.

DEEP LEARNING (DL) IN POLEN GRAIN ANALYSIS

Due to its capability to extract patterns from high-dimensional data, DL has been widely applied to complex tasks such as computer vision. Convolutional Neural Networks (CNNs) are a class of deep neural networks particularly well-suited for image classification tasks. Their powerful neural network architectures automatically extract mid-to high-level features from image datasets. A significant challenge for training CNNs from scratch is the need for massive datasets, which are often unavailable in specialized fields like palynology. Transfer learning

addresses this challenge by utilizing the low-level feature (e.g., edges, colors, textures) identification capability of pre-trained models and fine-tuning them for the specific task by using small training datasets. In this section, we will review the recent notable research (see Table 2) that employs CNNs and transfer learning for the analysis of pollen grains. Along with the newly developed ones, the used CNN models in these studies are AlexNet (Krizhevsky et al., 2012), VGG (Simonyan and Zisserman, 2014), MobileNetV2 (Sandler et al., 2018), ResNet (He et al., 2015), InceptionV3 (Szegedy et al., 2016), Xception(Chollet, 2017), SE-ResNeXt (Hu et al., 2018), NASNet (Wistuba et al., 2019), Vision Transformer (ViT) (Dosovitskiy et al., 2020), DenseNet (Huang et al., 2017), EfficientNet (Tan and Le, 2021), and ResNeSt (H. Zhang et al., 2022).

Sevillano et al. (2020) collected 19,500 images of 46 pollen types from New Zealand and the Pacific regions, applied transfer learning with AlexNet for feature extraction and trained a linear discriminant classifier for final classification. Using 10-fold cross-validation, they achieved 97.86% accuracy on the validation set. Before the training stage, cropping with minimum padding, resizing, and rotation data augmentation techniques were applied to the dataset. Classification was more challenging for taxa with fewer images. Tsiknakis et al. (2022) applied transfer learning with four CNN architectures: InceptionV3, Xception, ResNet, and Inception-ResNet along with ensemble learning (hard and soft voting) to combine the predictive power of base models on the Cretan Pollen Dataset (CPD), which comprises more than 4000 pollen-grain images of 20 pollen types. Zero padding to standardize the image sizes, randomly adding Gaussian noise, linearly adjusting contrast and brightness, rotating, and translating the image were also applied as pre-procesing and data augmentation techniques. Ensemble learning improved robustness and sensitivity. They achieved 97,5% accuracy as the best performance by an ensemble of all models with soft voting, but this model performed poorly (weighted average AUC score of 0.52) for pollen grains extracted from honey samples. The misclassifications of models on both datasets were mainly attributed to morphological similarities (Figure 2). On the other hand, the effect of other elements (sugar, proteins), probable state (joined, swollen), and the distribution of the grains in honey sediment were also commented as the probable factors causing the steep drop in the performance for the honey-based samples. Therefore, the importance of honey-based datasets and robust pre-processing techniques was emphasized.

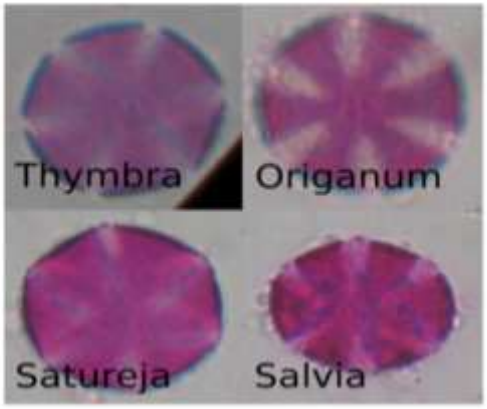

Figure 2. Pollen-grain images of pollen types with similar morphological characteristics (Tsiknakis et al., 2022)

Using 10,000 images of 40 pollen species of the Great Basin Desert, Nevada, USA, Rostami et al. (2023) applied transfer learning to pretrained CNN models, AlexNet, VGG-16, MobileNet-V2, ResNet, ResNeSt, SE-ResNeXt, and Vision Transformer (ViT) with various depths. An accuracy of 97.24% was achieved as the best performance by ResNeSt-110. They

also developed a new CNN-based model with six layers and achieved an accuracy of 91.87%. Z-stack imaging, cropping, resizing, noise removal, rotation, flips, zoom, rescaling, noise addition pre-processing and augmentation techniques were applied before the training stage.

Valiente et al. (2023) constructed a dataset of 16 types of 19.000 images obtained from Spanish citrus and rosemary honeys. They annotated the pollen images with a Java-based tool, HoneApp, which was also develeped within the scope of their study. Along with eight CNN models (VGG16, VGG19, InceptionV3, Xception, ResNet50, DenseNet201, MobileNetV2, EfficientNetV2M), they also developed, a new CNN-based model, PolleNetV1, with their dataset. InceptionV3 was the best-performing model with 97.99% accuracy. Their new model, PolleNetV1, which had significantly fewer parameters, faster, and achieved 95.66% accuracy. Another important aspect pointed out in this study is the 1–2% increase in accuracy performance for all the models before and after data augmentations of applying some affine transformations (rotation, translation, and reflection) to original images to increase the number of samples in the dataset. As the continuation of this study, Valiente et al. (2025) improved the dataset to 24 pollen and non-pollen classes of 32.285 images by including more Spanish honey types, and named it POLLEN24_SP. They also improved their PolleNetV1 model to PolleNetV2 and developed a lightweight variant of it (PolleNetV2.mobile). The best performance achieved by the nine CNN models (VGG16, VGG19, ResNet50, InceptionV3, Xception, DenseNet201, EfficientNetV2M, MobileNetV2, NASNet) was 98.03% by EfficientNetV2M. PolleNetV2 and PolleNetV2.mobile had 97.10% and 96.48% performances, respectively.

Rostami et al. (2025) applied nine CNN models: DenseNet201, EfficientNetV2S, InceptionV3, MobileNetV2, ResNet50, ResNet101, VGG16, VGG19 and Xception on 1.353 pollen images of 6 similar types collected from herbarium specimens at the University of Nevada, Reno Museum of Natural History (UNRMNH). All of the models achieved ≥ 96 % accuracy. The ResNet101 model had the best accuracy performance of 99%. Conversion to grayscale, thresholding, and morphological operations for noise removal, random rotations, flips, and zooming were used as pre-processing and data augmentation techniques.

Shamrat et al. (2024) used the New Zealand pollen dataset which has more than 19.000 images of 46 pollen types. They applied duplicate and poor-quality image removal, after which the number of images left was 19.612. Other pre-processing techniques applied were non-local means denoising and gamma correction to preserve fine texture and enhance contrast and normalize luminance, respectively. Along with four CNN models (AlexNet, MobileNet V2, VGG16, ResNet50) to be used with transfer learning, they also developed a new custom CNN model PollenNet, which achieved the best accuracy performance of 98.45%. The best performance achieved by the other models was 81.6% by MobileNet V2. They also applied Grad-CAM and saliency maps Explainable Artificial Intelligence techniques, to generate the heatmaps that reveal the regions and specific pixels with the most influence on the model's decision, respectively.

Figure 3 presents the correctly classified samples' Grad-CAM heatmaps, as red and blue regions representing the pixels which the model considers as the most and the least influential, respectively. This type of model explanation also serves of proof that models recognize and leverage distinct features of the images consistently, such as the red regions of central pollen structure in 'FucosporaFusca' and the edges in 'Kunzea'.

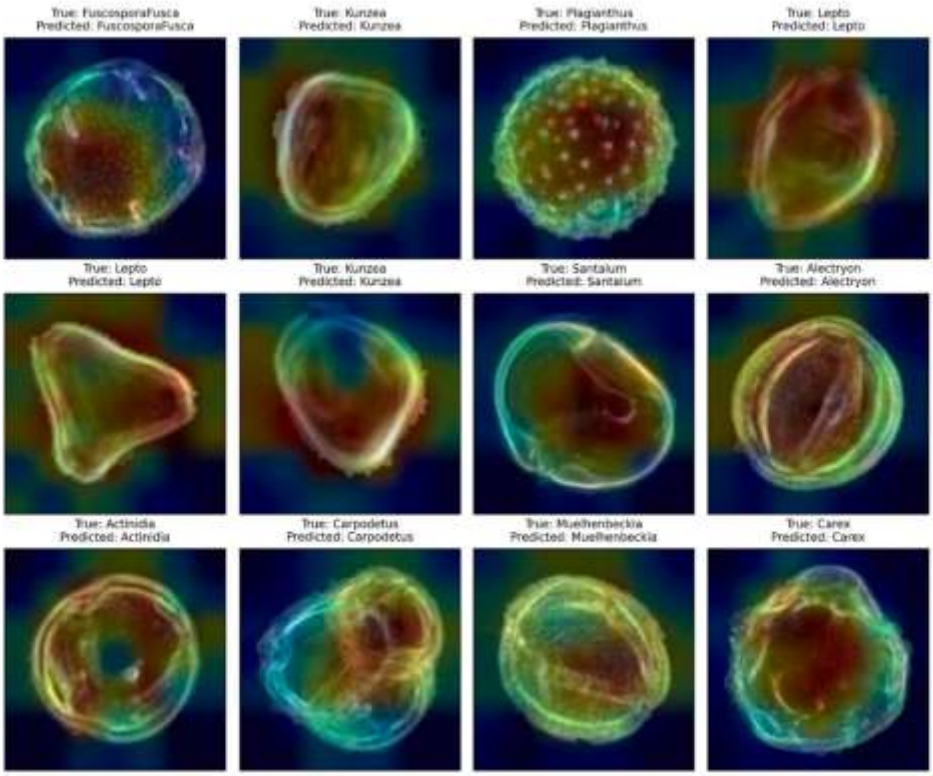

Figure 3: Grad-CAM heatmaps demonstrate PollenNet's accurate feature detection across various correctly classified pollen grains (Shamrat et al., 2024)

Figure 4 presents the Grad-CAM heatmaps of misclassified samples. Two of these cases, for Lepto and Kunzea with similar morphology, highlight that the misclassification might have been caused by the similarity that the model was unable to cope with to discern the types.

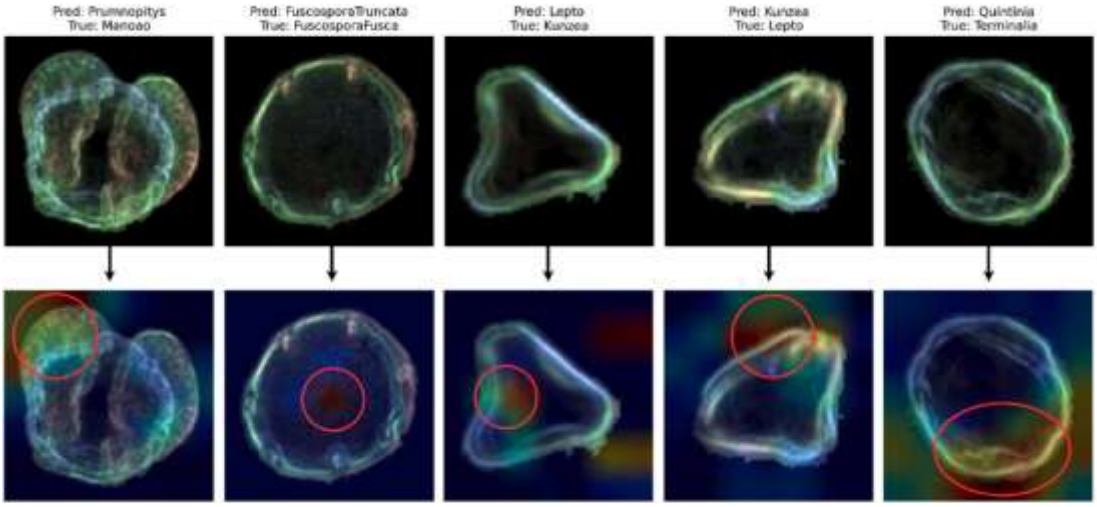

Figure 4: Grad-CAM heat maps on specific misclassified images by PollenNet showing regions impacting its predictions (Shamrat et al., 2024)

Table 2: Convolutional Neural Network (CNN) based Pollen Grain Analysis Studies

Ref.	**Dataset**	**Pre-processing and augmentation**	**Models**	**Best Performance**
(Sevillano et al., 2020)	46 types, 19.500 images (NZPD)	Cropping, resizing, rotation	AlexNet: feature extraction LDC: classification	(Acc.) 97.86%
(Tsiknakis et al., 2022)	4034 images, 20 types (CPD)	Zero padding, gaussian noise, contrast, brightness, rotating and translating.	Inception v3 , Xception , ResNet, Ensemble Learning	(weighted Auc) '-CPD:97.5 % -Honey polen-52%
(Rostami et al., 2023)	10,000 images of 40 types	Z-stack imaging, cropping, resizing, noise removal, rotation, flips, zoom, rescale, noise addition	AlexNet, VGG-16, MobileNet-V2, ResNet, ResNeSt , SE-ResNeXt, ViT, *new CNN*	(Acc.) -ResNeSt-110- 97.24%. -New CNN: 91.87%
(Valiente et al., 2023)	1900 images, 6 types	Image standardization, rotation, translation, reflection, zooom	VGG16, VGG19, InceptionV3, Xception, ResNet50, DenseNet201, MobileNetV2, EfficientNetV2M, *PolleNetV1*	(Acc.) -InceptionV3: 98% -PollentNet:95.66%
(Rostami et al., 2025)	1.353 images, 6 types (UNRMNH)	Grayscale conversion, noise removal, random rotations, flips, and zooming	DenseNet201, EfficientNetV2S, InceptionV3, MobileNetV2, ResNet50, ResNet101, VGG16, VGG19, Xception	(Acc.) - ResNet101: 99 %,
(Valiente et al., 2025)	24 types of 32,285 images (Pollen24_SP)	Image standardization, rotation, translation, reflection, zooom	VGG16, VGG19, ResNet50, InceptionV3, Xception, DenseNet201, EfficientNetV2M, MobileNetV2, NASNet	(Acc) -CNNs: 96–98 % -PolleNetV2: 97.1% -PolleNetV2.m:96.48 %
(Shamrat et al., 2024)	46 types, 19.500 images (NZPD)	Non-local means denoising and gamma correction	AlexNet , MobileNetV2, VGG16, ResNet50 and PollenNet (customized CNN)	(Acc) -MobileNetV2: 84.91 % -PollenNet: 98.45 %

Acc: Accuracy, Auc: Area Under the Curve, LDC: Linear Discreminant Classifier, CPD: Cretan Pollen Dataset, NZPD: New Zealand Pollen Dataset, GBDPD: Great Basin Desert Pollen Dataset, UNRMNH: Reno Museum of Natural History, NBC: Naturalis Biodiversity Center

DISCUSSION AND CONCLUSION

The accuracies of over 95% performance at pollen grain recognition make the CNN-based DL models strong candidates as effective solutions for honey botanical origin authentication. Several remarkable points contribute to the accuracy performances. Improved performance by ensembling the models contributed to the accuracy (Tsiknakis et al., 2022). For the studies, involving the development of a new CNN model (Rostami et al., 2023; Shamrat et al., 2024; Valiente et al., 2023, 2025), the only one with a better accuracy than the CNN and transfer-learning based approach was (Shamrat et al., 2024), which had a dataset of size greater than the others. In (Valiente et al., 2023), the significant increase (1-2%) in performance obtained after data augmentation of the same data set clearly highlights the importance of the factors contributing to the reliability, size, and diversity of the dataset. None of the pre-trained CNN models came into prominence, considering the studies evaluated. Morphological similarity of pollen grains was commented on as a significant challenge in all the studies. Tsiknakis et al. (2022) and Shamrat et al. (2024) emphasized this fact, by exposing the misclassified pollen types with similar morphologies. Multi-modal models, combining standard microscopic image data with other physicochemical or spectral measurements (data fusion), could be a good approach for distinguishing between species that have subtle morphological differences, as commented on by the studies in the domain other than the ones we evaluated (Rajendran & Mahadevan, 2025).

Explainable Artificial Intelligence (XAI) is another critical point that significantly enhances the reliability of deep learning (DL) models in pollen grain classification by assisting in confirming whether the deep learning model is making classification decisions based on the actual, relevant morphological features of the pollen grain, rather than irrelevant artifacts. Besides, the misclassification examples exposed in Shamrat et al. (2024), as Grad-CAM visualizations, highlight the capability of XAI-based approaches to indicate regions that a model fails and guide efforts to improve the model or the dataset.

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CONSERVED MOTIFS IDENTIFICATION OF THIONIN PROTEIN OF TWENTY-SIX PLANT SPECIES

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ABSTRACT

Thionins are an ancient family of plant-derived antimicrobial peptides, where their activity is dependent not only on their overall structure, but also on the presence of highly conserved sequence motifs. These motifs have been conserved through wide taxa and suggest strong evolutionary pressure to maintain critical domains possibly involved in membrane disruption and pathogen recognition. While the biological roles of thionins are well studied, there are few studies investigating molecular explanations for motif conservation. An exploration of these conserved elements would afford a better understanding of the structure-function correlation of thionins and their significance in adaptation in plant defense systems. In this study, a total of twenty-six thionin proteins sequences of plants family belonging to different orders were retrieved from NCBI with FASTA format. Amino acids sequence patterns were created using MEME (Multiple Em for Motif Elicitation; version 5.5.8) and MAST (Motif Alignment and Search Tool; version 5.5.8) programs on http://meme-suite.org. The program was designed to report the 10 most robust motifs of 10 to 30 width Amino acids, occurring zero or once per sequence, with detection spanning between 2 to 600 sites among proteins belonging to plants family. Based on the results, motif 1 (30aa, E-value= 9.9e-165) and motif 3 (19aa, E-value= 8.3e-064) obtained as the most common motifs, which were shared 24 plant species.

Motif 1: PDVHEYCKLGCASSVCGNJSTLQNSDASEI

Motif 3: MEVKSVIMCVLILGLVLGQ

These common conserved motifs among different plants' species confirmed thionin protein functional similarities.

Keywords: thionin protein; Bioinformatics; Conserved motifs; plants

INTRODUCTION

Thionins are a family of small, cationic antimicrobial peptides uniquely found in plants, serving as important components of crop innate immune responses against pathogens. Their rich disulfide bond network and stable tertiary structure have led to the evolutionary preservation of specific amino acid sequence arrangements -termed "conserved motifs"- which are critical for the potent antimicrobial activity of thionins and for their ability to interact with pathogen cell walls (Höng et al., 2021). Accurate identification of these motifs using modern bioinformatics applications allows the development of novel strategies for engineering disease-resistant plants.

Recent advances in motif analysis tools such as MEME Suite and comparative protein family platforms have enabled a comprehensive exploration into thionin motifs across diverse plant species (Bailey et al., 2009; Almaghrabi et al., 2019; Liu et al., 2025). These studies revealed that conserved thionin motifs maintain structural integrity while providing powerful antibacterial and

antifungal properties, frequently through interactions mediated by specific sequence fingerprints (Li et al., 2021; Stotz et al., 2013). While numerous investigations have characterized thionin physicochemical properties, systematic comparative motif analyses across botanical taxa remain underexplored (Höng et al., 2021).

From an applied standpoint, mapping and engineering key thionin motifs represent powerful tools for plant disease control. Incorporating thionin genes harboring functional motifs into susceptible crops via transgenic or genome editing approaches has been shown to boost resistance against major phytopathogens, including *Fusarium*, *Xanthomonas*, and *Pseudomonas* (Hoshikawa et al., 2012; Goyal and Mattoo, 2016; Khan et al., 2025). Integration of cutting-edge bioinformatics motif discovery methods with advanced genetic editing platforms such as CRISPRa can yield resistant varieties with minimal off-target genomics changes (Khan et al., 2025). This study aims to identify and compare conserved thionin motifs in selected plant species, analyze their structure–function relationships using modern bioinformatics tools, and suggest practical applications for development of resistant crops in plant protection programs and global food security initiatives.

MATERIALS AND METHODS

Thionin protein sequence retrieval

The Thionin protein Reference Sequences (RefSeq) of different plant species were downloaded from NCBI (http://www.ncbi.nlm.nih.gov) in FASTA format (date received: October 2024). Among the Thionin plant sequences, those with the range of 115-135 amino acids were designated by the NCBI filters, and one sequence from each plant's genus was selected as their representatives for further analyses. Total data consisted of twenty-six plant species, which are listed in Table 1 with their families, accession numbers, and abbreviations.

Index	Species	Abbreviation	Family	Order	Accession No.
1	*Aegilops tauschii subsp strangulata*	*Ats*	Poaceae	Poales	XP_020162640
2	*Arabidopsis thaliana*	*At*	Brassicaceae	Brassicales	NP_001318868
3	*Beta vulgaris subsp. vulgaris*	*Bv*	Amaranthaceae	Caryophyllales	XP_010669303
4	*Brachypodium distachyon*	*Bd*	Poaceae	Poales	XP_003561421
5	*Brassica napus*	*Bn*	Brassicaceae	Brassicales	XP_022561535
6	*Brassica rapa*	*Br*	Brassicaceae	Brassicales	XP_009134335
7	*Camelina sativa*	*Cs*	Brassicaceae	Brassicales	XP_019097238
8	*Cannabis sativa*	*Csa*	Cannabaceae	Rosales	XP_030480839
9	*Capsella rubella*	*Cr*	Brassicaceae	Brassicales	XP_023633900
10	*Cucumis melo*	*Cm*	Cucurbitaceae	Cucurbitales	XP_008461039
11	*Eutrema salsugineum*	*Es*	Brassicaceae	Brassicales	XP_006418673
12	*Gossypium hirsutum*	*Gh*	Malvaceae	Malvales	XP_016673430
13	*Gossypium raimondii*	*Gr*	Malvaceae	Malvales	XP_012484903
14	*Hordeum vulgare subsp vulgare*	*Hv*	Poaceae	Poales	XP_04495511
15	*Lactuca sativa*	*Ls*	Asteraceae	Asterales	XP_042757624
16	*Panicum hallii*	*Ph*	Poaceae	Poales	XP_025823867
17	*Panicum virgatum*	*Pv*	Poaceae	Poales	XP_039772495
18	*Raphanus sativus*	*Rs*	Brassicaceae	Brassicales	XP_018462798
19	*Setaria italica*	*Si*	Poaceae	Poales	XP_004987336
20	*Solanum lycopersicum*	*Sl*	Solanaceae	Solanales	XP_00423570
21	*Sorghum bicolor*	*Sb*	Poaceae	Poales	XP_002466811
22	*Syzygium oleosum*	*So*	Myrtaceae	Myrtales	XP_030464623
23	*Triticum aestivum*	*Ta*	Poaceae	Poales	XP_044389000
24	*Triticum dicoccoides*	*Td*	Poaceae	Poales	XP_037426657
25	*Vigna angularis*	*Va*	Faboideae	Fabaceae	XP_017409508
26	*Vitis vinifera*	*Vv*	Vitaceae	Vitales	XP_010662250

Table 1. The list of twenty-six thionin plant species analyzed in current study

Conserved motifs analyses

The motifs of protein sequences were created using the program MEME (Multiple Em for Motif Elicitation; version 5.5.8) and MAST (Motif Alignment and Search Tool; version 5.5.8) at website http://meme-suite.org/ (Bailey et al. 2009) to study the variation of thionin in different plants' species. Among tools for defining perceptible domains, MEME has some advantages, so that it is able to recognize smaller individual motifs and more divergent patterns. The program was set to report the 10 most robust motifs of 10 to 30 width amino acids, occurring zero or once per sequence, with 2 to 600 sites among the proteins belonging to thionin plants' species.

RESULTS AND DISCUSSION

In a group of protein-related sequences, a sequence pattern is observed repeatedly known as a conserved motif. Patterns and sequences analyses of thionin conserved motifs was done by MEME. MEME represents motifs as position-dependent letter-probability matrices, which assign the probability of each possible letter at each position in the pattern, though motifs in MAST are characterized as position-dependent scoring matrices and define the score of each possible letter at each position in the pattern. Ten conserved motifs of each thionin species was identified by MEME which their scores, E-values and the number of motifs' species are listed in Table 2.

Table 2. Characterization of ten motifs of different plants' thionin analyzed in this study

Index	Best possible match	Site	Width	E-value
1	PDAHEYCKLGCASSVCGNJSTLQNSDESEI	24	30	2.0e-166
2	EAKSCCPNTTARNCYNVCRFPGTSRP	14	26	1.6e-127
3	MEGKSVIMCVLVLGLVLGQ	24	19	8.0e-064
4	VERCTNACSRFCTKGSGKA	22	19	9.2e-054
5	LCGCKIISGPTCPPGYPKJ	13	19	8.5e-045
6	CALKCLKKCJIPKSNT	8	16	1.2e+000
7	EGCVDSCSETCAKK	5	14	1.0e+002
8	WEDCWILLPT	2	10	2.0e-002
9	KEKCEHHPFE	2	10	4.0e+003
10	FCLRFGGCPPGPP	2	13	8.3e+006

These findings also showed that all species shared at least four identical motifs (Figure 1).

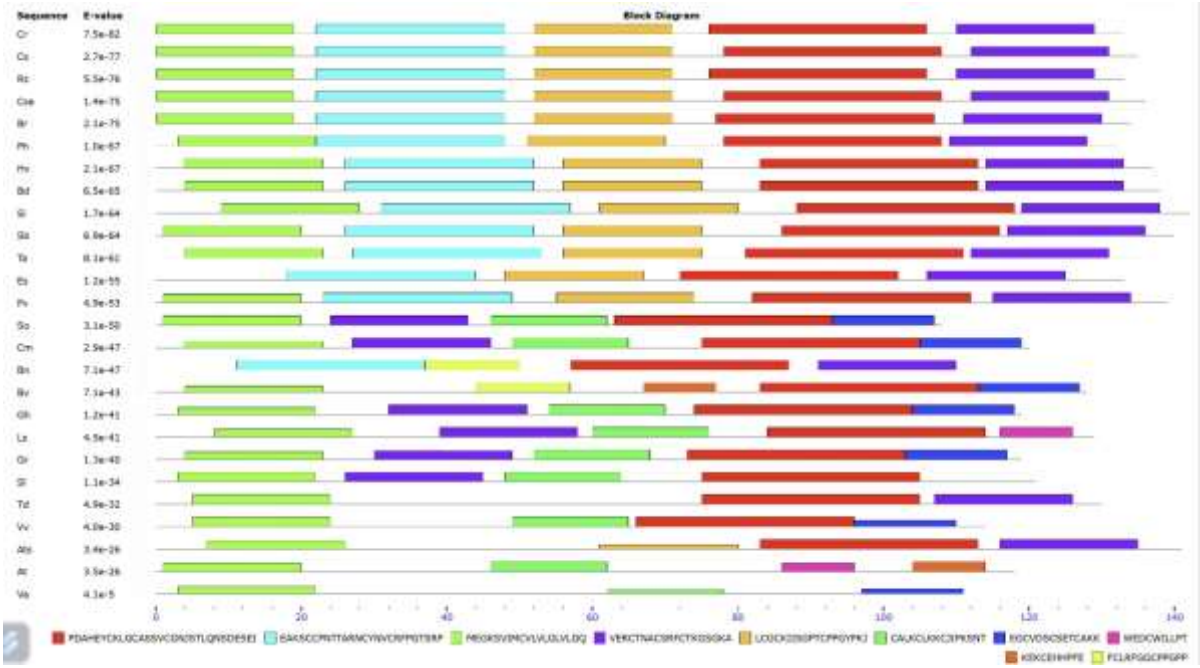

Figure 1. Motifs of thionin protein in plants. The MEME motifs are shown as different boxes. The E-value of a sequence is the expected number of sequences in a random database of the same size that would match the motifs as well as the sequence.

Nucleotide and protein sequences comprise patterns or motifs that have been preserved through evolution since they are crucial to the function or structure of the molecule. In proteins, these conserved sequences may be involved in the binding of the protein to its substrate or to another

protein, may contain the active site of an enzyme, or may determine the three-dimensional structure of the protein (Brejová et al., 2000). Members of protein families are often characterized by more than one motif (on average, each family has 3-4 conserved regions), which increases the certainty that a protein has been assigned to a precise family (Nevill-Manning et al., 1998).
These analyses showed that motifs 1 and 3 were common in 24 samples whose logos are shown in Figure 2, while motifs 8, 9, and 10 were only shared in two plant species. Moreover, the least observed motifs belonged to *Vigna angularis*, with only one motif.

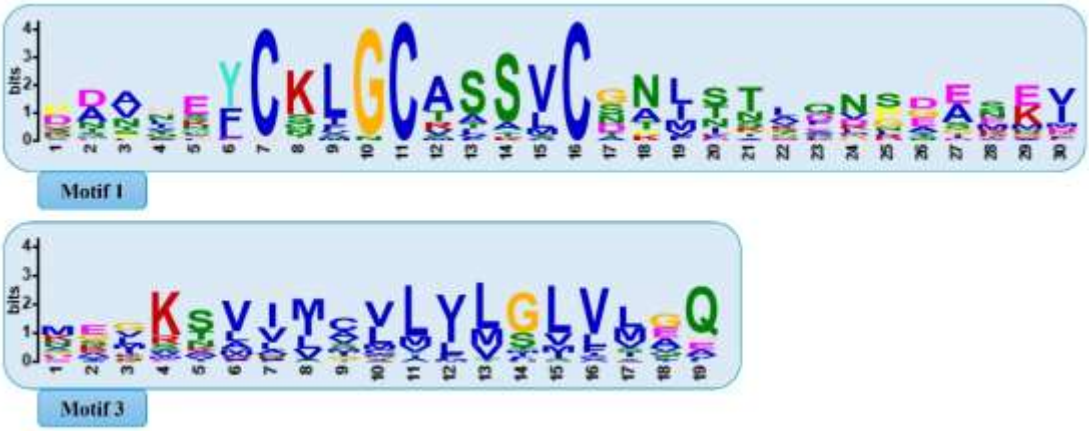

Figure 2. Motifs of thionin protein in plant species shared in 24 samples.

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BIOINFORMATICS STRUCTURAL ANALYSIS OF THIONIN PROTEIN IN SIX PLANT SPECIES OF BRASSICACEAE FAMILY

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ABSTRACT

Thionins are a family of small proteins exclusive to higher plants. These proteins typically consist of 45-48 amino acids, with 6–8 cysteine residues that form 3-4 disulfide bonds. They include phoratoxins and viscotoxins. Alpha- and beta- thionins are related to each other while gamma thionins, despite having a superficially similar structure, belong to a distinct category of proteins now identified as plant defensins. In this examination, the thionin protein reference sequences (RefSeq) from various plant species within the Brassicales order, including *Brassica napus, Brassica rapa, Raphanus sativus, Eutrema salsugineum* and *Capsella rubella* were obtained from the National Center for Biotechnology Information (NCBI) in FASTA format. Structural analyses of thionin protein were performed using online tools available on the EXPASY website. Primary and secondary structures of thionin proteins for each Brassicales species were estimated using ProtParam and SOPMA, respectively. The tertiary structure prediction and visualization of thionin were performed by PHYRE2 server. Thionin protein in all Plants ranged from 115 to 134 amino acids in length. The theoretical isoelectric points and molecular weight were calculated at a range of 5.36-8.75 and 12196.93-1431872 Da, respectively. A High aliphatic index suggested that thionin exhibit structural stability in Brassicales plant samples. The GRAVY values of this protein designated thionins are generally membrane-associated (hydrophobic) proteins in nature, except for *Arabidopsis thaliana*, which is globular (hydrophilic protein). The Instability index analysis revealed thionins in *Brassica rapa* and *Capsella rubella* were stable, whereas those in other Brassicales samples were classified as unstable. SOPMA analysis showed that alpha helices and random coils were the dominant secondary structural elements while extended strands were occasionally distributed in the proteins. Tertiary structure predictions using PHYRE2 indicated significant similar structure among plant thionins.

Keywords: Brasicales; ProtParam, SOPMA; Structural analysis; Thionin

INTRODUCTION

Thionins represent a crucial family of small, cysteine-rich peptide-like proteins with diverse functions, mostly in plants, in which they provide defense against entire sets of microbial pathogens, including bacteria and fungi. Through binding and disrupting membranes, these proteins are thought to mediate their antimicrobial activity and can be associated with the plant's defense arsenal. Structurally, thionins possess characteristic folds stabilized by specific arrangements of disulfide bonds that maintain their compact structure along with conserved α-helices and β-sheets that play vital roles in their bioactivity and stability (Stec, 2006).

The Brassicaceae family consists of economically important cruciferous plants with *Arabidopsis* and *Brassica* species that are known to generate diverse defense peptides, thionins, among others. Though thionins are known for their importance, the lack of profiled comprehensive analyses on bioinformatics level on their structural diversity and specificity across different Brassicaceae species limits our understanding of their evolutionary adaptations and molecular function (Stec, 2006).

Progresses in structural bioinformatics, largely driven by artificial intelligence and deep learning approaches, have revolutionized protein structure prediction and analysis. With the advent of bioinformatics methods, the near-experimental accuracy in predicting protein tertiary structures from amino acid sequences could be achieved, thereby accelerating structural studies at an unprecedented scale beyond the experimental paradigm (Jumper et al., 2021). This has opened the door to in-depth in silico studies of protein families with a focus on conserved structural motifs and residues required for function and to elucidate the potential impact of sequence variation on stability and activity (Huang, 2023).

Besides aiding in the prediction of protein structural classes, machine-learning algorithm integrations add more powerful data analysis tools for investigating sequence-to-structure relationships essential to explain molecular mechanisms of antimicrobial activity in thionins and other similar peptides (Zhu et al. 2021). Structural bioinformatics packages make possible detailed sequence and structure conservation analyses that further our ability to dissect functional determinants within protein families (Grant et al., 2021).

The work illustrates the contemporary interplay between structural bioinformatics and artificial intelligence in stimulating advances in plant molecular biology and protein engineering. The study's results provide useful information on thionin structural diversity and its evolution, pointing to a possible use of these proteins to develop novel antimicrobial strategies against phytopathogens.

MATERIALS AND METHODS

Thionin protein data received

In this research, the Thionin protein Reference Sequences (RefSeq) of different plant species were downloaded from NCBI (http://www.ncbi.nlm.nih.gov) in FASTA format (date received: November 2024). Due to the large number of sequences available for Thionin protein in plants, it is not possible to analyze all the sequences. Among the thioninplant sequences, those with the range of 115 to 134 amino acids were designated by the NCBI filters, and one sequence from each plant's genus was selected as their representatives for further analyses. Total data consisted of six plant species, which are listed in Table 1 with their families, orders, accession numbers, and abbreviations.

Table 1. The list of fourteen Thionin plant species analyzed in the current study

Index	Scientific Name	Family	Order	Abbreviation	Accession number
1	*Brassica napus*	Brassicaceae	Brassicales	*Bn*	XP_022561535
2	*Brassica rapa*	Brassicaceae	Brassicales	*Br*	XP_009134335
3	*Raphanus sativus*	Brassicaceae	Brassicales	*Rs*	XP_018462798
4	*Eutrema salsugineum*	Brassicaceae	Brassicales	*Es*	XP_006418673
5	*Capsella rubella*	Brassicaceae	Brassicales	*Cr*	XP_023633900
6	*Camelina sativa*	Brassicaceae	Brassicales	*Cs*	XP_019097238

Structural analyses

Several online web services and software were used for analyses of thionin in plants. Total number of amino acids, theoretical isoelectric point, instability index, aliphatic index, and grand average of hydropathicity (GRAVY) of plant samples were predicted by ProtParam (http://web.expasy.org/protparam/) (Gasteiger et al. 2005). Secondary structures were predicted by SOPMA server (http://npsa-pbil.ibcp.fr/cgibin/npsa_automat.pl?page=npsa_sopma.html) (Geourjon and Deleage 1995).

The tertiary structure prediction analysis of thionin protein families was predicted by Phyre2 server (http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index) (Kelley and Sternberg 2009) using profile-profile matching and secondary structure in representative plant species. Chimera 1.11 was used for 3D structure modeling of *Raphanus sativus* as a sample of plant's species (https://www.cgl.ucsf.edu/chimera/). The 3D model of thionin protein was created using the "c1ed0A" model (PDB Accession Code: 1ed0) with 99.7% confidence level.

RESULTS AND DISCUSSION

In order to investigate the structures of thionins, six species from different Brassicaceae species were analyzed by bioinformatics tools. The primary structure of selected thionins was analyzed by ProtParam. This server computes various physico-chemical properties that can be deduced from a protein sequence, including the instability and aliphatic index, and grand average of hydropathicity (GRAVY) (Table 2). Thionin protein in all Brassicaceae species ranged from 115 to 135 amino acids in length. Also, the molecular weight was calculated at a range of 12196.93-14318.72Da.

Table 2. Primary structure of thionin protein in six plant species

Index	Sample	Number of amino acids	Molecular weight	Theoretical pI	Instability index	Aliphatic index	GRAVY
1	*Bn*	115	12196.93	5.55	48.77	78.09	0.070
2	*Br*	134	13972.09	7.40	25.09	81.57	0.138
3	*Rs*	133	13912.96	7.38	43.89	82.86	0.101
4	*Es*	133	14318.72	7.40	41.90	76.39	0.058
5	*Cr*	133	13781.90	5.36	29.37	79.92	0.111
6	*Cs*	135	14167.45	7.90	38.71	84.52	0.128

Isoelectric Point is the pH at which a protein carries no net charge. Below the isoelectric point proteins carry a net positive charge, and above it, a net negative charge. The isoelectric point is of significance in protein purification since it is the pH at which solubility is often minimal and at which mobility in an electrofocusing system is zero, and therefore the point at which the protein will accumulate (Seddigh, 2019). In computing PI analysis, the most isoelectric point in thionin of selected Brassicaceaea species belonged to *Camelina sativa* (7.90), while the least was found in *Capsella rubella* (5.36). This analysis revealed that thionin proteins are different in nature and depend on the species, may be acidic or basic. In the current ana lysis, all the samples were basic except Bn and Cr, which showed acidic characteristics in nature. The aliphatic index of a protein is defined as the relative volume occupied by aliphatic side chains (alanine, valine, isoleucine, and leucine). The aliphatic index value of proteins from thermophilic bacteria was found to be significantly higher than that of ordinary proteins, and hence, it can serve as a measure of thermostability of proteins (Ikai, 1980). So, this is a positive indicator of globular protein thermostability. High aliphatic index in thionins indicated structural stability of this protein in plant samples.

The GRAVY value for a protein is calculated as the sum of hydropathy values of all the amino acids, divided by the number of residues in the sequence (Kyte and Doolittle, 1982). According to Kyte and Doolittle (1982), integral membrane proteins typically have higher GRAVY scores than do globular proteins. Though the GRAVY score is a helpful piece of information, it cannot reliably predict the structure without the help of hydropathy plots. Gravy index scores of proteins below 0 are more likely globular (hydrophilic protein), while scores above 0 are more probable membranous (hydrophobic protein). The GRAVY values of this protein, designated thionin Brassicaceae species to be membranous (hydrophobic protein) in nature.

The instability index is a measure of proteins, used to determine if they will be stable in a test tube or not. It is also known that an instability index less than 40 predicts a stable protein, while values higher than 40 denote a potentially unstable protein (Seddigh, 2019). The Instability index analysis of Brassicaceae thionin revealed that three of the representative plant samples, including *Brassica napus, Raphanus sativus,* and *Eutrema salsugineum* were unstable, and the rest of them were stable proteins.

The secondary structure of Brassicaceae thionin proteins was predicted by Self-Optimized Prediction Method with Alignment (SOPMA) server, which analyzes the percentage of alpha helix, extended strand, and random coil. The prediction of the secondary structure of thionin based on hierarchical neural network analysis (combet et al., 2000) revealed that the most plentiful structural elements of the secondary structure were alpha helices and random coils, while extended strands were occasionally distributed in the proteins. Also, there were just two exceptions among all samples, so that random coil structures were more than alpha helices, including *Brassica napus* and *Camelina sativa* (Table 3).

Table 3. Secondary structure of thionin protein in six plant species

Index	Sample	Alpha helix	Extended strand	Random coil
1	*Bn*	34.78	15.65	49.57
2	*Br*	49.25	8.21	42.54
3	*Rs*	45.86	9.77	44.36
4	*Es*	49.62	7.52	42.86
5	*Cr*	46.62	10.53	42.86
6	*Cs*	44.44	8.89	46.67

The key objective of protein structure prediction is the tertiary structural modeling, which is crucial for a full understanding of protein function. Also, structures are much more conserved than sequences, so their evaluations allow us to look even further back into biological prehistory (Orengo et al., 2003; Seddigh, 2019). So, three-dimensional modeling of six Brassicaceae samples was predicted by Phyre2. This server uses profile–profile matching and secondary structure for tertiary structure prediction (Kelley et al., 2015) and uses the alignment of hidden Markov models through HHsearch (Söding, 2005). Based on these analyses, the tertiary structure of these plant thionin proteins was very similar to each other, which confirmed the conserved structure of this protein (Figure 1).

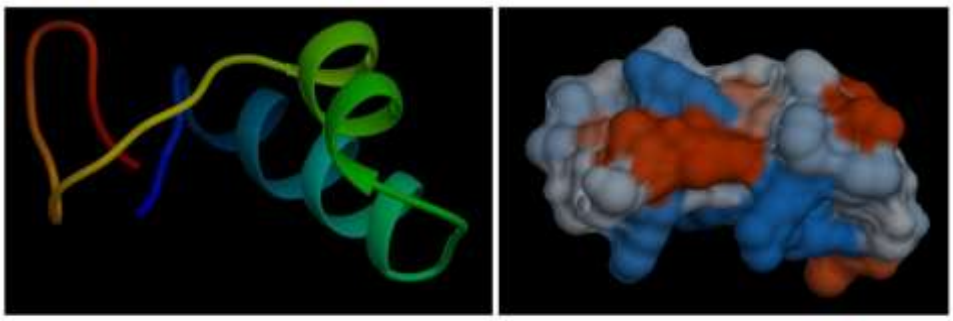

Figure 1. Tertiary structure of thionin protein in *Raphanus sativus* as the Brassicaceae representative sample (PDB accession code: 5szn) using Phyre2 server. (a) Ribbons model, alpha helices are shown with helix shape and beta sheets with arrows. (b) Hydrophobicity surface model using Chimera 1.11. Color from red for negative potential, to blue for positive potential.

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SUSTAINABLE TOURISM IN TÜRKIYE AND THE BALKAN COUNTRIES: POLICY EVOLUTION AND KEY CHALLENGES

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ABSTRACT

This study examines the development process of sustainable tourism policies implemented in Türkiye and the Balkan countries, along with the key challenges encountered, through a comparative approach. The historical development of the concept of tourism and the policies developed in line with sustainability principles are evaluated in terms of the economic, social, and environmental dimensions of current tourism activities in Türkiye and the Balkan countries. The study analyzes theoretical and practical aspects using secondary sources published between 2010 and 2025. The Balkan countries and Türkiye, sharing a close geographical proximity and common history, possess cultural, natural, historical, and geographical characteristics. With all these features and sustainable tourism practices, the Balkan countries have become a center of attraction for visitors from various countries around the world, particularly Türkiye. However, the wars and internal conflicts experienced in the recent history of the Balkan countries, management crises, and ongoing infrastructure and superstructure issues in certain regions hinder the substantial increase of tourism revenues. This situation continues to obstruct the sustainability of tourism in the region. The study highlights the importance of regional cooperation and the strategic objectives established by the European Union in developing sustainable tourism policies. Through a detailed comparison between Türkiye and the Balkan countries, this evaluation assesses the current state of sustainable tourism policies and proposes solutions to potential obstacles and issues. As a result of this research, it has been observed that sustainable tourism policies are generally determined by central authorities, with limited public participation in this process. The most significant barriers to achieving sustainable tourism goals have been identified as a lack of long-term planning, weak evaluation mechanisms, insufficient local capacity, and gaps in coordination among stakeholders. According to the findings obtained from the research, sustainable tourism policies in the Balkan countries are generally developed in accordance with their EU membership processes. Despite the pursuit of EU-compatible policies, bureaucratic obstacles are encountered in accessing the budget required for the implementation of these policies. Moreover, it is observed that decisions concerning policy-making are taken by central authorities. This research provides suggestions and recommendations to policymakers, researchers who will conduct studies on this topic, and all individuals and institutions concerned about sustainable tourism in Türkiye and the Balkan countries.

Keywords: Tourism Enterprises, Sustainable Tourism, Tourism Policy.

1. INTRODUCTION

Tourism is one of the sectors that has the highest potential for job creation and generating foreign income in the world *(Alam et al., 2022)*. Due to these characteristics, the tourism industry makes significant contributions to regional economic development, intercultural communication, and optimal resource utilization. For all these stated activities to continue to take place in the long term, there is a necessity for tourism practices to be sustainable. In sustainable tourism practices, efforts are expected to be made to ensure the protection of resources such as the natural, historical, and cultural heritage that countries possess.

Türkiye and the Balkan countries, due to their closely located geographical regions, offer highly attractive prospects for visitors seeking sustainable tourism practices. On the other hand, the institutional capacities, sustainable tourism policies, and regional cooperation ideas of Türkiye and the Balkan countries, which are geographically and culturally so close to each other, exhibit significant differences (İşgüven and Şeker, 2024). Among the Balkan countries, Serbia, Albania, Montenegro, Bosnia and Herzegovina, Kosovo, and North Macedonia, which are not yet EU members, are striving to align their national tourism policies on sustainable tourism with the strategies of the European Union, similar to the EU member states of Bulgaria, Greece, Romania, Slovenia, and Croatia. Although the membership processes and funding for these non-EU Balkan countries have accelerated regional initiatives, it has been observed that several obstacles and limitations persist (Kamberi & Shkreli, 2023).

2. METHODOLOGY

This study was conducted using a review method mainly based on academic articles, policy documents, official reports, and sectoral analyses published between 2015 and 2025. Within the scope of the research, the development process of sustainable tourism policies has been examined through a comparative approach, focusing on both Türkiye and the Balkan countries. The literature review was carried out using international databases such as Web of Science, Scopus, and Google Scholar; the selected sources were evaluated in terms of their compliance with sustainability principles across economic, social, and environmental dimensions. Additionally, European Union strategy documents and regional development plans were included in the analysis process to elaborate on policy orientations and implementation challenges. Throughout the research process, the determining factors of sustainable tourism policies, implementation barriers, and regional differences were identified through comparative analysis.
In the research process, the determinants of sustainable tourism policies, implementation barriers, and regional differences have been revealed through a comparative analysis method. Considering the structural, historical, and administrative differences between Türkiye and the Balkan countries, a comprehensive evaluation has been conducted through policy documents and academic studies. This method has brought together both theoretical and practical perspectives; the current situation of sustainable tourism and future recommendations have been presented within a holistic framework.

2.1. The Development of Sustainable Tourism Policies in the Balkan Countries

The Western Balkan countries are noted for their rich cultural history, which includes historical artifacts and areas, traditional crafts, and unique customs and traditions. Sustainable tourism helps to preserve this heritage by promoting the introduction and safeguarding of cultural values among visitors. Furthermore, sustainable tourism is regarded as promoting social inclusion and respect

for diversity. It encourages cultural connection and mutual exchange between visitors to sustainable tourism sites and the local inhabitants. This also helps to break down boundaries between different cultures and communities, improving mutual understanding (Mulita, 2024). Sustainable tourism is considered to be the key to the success of tourism destinations, and in this context, Bosnia and Herzegovina, one of the Balkan countries, has been making intense efforts in recent years to develop sustainable selective tourism through various collaborations, initiatives, and organizations. Given that tourism relies on the cooperation between numerous sectors, it is essential to involve tourism businesses from the private sector, public and civil society organizations, consumers, development agencies, and local communities in the process to ensure the success of the destination (Pobrić & Osmanović, 2024).

In the 1980s, the increasing global awareness of environmental issues and the concept of sustainable development contributed to the emergence of sustainable tourism. The Brundtland Report, prepared in 1987, defines sustainable development as 'meeting the needs of the present without compromising the ability of future generations to meet their own needs' (United Nations, 1987). This understanding necessitates the simultaneous consideration of economic, environmental, and social dimensions in tourism planning. Sustainable tourism practices worldwide have diversified through the implementation of environmental management systems, eco-certifications, programs that promote the participation of local communities, and responsible tourism initiatives. In Europe, many countries have implemented sustainable tourism policies at both national and regional levels that prioritize the preservation of natural and cultural heritage. (Serio, Dickson, de Graaff & Pels, 2024).

Tourism began as an activity solely aimed at rest and recreation throughout history, evolving over time into a comprehensive sector with economic, social, and cultural dimensions. In the early 20th century, tourism was primarily focused on economic gain and recreation; however, the rapid and unregulated growth of the sector has also led to negative consequences such as environmental degradation, erosion of cultural values, and socio-economic imbalances (Mihalic, 2016; Hall, C. M., Gössling, S., & Scott, 2015). These phenomena have laid the groundwork for the development of sustainable tourism.

2.2. Challenges Encountered in Sustainable Tourism Policies in the Balkan Countries

Europe, one of the continents with the largest share in global tourism revenues and mobility, possesses considerable tourism potential within the Balkan geography, enriched by its rich cultural and natural heritage. However, the countries in the region face specific challenges unique to their respective nations in implementing sustainable tourism policies and aligning with European Union standards in this region.

First, deficiencies and inadequacies in institutional capacity and governance are among the prominent issues. The EU plays a role in coordinating, guiding, and shaping the national policies of member states within the framework of sustainability principles (Yıldırım and Keleş, 2022). Both Balkan countries that are EU members and those that are candidates for EU membership struggle to ensure effective coordination between central and local governments. For instance, in EU member states as Romania and Bulgaria, bureaucratic obstacles and inadequate transparency in the distribution and oversight of resources allocated for EU funds and sustainable tourism projects complicate the effective implementation of policies on the ground.

Another significant challenge related to the Balkan countries is the inconsistencies in legislation and the problems encountered in implementation. The EU's policy areas, such as Environment, Transport, and Regional Development, indirectly influence sustainable tourism (Gürsoy, 2017; Yıldırım and Keleş, 2022). In coastal regions, intense construction and environmental pollution contradict the principle of natural resource conservation and undermine the goals of sustainable development. For instance, the excessive tourism pressure experienced on Greece's Santorini Island and in Croatia's Dubrovnik city strains the infrastructure and leads to a deterioration in the quality of life for the local population and the environmental balance (Çam & Çelik, 2022).
Sustainable tourism practices (such as energy efficiency and waste management systems, etc.) often require a high initial investment cost. According to TÜSİAD's Sustainable Tourism Report (2012), it is known that businesses lack the financial resources or technical knowledge necessary to undertake this transformation due to their short-term profit-oriented perspective. It can be observed that the Balkan countries pursue distinct campaigns and strategies for their national tourism promotions (Porfido, 2020);

Table 1: Tourism Policies and Promotion Campaigns of Balkan Countries. (Porfido, 2020)

Country	Recent Tourism Strategies and Policies	Recent Tourism Promotion Campaign
Albania	Sustainable Tourism Development Strategy 2019–2023 (Ministry of Tourism and Environment, 2019)	Albania – Go your own way
Bosnia and Herzegovina	Tourism Development Strategy for the Federation of Bosnia and Herzegovina 2008–2018 and Republika Srpska Tourism Development Strategy 2010–2020 (OECD, 2018)	Bosnia & Herzegovina – The heart-shaped land
Croatia	Tourism Development Strategy of Croatia until 2020 (Government of Croatia, 2013)	Croatia – Full of life
Kosovo	No officially approved or drafted national tourism strategy	Be in Kosovo – the young Europeans (unofficial)
Montenegro	Tourism Development Strategy of Montenegro until 2020 (Ministry of Tourism and Environment, 2008)	Montenegro – Wild beauty
North Macedonia	National Tourism Development Strategy 2016–2021 (Government of North Macedonia, 2016) – only in draft form	Macedonia Timeless
Serbia	Tourism Development Strategy of Serbia 2016–2025 (Government of Serbia, 2016)	Serbia - The place to be

As can be seen in the table above, tourism promotion and marketing activities in the Balkan countries are still focused on mass tourism movements. Areas related to niche and sustainable tourism, such as cultural, ecological, or rural tourism, are not being effectively promoted and marketed. In the context of the relationship between foreign policy and tourism, the bilateral agreements made (Aydemir and Çözeli, 2016) primarily focus on increasing visa facilitation and transportation connections, while the promotion of sustainable tourism products remains a

secondary concern. This situation also hinders the increase in revenue that the Balkan countries could generate from sustainable tourism.
The Balkan countries are adopting the EU's sustainable tourism narrative and policies. However, during the implementation phase of these policies, they face serious challenges regarding institutional capacity, effective legislation enforcement, access to financing, and marketing strategies. Overcoming these challenges will only be possible through strong political will, effective participation of all stakeholders in the sustainable tourism process, and ensuring the purposeful and transparent use of EU funds.

2.3. The Development of Sustainable Tourism Policies in Türkiye

After the COVID-19 pandemic, which affected all sectors worldwide, Türkiye prioritized sustainability in its tourism sector and entered a comprehensive restructuring process in its tourism policies. The Ministry of Culture and Tourism has identified sustainable tourism as a strategic priority in the Updated Tourism Master Plan for the period of 2022–2024; through initiatives such as the 'Safe Tourism Certification,' it has facilitated the integration of COVID-19 safety practices into the tourism process, thereby strengthening certification and hygiene standards within the sector (OECD, 2022). During the same period, the Turkish Tourism Promotion and Development Agency (TGA) developed the 'National Sustainable Tourism Program' in collaboration with the Global Sustainable Tourism Council (GSTC) to align Türkiye's tourism sector with international sustainability criteria. Thus, Türkiye has gained a significant position in international sustainability efforts (Bergen, 2022).

Türkiye was included in the 'Green Travel List' of Wanderlust magazine in 2025, thereby starting to become more prominent in international ecotourism. A notable example of this recognition is the emphasis placed on Mount Nemrut, which is listed as a UNESCO World Heritage Site and highlighted in Wanderlust. With its monumental stone sculptures and ancient hill tombs, Mount Nemrut not only possesses extraordinary cultural and historical value but also demonstrates Türkiye's increasing commitment to the integration of ecologically conscious principles in heritage site management and sustainable tourism practices. (Sarkar,2025).

2.4. Challenges in the Implementation of Sustainable Tourism Policies in Türkiye

Although Türkiye has a strong potential for sustainability, there are several challenges that hinder the implementation of sustainable tourism policies. Foremost among these is environmental sustainability: poor management of natural resources, deficiencies in waste control, and environmental pollution remain major issues within the tourism sector. Furthermore, in some regions, infrastructure and transportation services are insufficient to accommodate tourist density. This inadequacy can negatively affect visitor experiences, leading to dissatisfaction. **Greenwashing practices presented as sustainability:** The prevalence of greenwashing practices in the tourism sector raises questions about the industry's credibility. For instance, hotels boasting about sustainability by producing only 10 kilograms of cucumbers annually in their gardens illustrate how the industry often misunderstands the concept of sustainability. Collectively, these challenges represent significant obstacles to Türkiye’s sustainable tourism vision (Akgül, 2024)

Although efforts towards sustainable tourism opportunities in Türkiye are intense, issues such as inadequate infrastructure, lack of coordination, and the absence of policies based on long-term planning remain primary concerns. For instance, excessive development in many coastal destinations and the unconscious use of natural resources jeopardize sustainability; these problems result in even more negative outcomes due to short-term profit-oriented approaches that harm the environment. Furthermore, the inability of non-governmental organizations, local communities, and other stakeholders directly affected by the policy to participate in the formulation process of sustainable tourism policies poses a significant issue. Additionally, the lack of coordination between the central government and local authorities represents another barrier to implementing sustainable tourism policies.

3. CONCLUSION AND DISCUSSION

This study provides a detailed analysis and comparison of sustainable tourism practices in Türkiye and the Balkan countries, addressing the barriers and challenges that arise during policy implementation. Both Türkiye and the Balkan countries share similar geographical features, as well as a common history and cultural heritage. Moreover, given their natural characteristics, both regions possess significant potential for sustainable tourism. Despite their high potential for sustainable tourism practices, Türkiye and the Balkan countries converge on certain issues while diverging on others during the policy implementation process.

The adoption of sustainable tourism practices in Balkan countries can offer several advantages to stakeholders involved in these initiatives. While enhancing the resilience of destinations and businesses, it will positively impact local stakeholders' access to all public services. Some of the advantages that sustainable tourism can provide to its stakeholders include: being prepared for future adversities, becoming resilient in challenging conditions, attracting interest from domestic and foreign investments, reducing negative environmental impacts and rehabilitating affected areas, lowering operational costs, promoting the development of local regions, creating quality jobs and learning opportunities for local communities, meeting changing consumer demands, raising awareness among visitors and potentially altering their behavior, and offering individual and unique experiences to regional visitors, among others. Some of the issues encountered regarding sustainable tourism practices in the Balkan countries are as follows: deficiencies in infrastructure, political instability, legal regulations and legislative issues, the degradation of ecosystems due to sustainable tourism, over tourism, pressure on physical infrastructure (for example, excessive utilization of waste management systems, public transportation, traffic congestion, etc.), rapidly rising living costs or insufficient housing, and the intensive use issues concerning regional resources such as water, electricity, and food (Chavez et al. 2023).

Decision-makers in the Western Balkan countries should consider the following aspects while formulating tourism policies;

EU Compliance Requirement: Initiatives such as the EU's Green Deal and New Generation EU, which are the main markets and economic partners, directly influence regional policies.

Need for Transformation: Green and digital transformation, carbon neutrality targets, and circular economy models are no longer an option but a necessity.

Changing Demand: Tourists are now seeking sustainable, authentic, and technology-infused experiences, which necessitate tourism diversity.

Regional Cooperation: Despite historical issues, a 'Western Balkans' brand created through cooperation instead of competition will provide a much stronger positioning in the global market (Knežević et al., 2024).

The Balkan countries need to create a new strategic roadmap that will adapt to current market dynamics and center around sustainable tourism principles. In this context, increasing the diversity of existing tourism products (Porfido, 2020) and spreading tourism investments to new destinations to balance regional development are among the primary goals.

The COVID-19 pandemic, which has profoundly affected all sectors around the world, particularly the tourism industry, has changed the way business is conducted globally. Following the pandemic, sustainable tourism has gained substantial importance in Türkiye's tourism sector and has begun to be prioritized by businesses and managers. Programs such as the "Safe Tourism Certificate" and the "National Sustainable Tourism Program" developed in collaboration with the Global Sustainable Tourism Council have opened a significant new chapter in the tourism sector, with hotel businesses making intensive efforts to stay ahead of their competitors. For instance, prominent enterprises in Turkish hospitality, such as Hilton Istanbul Bosphorus and Conrad Istanbul Bosphorus Hotels, are reusing 120 tons of water in one day through a greywater system to obtain sustainability certifications issued by the Global Sustainable Tourism Council, and they are implementing effective waste management, aiming to reduce their carbon footprint by 50% by the year 2030 (Turizm Proje Dergisi, 2023).

Proposals for the development of sustainable tourism policies in Türkiye:

Environmental Sustainability and Management of Existing Natural Resources

- Increased utilization of green technology practices for waste management and energy conservation.
- Tourism planning in accordance with the carrying capacities of natural areas.
- Promotion of renewable energy use in tourism businesses.

Ensuring Community Participation

- Ensuring the active involvement of local communities in the decision-making processes related to sustainable tourism practices.
- Increasing local economic benefits and enhancing points of sale for local products.
- Raising public awareness regarding sustainable tourism practices through education and seminars.

Improving Infrastructure and Sustainable Solutions in Individual and Public Transport

- Development of environmentally friendly transportation systems, such as bike paths and electric public transport in tourism regions.
- Planning sustainable infrastructure investments for access to cultural and natural heritage areas

Development of Policy and Legal Framework

- Making environmental certification systems mandatory for all tourism businesses
- Enhancing inspection and enforcement mechanisms. Education and Capacity Development
- Programming sustainability-focused training for employees in businesses operating within the tourism industry.
- Incorporating lessons on climate change, green economy, and sustainability into school curricula at all educational levels, from primary school to university. International Cooperation and Effective Resource Utilization
- Effectively utilizing sustainable tourism p-rojects and grants supported by the EU and UN

- Strengthening regional cooperation by developing joint destination packages with Balkan countries.

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SMART MATURITY MONITORING IN OLIVE ORCHARDS: UTILIZING VIS/NIR TOOLS FOR OPTIMAL HARVEST DECISION-MAKING IN ALBANIA

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ABSTRACT

The determination of olive harvest maturity is essential for optimizing yield quality and enhancing economic returns, particularly for cultivars designated for extra virgin olive oil production. This study presents an integration of non-destructive methods into standard maturity assessment protocols, signifying a substantial advancement for agricultural producers and oil processors. This research specifically examines the Albanian olive cultivar 'Kalinjot' and the well-known Italian Leccino, and evaluates the effectiveness and practical implications of non-destructive technologies, namely the Color Index (CI) and the Index of Absorbance Difference (IAD), using Vis/NIR devices (DA-Meter® and Kiwi-Meter®). These methodologies facilitate rapid, in situ evaluations of fruit ripeness without inflicting tissue damage, thereby offering a reliable and cost-effective framework for continuous monitoring throughout the ripening process. Statistical analyses indicate strong correlations between the non-destructive indicators (IAD, CI) and critical oil quality metrics, including oil content and polyphenol concentration. The results empower producers to identify the optimal harvest window, particularly during mid to late October for 'Kalinjot' cultivar and two weeks earlier for Leccino, thus mitigating the resource demands associated with laboratory-based destructive analyses. For olive oil processors, these advancements contribute to better planning of harvest and milling workflows, reducing the over-ripening risk and improving consistency in product quality and classification. The adoption of non-destructive methodologies represents a significant evolution toward precision agriculture within olive cultivation, enhancing traceability, sustainability, and competitive positioning in the olive oil sector.

Keywords: Non-destructive methods, olive ripeness, harvest timing, IAD, olive oil quality.

INTRODUCTION

Albania has an ancient tradition in the production of olives and olive oil, which is an important part of the typical Mediterranean diet, providing the already known positive effects on human health. The olive sector in our country exhibited great developments last year, with a significant increase in olive production reaching 158thousand tons, and olive oil approximately 28 thousand tons. In Albania, olives have spread along the Ionian and Adriatic coasts up to 100 km in land (INSTAT, 2023).

The Kalinjot olive,noted for its late fruit ripening,is an important native cultivar covering about 46% of the olive plantations in the country, while from the foreign cultivars, Leccino has

been successfully adapted in Albania, with early ripening, stable production and high oil quality (Ismaili H., 2018). Fruit maturity stage is one of the most important factor affecting the physical, chemical, and organoleptic characteristics of the oil, as well as the yield (Famiani F., et.al, 2022).

Harvesting too early negatively affects the yield since the oil biosynthesis is not completed and its extraction becomes difficult. But harvesting from very ripe olives can also present disadvantages in the quality of the oil. During fruit ripening, a series of metabolic processes occur that lead to the production of phenols that affect the quality, sensory properties, oxidative stability, and nutritional value of the oil (International Olive Council, 2007). The maturity assessment has usually been based on destructive methods to evaluate maturity indexes. The most common is the color assessment method, known as the Jaén index or maturity index (MI), which indicates the degree of fruit ripening on a scale of fruit pigmentation ranging from zero (green) to seven (pigmentation of the whole epicarp and mesocarp). However, fruit color-based MI does not always evolve linearly over time and is affected by the cultivar and environmental factors (Cinosi N., et al., 2023). These methods have limitations, such as being time-consuming, requiring specialized laboratory equipment, and destroying the fruit samples, which prevents continuous monitoring throughout the ripening process (Garcia J.M., 2005).

Recently, there has been increasing interest in applying non-destructive techniques for assessing olive fruit maturity. These methods do not require fruit destruction, offer several advantages, including the ability to continuously monitor fruit characteristics over time without damaging the samples. Non-destructive methods can measure parameters such as fruit colour and moisture content, providing valuable insights into fruit ripeness and harvest suitability.

Instrumentation advances promise lower cost and easier use of Vis-NIRS within postharvest applications (Walsh K., 2020).Techniques such as the Color Index (CI) measured by a colorimeter, as well as the use of the Index of Absorbance Difference (IAD) with instruments like the Kiwi-Meter® and standard DA-Meter®, have been explored as potential tools for assessing olive fruit maturity directly in the field (Cinosi N., et al., 2023).These non-destructive techniques provide real-time, in situ measurements, which could greatly improve the efficiency and accuracy of harvest timing, leading to better-quality oil production and reducing waste. The objective of this study is to determine the optimal olive harvest stage of “Kaninjot” and “Leccino” cultivars based on a combination of maturity indices using destructive and non-destructive methods.

This study was focused on evaluating the maturity indexesduring the ripening process, such as the diameter, fresh weight, maturity index (MI), flesh firmness, fruit detachment index (DI), oil, moisture, and total polyphenols content using destructive methods. Maturity indices such as color index CI and IAD measurements with DA-Meter(R) and Kiwi-Meter(R)were evaluated using non-destructive methods. The possible use of nondestructive methods such as Color Index with colorimeter and IAD with Kiwi-Meter®andDA-Meter® devices for maturity indexes of olive directly in the field was tested for the first time, for the under-study cultivars at the Vlora region.

MATERIAL AND METHOD

Maturity index measurements were carried out during the September-December 2024 period, at 10-day intervals, on 6 randomly selected trees for the under-study cultivars on 6/9, 17/9, 27/9, 8/10, 18/10, 28/10, 8/11, 18/11, and 3/12 (abbreviated H1-H9). Samples of 3 kg were taken in each harvest, 100 fruits of which were used for maturity indices, and a part for laboratory

analysis of the content of oil, water, and total polyphenols. The evaluated parameters in this study were as follows:

1. Maturity index (MI)

For the MI measurements, 100 fruits were taken and divided according to the color scale. The number of fruits in each color category is multiplied by the number of that category. Pigmentation was determined using the MI, ranging from 0 to 7, with 0 for green olives and 7 for olives with pigmentation up to 100% of the epicarp and 100% of the mesocarp (International Olive Council, 2007; Amon D., et al. 2011; Uceda M., 1998).

2. **Color index (CI)**

This is a non-destructive method for assessing fruit color. The CIE L*, a*, and b* values of olives were measured using a colorimeter (Model CR-400, Konica-Minolta, Osaka, Japan). Measurements are taken from both sides of the fruit at the axis area (Gamli O.F., et al., 2017). The Color Index (CI) is calculated using the formula:

$$CI = L \times \frac{b-a}{100}$$

3. Flesh firmness (FF)

The firmness of the fruit flesh was measured using a handheld Push-Pull Dynamometer (Model FD 101, TR Turoni, Forlì, Italy), which has a measurement range of 0 to 1000 g and a resolution of 10 g. The plunger is made of steel and has a diameter of 1.5 mm. Firmness is expressed in Newton (N) (Costa et al., 2009; Cinosi et al., 2023).

4. Detachment Index (DI)

The Detachment Index is calculated by dividing the traction force by the fresh weight of the selected olive fruits (Gamli O.F., et al., 2017). The traction force was measured using a hand-held held Push - Pull Dynamometer (Model FD 101, TR Turoni, Forlì, Italy) with a range of 0 to 1000 g and a resolution of 10 g.The Detachment Index is expressed in Newton per gram (N/g).

$$DI\,(N/g) = \frac{F}{FW}$$

Where:
DI =N/g, F= Detachment force (N), FW =Fresh weight(g).

5. Index of Absorbance Difference (IAD - A non-destructive method

The DA-Meter(R) and Kiwi-Meter(R)are portable Vis/NIR devices designed to measure the Index of Absorbance Difference (IAD)and were used to monitor ripening in the field directly on the tree. The IAD for the DA-Meter® is determined by calculating the difference between absorbance values at 670 nm and 720 nm for chlorophyll. In contrast, the Kiwi-Meter® measures anthocyanin at wavelengths from 545 to 640 nm, using 750 nm as the reference point (Costa et al., 2009; Cinosi et al., 2023).

6. Oil Content Determination (Soxhlet Method)

Oil content was determined using the Soxhlet extraction method (AOAC., 2000). Approximately 5–10 g of dried and ground olive sample was placed in a cellulose extraction thimble and extracted with petroleum ether (boiling point 40–60°C) for 6–8 hours. After

extraction, the solvent was evaporated, and the remaining oil was weighed. The oil percentage was calculated based on the initial dry weight of the sample.

7. Total phenolic contents.

Total polyphenol content was determined using the Folin–Ciocalteu colorimetric method (Farinelli D., 2002), with slight modifications. Briefly, 1 ml of each extract (in the concentration of 1 mg ml−1) was mixed with 5 ml of Folin-Ciocalteu reagent (previously diluted tenfold with distilled water) and allowed to stand at room temperature for 10 min. Then, 4 ml of sodium bicarbonate solution (75 g/l) was added. The mixture was allowed to stand for a further 30 min in the dark at room temperature, after which absorbance was measured at 765 nm using a UV/VIS spectrophotometer. Total phenolic contents were quantified using a calibration curve obtained from measuring the absorbance of four known concentrations of gallic acid (GA) standard (25–50–70–100–200 µg/l). The concentrations are expressed as milligrams of gallic acid equivalents (GAE) per gram of dry extract.

Statistical Analysis

Statistical analyses were conducted using Statistics 10 (Analytical Software, Tallahassee, FL, USA). The one-way analysis of variance (ANOVA) was used to assess the effect of harvest date on the physicochemical parameters, followed by Turkey's HSD test for post-hoc comparisons at a significance level of $p < 0.05$. Pearson correlation coefficients (r) were calculated to examine linear relationships between variables.

RESULTS AND DISCUSSION

Physicochemical changes of olive fruit during ripening

The study findings provide insights into the dynamic changes in fruit characteristics, supporting evidence-based decisions for harvest scheduling. Table 1 presents data obtained from both destructive and non-destructive measurements, including fruit diameter, fresh weight, maturity index (MI), color index (CI), flesh firmness, detachment index (DI) and IAD of standard DA-meter for green and DA-kiwi-meter for red pigment.

Fruit diameter and fresh weight of "Kalinjot" olives progressively increased until H8 reaching 18.54 mm and 3.70 g respectively (Table 1), while Leccino olives showed an increase until H5 and the stabilize **(**table 2)due to ongoing tissue growth and potential oil accumulation. These findings are consistent with observations in other olive cultivars (Baccouri, O., et al., 2008).

The Maturity Index (MI) of "Kalinjot" olives remained very low during the early stages (0.00–0.25), but steadily increased, at harvest stage 9 (H9). The Maturation Index (MI) of "Leccino" in contrast to the "Kalinjot" cultivar showed a rapid increase until the H4 harvest and after that values were stable until the last harvest.This reflects the typical change in skin color from green to dark purple/black (Beltran G., et al., 2008).

The "Kalinjot" olives Color Index (CI) showed a slight increase during mid-ripening (H6–H7) but declined at H9, likely due to color changes or over-ripening effects. On "Leccino olives the decrease of CI wassharp and very quick from26.11 in the first harvest (H1)to-0.95in the 4th harvest, this because of the fact that "Leccino" is early cultivar and high temperatures were prevailing during the season.

Table 1. Assessment of olive fruit characteristics (destructive and non-destructive) from the Kalinjot cultivar at different harvest stages

Harvest	MI	CI	Flesh firmness (N)	DI (N/g)	IAD (DA-kiwi meter)	IAD (DA Standard	% of Oil Content	Total Polyphenol Content (mg/kg gallic acid)
H1	0.0 ± 0.0[a]	29.90 ± 2.8	5.73 ± 0.4	2.09 ± 0.3	0.83 ± 0.3	2.99 ± 0.7	16.17 ± 0.6	1175.20 ± 1.6
H2	0.14 ± 0.3	31.73 ± 2.9	4.06 ± 0.3	1.75 ± 0.3	1.16 ± 0.2	3.42 ± 0.2	16.77 ± 0.0	1134.08 ± 1.2
H3	0.25 ± 0.4	30.17 ± 3.1	3.77 ± 0.3	1.69 ± 0.3	1.19 ± 0.2	3.08 ± 0.3	17.78 ± 0.0	1027.66 ± 0.0
H4	1.55 ± 0.9	23.23 ±16.3	2.87 ± 0.4	2.16 ± 0.6	1.21 ± 0.2	3.17 ± 0.4	17.88 ± 0.1	1433.10 ± 0.9
H5	1.75 ± 0.6	34.47 ± 6.3	2.76 ± 0.2	1.17 ± 0.2	1.26 ± 0.2	3.17 ± 0.2	18.88 ± 0.2	1722.31 ± 1.1
H6	1.80 ± 0.6	36.43 ± 5.5	2.60 ± 0.3	1.20 ± 0.2	1.26 ± 0.1	3.13 ± 0.3	21.63 ± 0.6	1820.89 ± 13.6
H7	1.80 ± 0.6	36.84 ± 4.7	2.51 ± 0.4	1.20 ± 0.2	1.27 ± 0.1	3.13 ± 0.3	23.38 ± 0.1	1095.48 ± 3.5
H8	2.15 ± 0.5	26.80 ± 4.1	2.44 ± 0.3	1.08 ± 0.1	1.42 ± 0.1	2.74 ± 0.4	23.52 ± 0.0	1161.75 ± 0.7
H9	3.25 ± 0.4	-0.85 ± 5.6	2.03 ± 0.2	0.99 ± 0.2	1.62 ± 0.3	1.83 ± 0.3	25.42 ± 0.1	1171.49 ± 0.9
Mean	1.41	27.75	3.20	1.48	1.25	2.96	20.158	1221.4
SD	1.16	13.46	1.13	0.51	0.27	0.57	3.2121	244.55
Variance	1.34	81.06	1.27	0.26	0.08	0.32	10.318	59804
C.V.	81.90	48.48	35.30	34.75	21.98	19.13	15.935	20.022
Minimum	0.00	-5.33	1.60	0.80	0.50	0.00	15.430	971.39
Maximum	4.00	42.78	6.40	3.90	2.00	3.80	25.650	1842.6

a- Mean, SD ±

Flesh firmness and the Detachment Index (DI)exhibited a consistent decreasing trend for both cultivars, indicating fruit softening as maturity and ripening progressed. This is a physiological change linked to cell wall degradation.

The Index of Absorbance Difference (IAD) measured by the standard DA-Meter declined, indicating internal changes and chlorophyll breakdown, while IAD measured by the Kiwi-meter increased due to anthocyanin formation. These results confirm that non-destructive DA-meter measurements can serve as reliable maturity indicators (Camposeo S., et al., 2021; Grassi S., et al., 2021). The oil content of "Kalinjot" olives steadily increased from 16.17% at harvest stage H1 to 25.42% at harvest stage H9, with the most significant increase occurring between H5 and H9 (Table 1).

The oil content of "Lecino" olives increased from 11.8% at harvest stage H1 to 24.15% at harvest stage H8 (Table 2). This suggests a heightened lipid biosynthesis during the final stages of ripening. These results are consistent with those reported by Beltrán et al., 2004, confirming that peak oil accumulation occurs in fully ripe olives. However, delaying the harvest for extended periods can adversely affect oil quality due to fruit softening and oxidative degradation.

Total phenolic compounds of "Kalinjot" olives followed a bell-shaped curve, peaking at 1820.89 mg/kg in H6, and then sharply declining in H7. The high variability (C.V. = 20.02%) highlights the sensitivity of phenolic accumulation to the ripening stage. These results are in line with prior studies (Servili M., 2002), Aparicio and Luna (2002), which showed that phenolic levels reach their maximum before full ripeness and then decline due to enzymatic activity and tissue dilution.

Total phenolic compounds of "Leccino" olives steadily increased up to H7 and started to decrease at full ripening stage.

Overall, the data suggest that harvest interval H5–H6 corresponding to the period from mid October to early Novemberfor the "Kalinjot" olives, provide the optimal balance between maturity and technological quality for oil production (Figure 1). Conversely, later harvests (H8–H9) may lead to over-ripening, which is associated with excessive softening and pigment loss.

The harvest interval H4–H5 corresponding to the period from early to mid-Octoberfor the "Leccino" olives provide the optimal balance between maturity and technological quality for oil production (Figure 1).

Study results support the use of destructive and non-destructive methods, particularly IAD and flesh firmness, as reliable olive maturity indices, in line with findings in both table and oil olives.

Table 2. Assessment of olive fruit characteristics (destructive and non-destructive) from the 'Leccino' cultivar at different harvest stages

Harvest	MI	CI	Flesh firmness (N)	DI (N/g)	IAD (DA-kiwi meter)	IAD (DA Standard	% of Oil Content	Total Polyphenol Content (mg/kg gallic acid)
H1	0.5 ± 0.5[a]	27.13 ± 2.5	4.99 ± 0.5	2.67 ± 0.2	1.29 ± 0.2	3.14 ± 0.2	11.16 ± 0.1	831.41 ± 1.00
H2	2.11 ± 0.7	4.56 ± 4.6	2.97 ± 0.3	1.75 ± 0.3	1.28 ± 0.1	3.07 ± 0.2	11.66 ± 0.3	663.24 ± 0.4
H3	3.20 ± 0.8	-0.79 ± 1.4	2.56 ± 0.5	1.79 ± 0.3	1.56 ± 0.2	3.03 ± 0.3	14.26 ± 0.3	956.92 ± 2.2
H4	3.80 ± 0.4	-0.51 ± 1.4	1.71 ± 0.2	1.57 ± 0.3	1.63 ± 0.2	2.85 ± 0.2	14.70 ± 0.0	687.74 ± 0.8
H5	3.9 ± 0.3	-1.06 ± 0.6	1.73 ± 0.3	1.35 ± 0.2	1.62 ± 0.2	2.72 ± 0.2	15.13 ± 0.2	743.73 ± 1.3
H6	4.20 ± 0.4	-0.94 ± 0.8	1.53 ± 0.2	1.28 ± 0.2	1.89 ± 0.4	2.73 ± 0.2	18.12 ± 0.2	1000.5 ± 3.2
H7	4.00 ± 0.0	-1.10 ± 0.5	1.47 ± 0.2	0.74 ± 0.1	1.92 ± 0.2	2.73 ± 0.3	19.96 ± 0.1	1302.3 ± 3.6
H8	4.30 ± 0.5	-1.06 ± 0.5	1.43 ± 0.2	0.68 ± 0.1	2.19 ± 0.2	2.59 ± 0.3	19.93 ± 0.1	1481.2 ± 5.1
Mean	3.36	2.35	2.19	1.47	1.69	2.85	15.79	963.32
SD	1.22	8.32	1.1	0.64	0.37	0.3	3.17	3.6
Variance	1.50	69.28	1.16	0.41	0.13	0.1	10.04	80.38
C.V.	36.38	54.57	49.02	43.62	21.74	10.55	20.07	29.43
Minimum	0.00	-2.4	1.1	0.54	1	2	11.08	662.95
Maximum	5.00	30.78	5.78	3.1	2.8	3.5	20.08	1488.2

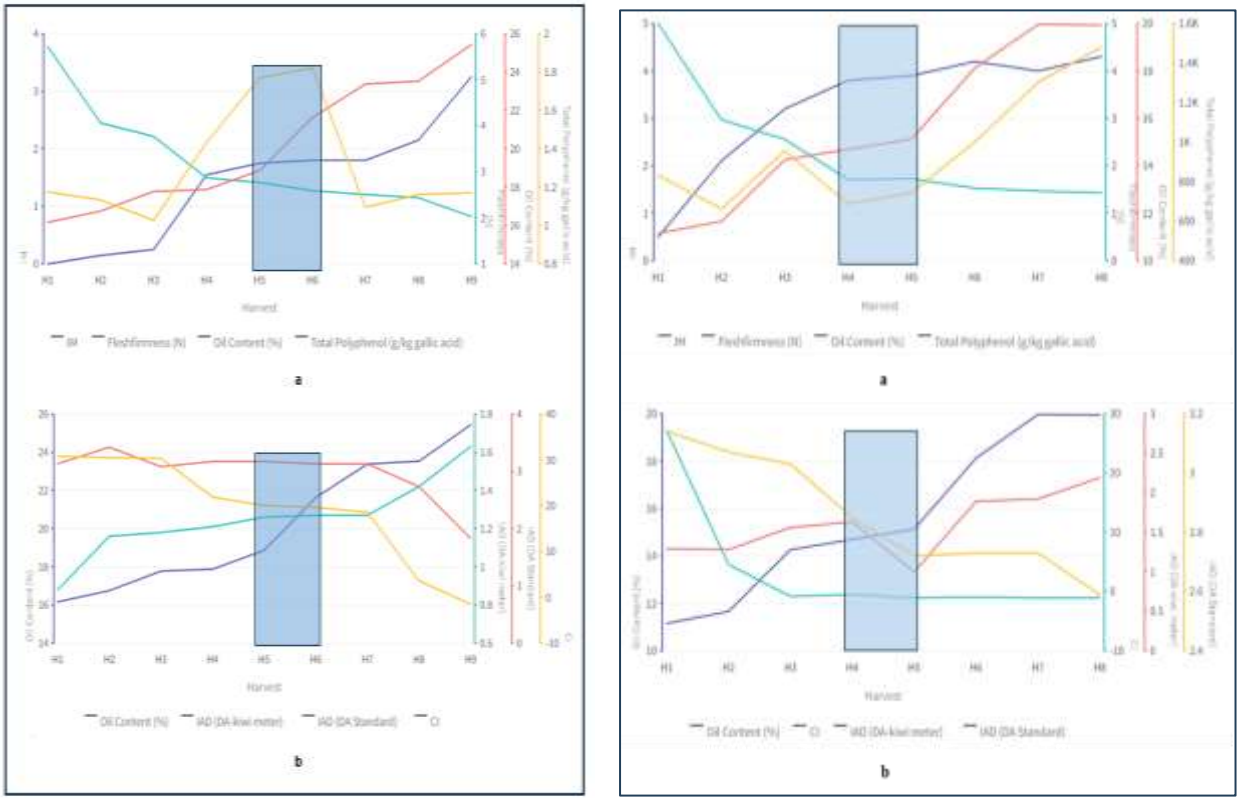

Figure 1. *Optimal harvest interval of 'Kalinjot' and Leccinocultivar based on a) destructive and b) non-destructive methods.*

Statistical Interpretation of Ripening Parameters

A General Linear Model ANOVA (GLM ANOVA) demonstrated that all assessed parameters varied significantly across the different harvest dates, with high statistical significance ($p < 0.0001$). This indicates a strong temporal effect on both physical and chemical attributes of 'Kalinjot' and "Leccino" olives during the ripening period, supporting the reliability of using time-based indicators for harvest decision-making.

Tukey HSD Post-Hoc Results

Post-hoc analysis using Tukey's Honest Significant Difference (HSD) test further clarified the nature of these differences by grouping statistically similar means:

- The Maturity Index (MI) consistently increased, while the Color Index (CI) decreased over time.

- The firmness parameter significantly decreased with ripening, showing strong group separation after H5, which suggests advanced softening in the later stages.

- IAD values (DA-Kiwi-meter values increased while DA-Standard values decreased) were strongly correlated with the progression of maturity and formed distinct groups that paralleled changes observed in destructive indices

- Oil content increased significantly until H6, after which the differences stabilized. This suggests that there is an optimal oil accumulation plateau around mid-late October (H5–H6) for “Kalinjot” and around early-mid October (H4-H5) for “Leccino” olives.

Pearson Correlation Results

Pearson correlation analysis (r) revealed several strong and biologically meaningful associations:

“Kalinjot” cultivar:

- The Maturity Index (MI) was positively correlated with the IAD index as determined by the DA-Kiwi-meter (r=0.874), indicating their concurrent increase as the fruits mature and ripen. Conversely, MI was negatively correlated with the IAD of the standard DA-meter (r = -0.697).

- The Maturity Index (MI) showed a negative correlation (r = -0.566) with the Color Index (CI) due to a delayed color change over time.

- A significant positive correlation was observed between IAD and oil content, with a correlation coefficient (r) of 0.841 for the DA-Kiwi meter and -0.690 for the DA standard. This reinforces their validity as non-destructive maturity indicators.

“Leccino” cultivar:

- The Maturity Index (MI) was positively correlated with the IAD index as determined by the DA-Kiwi-meter (r=0.897), indicating their concurrent increase as the fruits mature and ripen. Conversely, MI was negatively correlated with the IAD of the standard DA-meter (r = -0.838).

- The Maturity Index (MI) showed a negative correlation (r = -0.613) with the Color Index (CI) due to a delayed color change over time.

- A significant positive correlation was observed between IAD and oil content, with a correlation coefficient (r) of 0.947 for the DA-Kiwi meter and -0.834 for the DA-meter standard. This reinforces their validity as non-destructive maturity indicators.

- Flesh firmness and Detachment indexes were decreased and negatively correlated with MI, IAD (DA kiwi), and oil content, highlighting an inverse relationship with maturity progression for both cultivars.

These statistical findings confirm that both destructive and non-destructive measurements evolve in coherent and predictable patterns throughout the harvest season.They can reliably inform the optimal harvest window for the 'Kalinjot' and Leccinocultivars in the Vlora region.

CONCLUSIONS

1. A strong correlation is observed between the maturity index IM (increase), and the color index CI (decrease).
2. A very important finding was the correlation of IAD (anthocyanin absorbance) with the maturity index (MI), a maturity index widely used in olives.
3. Non-destructive methods, such as the Color Index (CI) and IAD via Kiwi-Meter®, offered rapid evaluations, while destructive methods yielded accurate measurements for detailed physical and chemical analysis.

4. From the data analysis, the harvest intervals were determined when the olives reach their maximum weight, high oil content, and high polyphenols.

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COMPLIANCE OF ALBANIAN FISHERIES LEGISLATION WITH EU REGULATIONS: A CRITICAL ASSESSMENT AND HARMONIZATION PERSPECTIVE

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ABSTRACT

The Albanian fisheries sector is currently grappling with significant structural and legal challenges that hinder its alignment with European Union standards. This analysis aims to assess the discrepancies between Albanian legislation and EU regulations regarding the production and trade of fishery and aquaculture products, while also identifying key measures for harmonization. Drawing on national strategic frameworks and pertinent EU regulations (notably Regulations 2020/2235, 2020/692, and 2021/617), the findings highlight that Albania has made strides in areas related to food safety and veterinary oversight. However, considerable deficiencies persist within the legal framework, particularly regarding molluscs, crustaceans, international transport, and aquaculture health management. These deficiencies pose significant barriers to export opportunities and the sustainable advancement of the sector. Statistical analyses indicate a marked decline in lagoon production and the cultivation of Mytilus galloprovincialis, exacerbated by high pollution levels in coastal waters linked to unregulated urbanization and tourism pressures. To address these challenges, this study advocates for the enhancement of institutional capacities, the modernization of the fishing fleet and port infrastructure, and the establishment of a sustainable legal framework by 2026, thereby aligning with the EU acquis.

Keywords: Fisheries, aquaculture, Albanian legislation, EU compliance, water pollution, molluscs, export

INTRODUCTION

In 1992, the Trade and Cooperation Agreement between Albania and the European Economic Community marked a key step towards EU integration through essential reforms (1). The

enactment of the first consumer protection legislation in 1997 demonstrated a commitment to ensuring that products are safe for consumers.

The Stabilization and Association Agreement signed in June 2006 further advanced consumer protection by aligning Albania's laws with the Internal Market Acquis. A significant development in food safety is Law No. 9863, the "On Food" law, established on January 28, 2008, which provides a framework to safeguard human health and consumer interests in the food supply chain (1).

Albania's legislative framework has gradually aligned with EU standards. The European Commission's 2020 enlargement methodology highlights the importance of regional collaboration. Additionally, Decision No. 749 by the Council of Ministers on 19 December 2019 established the State Body responsible for leading Albania's EU accession negotiations.

The Law on Agriculture and Rural Development established the Agriculture and Rural Development Agency (ARDA), which serves as the primary framework for implementing agricultural and rural development policies. Key strategic documents, such as the National Strategy for Development and Integration (NSDI), outline objectives in tandem with farming and rural development initiatives.
The European Commission (2) has set its main priorities, focusing on the standards that candidate countries must meet, including traceability, quota management, and control of IUU (illegal, unreported, and unregulated) fishing, while also encouraging the inclusion of Western Balkan countries in the EU's common fisheries policies.
The FAO also recommends ways in which Albania, Montenegro, and other countries can harmonize their national legislation with EU Regulation No. 1380/2013 on the CFP, analyzing current legal gaps and implementing best practices for gradual inclusion in the EU system.
Various authors in scientific studies (4) suggest the Croatian model as a successful example of compliance with the acquis and how Western Balkan countries can benefit from access to EU funds for aquaculture and sustainable fisheries management.
Despite the setbacks and difficulties (5), EU Candidate Countries, including our country, have made progress in adopting regulations on fleet control, traceability, reporting, and institutional capacity.
But the European Court of Auditors (6) also criticizes and recommends how EU funds can be better used in countries like Albania for: Building monitoring systems (VMS, AIS), Modernizing fishing ports, Inspection and traceability capacities.
The Ministry of Agriculture and Rural Development of Albania, in order to improve the situation and approximate the standards and legislation of our country with the EU, has drafted the "National Fisheries Strategy" (7), which includes objectives for compliance with the EU CFP and plans to build a stable legal framework, as well as investments in infrastructure and technology.

MATERIAL AND METHODS

This study adopts a comparative legal and institutional framework to conduct a gap analysis between Albanian legislation and European Union directives in the fisheries and aquaculture domains. Key primary sources examined include existing Albanian laws relevant to fisheries, aquaculture, conservation of aquatic ecosystems, and food safety.

The analysis also incorporates pertinent European Regulations, such as Regulation (EU) 2020/2235, which delineates health requirements and certification; Regulation (EU) 2020/692, which specifies conditions for the importation of aquatic organisms; and Regulation (EU) 2021/617, focused on disease management in aquaculture. Additionally, national strategic frameworks, particularly the Fisheries and Aquaculture Strategy for 2021–2027, have been taken into consideration.

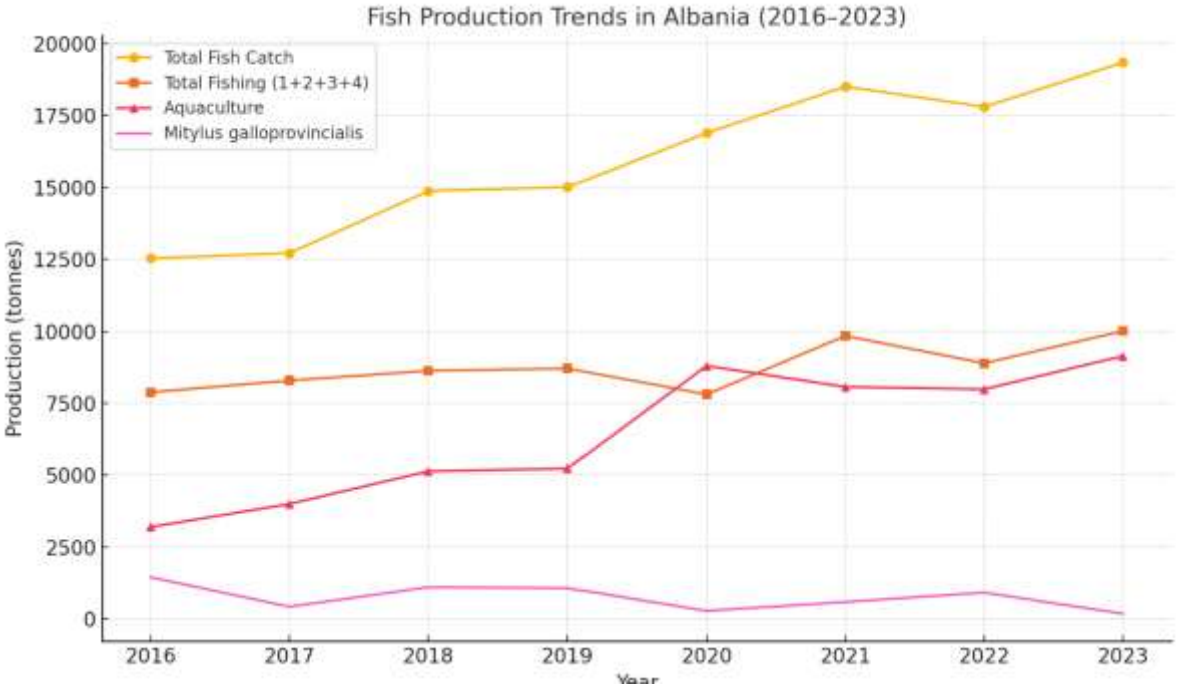
Employing a deductive methodology, this study commenced with a review of EU regulatory mandates, followed by an appraisal of their integration into Albanian legal provisions. A comprehensive documentary analysis was conducted in conjunction with a qualitative assessment of the legal frameworks. Findings were systematically reported, pinpointing each identified gap with specific recommendations regarding transposition timelines and requisite improvement measures.

A detailed examination of each component outlined in the EU regulations was conducted, with a focus on evaluating compliance within the Albanian legal framework. Instances of non-compliance were categorized as "NA." The analysis uncovered notable deficiencies, including the absence of health plans for aquaculture, inadequate regulatory frameworks governing the international transport of aquatic species, and significant shortcomings in the traceability and health registration systems for marine organisms.

Furthermore, statistical data were gathered to substantiate the findings. Annual fish production trends, categorized into lagoons, coastal, and aquaculture, were sourced from INSTAT and analyzed. This evaluation also took into account external factors, such as the repercussions of the COVID-19 pandemic, pollution, and urban development, on the sustainability and resilience of the fisheries and aquaculture sectors.

RESULTS AND DISCUSSION

Recent data from the Institute of Statistics (INSTAT) in Albania indicates a substantial increase in the fishing sector for 2023, with the total fish catch from diverse aquatic categories reaching 19,338 tons. This represents an 8.65% rise from 17,799 tons recorded in 2022 (Figure 1).
Notably, the sector experienced significant fluctuations in catch volumes during 2020 and 2021, underscoring its vulnerability to external factors, including disruptions caused by the COVID-19 pandemic and varying climate conditions. Compounding these instabilities are an inadequately stable maritime fleet and pervasive informal fishing practices that undermine regulatory efforts.
When examining specific species, concerning trends emerge. After reaching a peak catch of 5,537 tons in 2018, total captures have seen a consistent decline, dropping to 4,259 tons by 2023. The data indicate a significant decline in lagoon fishing, which plummeted from 598 tons in 2016 to a mere 218 tons in 2023, marking a decrease of approximately 63%. This downward trend raises critical concerns regarding the long-term viability of these fisheries. Coastal fishing also exhibits high volatility, with catches showing considerable fluctuations. For instance, 2018 recorded a catch of 315 tons, while 2022 saw a sharp increase to 984 tons, highlighting the instability of coastal fish stocks.



Source: *INSTAT*

Figure 1. Fish Production Trends in Albania.

The degradation of lagoon and coastal ecosystems is becoming increasingly severe, driven by several anthropogenic factors. Key contributors include urban encroachment, unchecked tourism development, and escalating water pollution, all of which pose significant threats to the ecological balance and the sustainability of fisheries in these regions.

The decline in mussel cultivation, particularly of the species Mytilus galloprovincialis, is particularly alarming. From 1,450 tons in 2016, production plummeted to a mere 186 tons in 2023, reflecting a staggering decrease of over 85%.

The Albanian fishing industry faces numerous critical challenges. Coastal water pollution poses a significant threat to marine biodiversity, which is exacerbated by a lack of export certification that restricts market access. Furthermore, inadequate quality control in laboratories intensifies issues related to product safety and compliance with market standards.

Key issues within the Albanian fishing sector include the overexploitation of marine resources, resulting in a significant decline in biodiversity, an aging fishing fleet, and outdated technologies that hinder operational efficiency. Additionally, the absence of effective pollution control measures and designated marine protected areas further threatens the sustainability of both the industry and this vital sector.

Based on the our surveying of the complete Albanian legislation in the field of the fishing industry and in comparison with the requirements of EU legislation (e.g. Regulation 2020/2235, 2021/617, 2020/692, etc.), the following scientific interpretation of the gaps (gap analysis) between Albanian and European legislation in the field of fishing and aquaculture can be carried out: Albania has made noteworthy strides in aligning its regulatory framework with European Union standards in several critical areas related to food safety and public health. Key achievements include the development of comprehensive legislation concerning Hygiene and Sanitary Conditions and the

Hazard Analysis and Critical Control Points (HACCP) principles, embodied in Law no. 9863/2008 and corresponding directives from the Minister of Agriculture.

However, despite these advancements, significant deficiencies remain in Albania's legal landscape when compared to EU norms.

Moreover, the nation lacks comprehensive health management plans for notable diseases affecting aquaculture. This shortfall poses challenges in ensuring the safety and health of aquatic organisms susceptible to these diseases, which could impede trade and exports.

Another area of concern is the absence of regulations governing the international transport of aquatic animals; Albania risks falling short of compliance with Articles 167-168 of Regulation (EU) 2020/692.

Additionally, the country's approach to registering the health of aquatic animals lacks completeness and harmonization. Current structures do not provide a clear and integrated system for monitoring key aspects such as mortality rates, quarantine procedures, and health status separation. As a result, this deficiency leads to violations of critical requirements outlined in Regulations (EU) 2016/429 and 2020/692, which are essential for maintaining sanitary control and ensuring robust biosecurity measures.

In summary, while Albania has made commendable progress in certain legislative areas, significant challenges must be addressed to fully align with EU standards and ensure the safety and health of its aquatic life and related exports.

CONCLUSION AND RECOMMENDATIONS

The fishing sector experienced fluctuations in catch volumes during 2020 and 2021, highlighting its vulnerability to external factors, such as the disruptions caused by the COVID-19 pandemic and varying climate conditions. These instabilities are further compounded by an inadequately stable maritime fleet and widespread informal fishing practices that undermine regulatory efforts.

Our review of Albanian fishing legislation shows gaps compared to EU standards (e.g., Regulations 2020/2235, 2021/617, and 2020/692). While Albania has made progress in aligning its framework with EU food safety through Law No. 9863/2008, which addresses hygiene and HACCP principles, the country still faces significant challenges. It has established regulations on microbial loads (Order No. 15/2021) and developed a national residue monitoring plan (Order No. 186, March 29, 2023).

However, critical deficiencies persist, particularly regarding legislation for live mollusks and crustaceans, which obstructs exports to the EU. Furthermore, there is a lack of comprehensive health management plans for aquaculture diseases and regulations governing the international transport of aquatic animals. The current system for registering aquatic animal health is incomplete, risking violations of essential biosecurity measures outlined in EU Regulations (2016/429 and 2020/692).

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IMPLEMENTATION OF A SMART IRRIGATION SYSTEM FOR WATER AND ENERGY WASTE REDUCTION USING ARDUINO

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ABSTRACT

Water scarcity and inefficient irrigation practices pose critical challenges to sustainable agriculture, especially in regions lacking access to advanced infrastructure. This study presents the development and implementation of a smart irrigation system designed to optimize water and energy usage using Arduino-based automation system. The system integrates soil moisture sensors and an ultrasonic water level sensor with an Arduino Uno microcontroller to monitor real-time environmental conditions. When the soil moisture falls below a predetermined threshold, and sufficient water is available, the system activates a water pump through a relay module to irrigate crops effectively. An LCD screen displays instant feedback such as soil moisture, water level in tank and pump status. Tests in real-world conditions have shown that the system reduces water consumption by more than 40% and maintains optimal soil moisture levels while minimizing energy usage. With its low-cost and scalable structure, this solution contributes to environmental sustainability by promoting precision agriculture. The study serves as a strong example of how simple and accessible technologies can address resource management challenges in small and medium-sized agricultural enterprises.

Keywords: Sustainable Agriculture, Irrigation System, Arduino, Soil Moisture

INTRODUCTION

Water scarcity has become one of the most critical environmental and socio-economic challenges of the 21st century, particularly in agricultural regions where water demand is exceptionally high. Traditional irrigation methods — such as manual watering or time-based sprinkler systems — often lead to the overconsumption of water, inefficient energy usage, and inconsistent crop hydration. These systems lack the responsiveness and adaptability required to match the actual water needs of crops, which vary based on soil type, weather conditions, and plant species. As a result, a significant portion of the water used in irrigation is either lost through evaporation, runoff, or applied when it is not needed, leading to substantial waste.

In parallel, the energy required to operate pumps and irrigation systems — especially in regions relying on electric or fuel-powered devices — contributes to the overall inefficiency and cost of traditional irrigation practices. In many agricultural areas, especially rural or underdeveloped zones, farmers may also lack access to data-driven tools or systems to monitor water levels, soil conditions, or irrigation schedules. This not only affects operational sustainability but also reduces crop yields and impacts long-term soil health.

The lack of intelligent, automated, and feedback-based irrigation systems creates a gap between the real needs of plants and the supply of water. Furthermore, climate change and increasing variability in rainfall patterns demand smarter and more adaptive irrigation solutions that can optimize water use while ensuring consistent agricultural productivity.

This study addresses these critical issues by developing an automated irrigation system that uses soil moisture sensors and Arduino-based control logic to apply water only when and where it is needed. By

doing so, the system aims to eliminate unnecessary irrigation, reduce energy usage, and increase the efficiency of water application. In essence, the project seeks to solve the dual problem of **waste in water consumption** and **inefficiency in irrigation energy usage**, while also providing a scalable solution that can be implemented in small- to medium-scale farming operations. The proposed system bridges the gap between traditional agricultural practices and modern, sustainable farming techniques using low-cost automation and real-time data collection.

The proposed study is situated within the **agricultural sector**, particularly in **rural and semi-rural farming environments** where access to advanced, high-cost irrigation infrastructure is limited. The solution is tailored for small to medium-sized farms, greenhouses, and potentially even home gardening systems. The agricultural industry is under increasing pressure to adopt sustainable practices in response to climate change, water scarcity, and rising energy costs. This project fits within the broader movement toward **smart agriculture (AgriTech)**, where automation, IoT, and real-time data collection are leveraged to improve the efficiency of farming operations. By introducing intelligent irrigation systems, farmers can optimize resource use, reduce operational costs, and increase yield, thus contributing to both economic and environmental sustainability.

Daniel H. Ngoma et al (2025) tested an IoT-based drip fertigation system for tomato farmers in Tanzania; the system optimizes water and fertilizer consumption by monitoring environmental parameters. The IoT-controlled fertigation system developed by Bariman et al. (2024) aims to improve crop quality by controlling irrigation and fertilization processes in a dosable mode. Users can monitor tank water, fertilizer mix, and irrigation times via their smartphones. The system introduced by Karar and Al-Rasheed (2020) uses an Arduino + IoT + MLP neural network to evaluate temperature, humidity, and soil moisture and optimize irrigation scheduling. This approach promises 30–50% water savings. Rehman et al. (2022) provide significant efficiency in both water and fertilizer use through precision fertigation; they also enable data analysis on cloud/IoT platforms. Satra et al. (2023) focuse on addressing the need to automate irrigation systems for efficient and sustainable agriculture practices. The use of soil moisture sensors helps to detect the moisture content of the soil and control the pumping motor to turn on or off accordingly.

SYSTEM DESIGN AND COMPONENTS

The primary method is the integration of **Internet of Things (IoT)** elements using an Arduino UNO microcontroller and soil moisture sensors. These components form the core of an automated decision-making system. The system operates on a programmed algorithm written in C++, which evaluates moisture data and controls water pumps accordingly.

System Design

(I) Real-Time Decision Making

Continuously read soil moisture levels, compare those values to a threshold and decide **when to turn on or off** the water pump

(II) Sensor Integration

Soil moisture sensors, ultrasonic sensors for water level detection and temperature or light sensors (maybe added later)

(III) Pump Control

Activates the pump based on moisture data, keeps track of how long it's running and prevents overwatering and energy overuse

(IV) Smart Feedback System

LCD display code to show current moisture level, tank status, and pump state and serial communication to monitor or debug the system from a laptop. This makes the system not only smart but also user-friendly and diagnostic-capable.

(V) Sustainability & Efficiency

Water waste is reduced through accurate, moisture-based decisions. Energy consumption is minimized by running pumps only when necessary. The system is low-cost and scalable, perfect for small to medium farms and can be used widely.

The study also incorporates Green Engineering principles by ensuring minimal water usage and reduced energy consumption. The method includes simulating and testing different irrigation scenarios to compare water usage in traditional vs. smart systems. Additionally, elements of Lean Manufacturing (waste reduction) are applied by eliminating non-value-added steps such as unnecessary manual irrigation and water overuse.

System Components:

(I) Hardware: The Arduino board, which is a microcontroller that can interact with the physical world through sensors and actuators. It has inputs (e.g., sensors) and outputs (e.g., lights or motors), and you can connect it to various components to create projects.

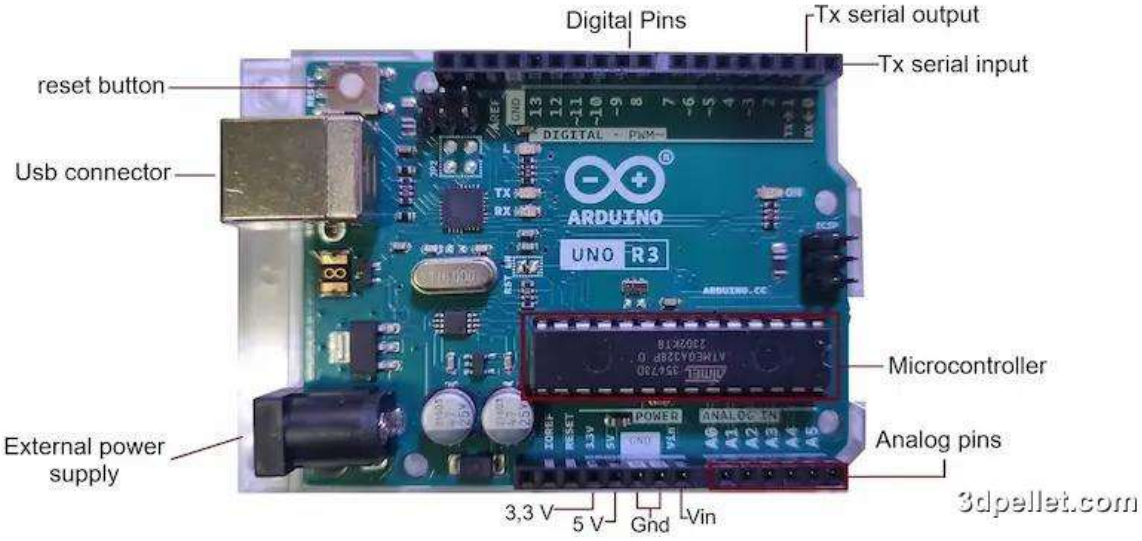

Figure 1. The Arduino board

(II) Software: The Arduino IDE (Integrated Development Environment), which is used to write and upload code to the Arduino board. The code is written in C++ but simplified for ease of use.

Arduino is widely used by hobbyists, students, and engineers for building prototypes, DIY projects, and even final products. Its simplicity and versatility make it a popular choice for learning about electronics and programming.

Table 1. Component List for smart irrigation using Arduino with Specifications:

Component	Specification	Function
Arduino UNO	ATmega328P, 14 digital I/O pins, 5V logic	Main microcontroller that processes sensor data and controls outputs

Component	Specification	Function
Soil Moisture Sensor	Analog output, 3.3–5V operating voltage	Measures the moisture level in the soil
Ultrasonic Sensor (HC-SR04)	2cm–400cm range, 5V, accuracy ±3mm	Measures the water level in the tank using sound waves
Water Pump	3–6V DC, 120L/H flow rate	Delivers water to the soil when irrigation is needed
Relay Module	5V trigger, 10A 250V AC/10A 30V DC	Electrically isolates and switches the pump on/off
LCD Display (16x2)	16 characters × 2 lines, I2C or parallel	Displays real-time system data like moisture and pump status
Power Supply	9V battery or USB 5V input	Powers the Arduino and connected modules
Jumper Wires & Breadboard	Standard male-male wires, prototyping board	Used for making safe and flexible connections during testing
Resistors (if needed)	220–330Ω (for LEDs or protection)	Used for current limiting in optional components

SYSTEM LOGIC

The workflow of the smart irrigation system is designed to enable real-time, autonomous decision-making based on soil moisture and water availability. The system continuously monitors the soil's condition and responds dynamically using a predefined algorithm. The logic is implemented in C++ and uploaded to the Arduino Uno microcontroller. Below is a description of the workflow:

System Workflow Steps:

Step 1 : Start the system

Arduino initializes all connected components (sensors, pump, LCD).

Step 2 : Read soil moisture level :

The Arduino receives an analog signal from the soil moisture sensor. This raw value (0–1023) is converted to a percentage (e.g., 0% = dry soil, 100% = saturated soil).

Step 3 : Compare moisture to threshold

If soil moisture is **below 30%**, proceed to irrigation. If soil moisture is **above 30%**, no action is taken. This dynamic response to moisture levels helps prevent both **overwatering** and **underwatering**, ensuring plants get exactly what they need.

Step 4 : Check water tank level

Before activating the pump, the ultrasonic sensor checks if the water tank has sufficient water. If the water level is **adequate**, continue. If **low**, show a warning on the LCD and stop the process.

Step 5 : Activate pump

The relay module triggers the water pump. Water is supplied to the soil for a fixed duration or until moisture reaches an acceptable level. Total water usage over time, system responsiveness to

environmental changes and energy consumption patterns data are stored for analyzing. This helps in assessing the **efficiency** and **sustainability** of the system

Step 6 : Update LCD display

The LCD screen continuously displays real-time values: Current soil moisture (%), Tank water level and Pump status (ON/OFF)

Step 7 : Loop and repeat

After each cycle, the system waits for a short delay (e.g., 10 seconds) before repeating the monitoring process.

The system continuously monitors soil moisture levels. When the moisture falls below a user-defined threshold (indicating dry soil), it checks the water tank level. If sufficient water is available, the relay triggers the pump to irrigate the soil. Once optimal moisture is restored, the pump is automatically turned off.

The LCD displays live data on:

- Current soil moisture percentage
- Water tank level (in centimeters)
- Pump status (ON/OFF)

This interactive feedback enhances usability and system monitoring for farmers.

TESTING & VALIDATION

To ensure the effectiveness and reliability of the smart irrigation system, several tests were conducted under controlled and real-world conditions. The objective was to evaluate the system's ability to accurately sense soil moisture, trigger irrigation at appropriate thresholds, and reduce water and energy consumption.

Test Conditions

The system was tested over a period of two weeks in an outdoor garden with varying weather conditions (sunny, cloudy, and partially rainy days). Soil type was loamy, and the plants used for testing included tomato and basil, both of which require moderate moisture levels.

Threshold Calibration

Initial tests were run to determine the most suitable soil moisture thresholds for irrigation. Through experimentation, the following values were established:

Moisture Level (%)	System Response
< 30%	Start irrigation
30% - 60%	Maintain current state
> 60%	Stop irrigation

These thresholds were chosen based on typical moisture needs for vegetable crops and observed sensor readings.

Parameters Measured

During testing, we measured:

- **Soil moisture level** before and after irrigation

- **Water consumption** per irrigation cycle
- **Energy consumption** of the pump (using a wattmeter)
- **Duration of irrigation cycles**

We also manually verified sensor readings with analog soil moisture probes to assess sensor accuracy.

Table 2. Test Results

Date	Soil Moisture Before (%)	Soil Moisture After (%)	Water Used (L)	Energy Used (Wh)	System Action
Day 1	28	53	1.5	12	Irrigation ON
Day 4	35	58	1.2	11	Maintained
Day 6	65	65	0	0	Irrigation OFF
Day 10	27	50	1.4	12	Irrigation ON

Observations

The system accurately responded to soil moisture changes and consistently activated/deactivated the pump based on the predefined thresholds.

Water usage was reduced by an estimated **40%** compared to a manual irrigation routine.

The pump's energy use per session was relatively low (average ~11 Wh), confirming the system's efficiency.

Cost Justification

The total cost of the prototype is under **$50**, making it a highly accessible solution for small-scale farmers or home gardeners. In larger implementations, bulk purchasing and PCB integration could further reduce the overall system cost. Compared to traditional automated systems which can cost several hundred dollars, this solution offers:

- Low initial investment
- Minimal maintenance cost
- Potential water and energy savings, which reduce long-term operational costs

Scalability Potential

Because the system is modular, it can easily be scaled:

- Add more sensors for wider coverage
- Use higher-power relays for multiple pumps
- Integrate solar panels for off-grid operation in rural areas

The affordability and flexibility make this solution suitable for widespread deployment in water-scarce agricultural regions.

Comparison: Traditional Irrigation vs. Arduino-Based Smart Irrigation

To better understand the benefits of using an Arduino-based smart irrigation system, we compared it with a traditional manual irrigation approach over a similar period and under similar environmental conditions. The implementation of the Arduino significantly improved irrigation efficiency by automating decision-making and reducing waste. This not only conserved water and energy but also minimized human effort, making it a practical and scalable solution for farmers and gardeners alike.

Table 3 Comparison: Traditional Irrigation vs. Arduino-Based Smart Irrigation

Item	**Traditional System**	**Arduino Smart system**	**Assessment (%)**
Water Usage (daily avg.)	2.5 L	1.4 L	%56 Decrease in Water consumption
Energy Consumption	~20 Wh/day (wasteful) Manual effort	11–12 Wh/day	%55 Decrease in Energy usage
Labor Requirement	High	Very low	Improved
Overwatering Incidents	Frequent	Rare	Improved
Soil Monitoring	None	Real-time, automatic	Improved
Plant Health	Inconsistent	Improved & stable	Improved
Sustainability	Poor	High	Improved

CONCLUSION

Testing demonstrated that the smart irrigation system is effective in conserving water and energy while maintaining appropriate soil moisture levels. Further improvements could include integrating weather forecasts and remote monitoring for more advanced control. If real-world environmental sensors are not available, the system can simulate weather conditions using software tools. For instance, varying virtual conditions such as **temperature**, **humidity**, or **rain** can be simulated to test system performance. This kind of testing is useful during the **prototype stage** to validate system behavior without relying on real-world conditions.

Simulated data also allows developers to test edge cases, such as drought conditions or rapid moisture fluctuations.

All collected data (moisture levels, pump activity, water levels) can be logged to an SD card or sent via serial communication to a computer. This logged data can be used for:

- System performance analysis
- Generating charts and visual reports
- Optimizing thresholds and settings for different plant types or regions

Furthermore, soil structure can be analyzed and analyzed using new sensors added to the system in the future. In this way, the soil's needs for elements can be met through fertilizers and other additives, and smart systems can be developed for this purpose.

This system aligns with the goal of integrating **data-driven decision-making** into agriculture, a key principle of smart farming (AgriTech).

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FIRST RECORD OF A PROTIST PATHOGEN OF *Labidostomis rufa* (COLEOPTERA: CHRYSOMELIDAE) (WALTL, 1838) IN ISPARTA, TÜRKİYE

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ABSTRACT

The family Chrysomelidae is one of the main families of the order Coleoptera, which is very important in terms of species number and species diversity. Both adults and larvae feed on cultivated plants as well as some useful wild plants or shrubs. The protection of agricultural products is very important both in terms of ensuring food security and contributing to the national economy. Interest in the use of entomopathogens (EPO) as an alternative to chemical insecticides in plant pest control strategies is increasing day by day. Therefore, this study aimed to investigate *Labidostomis rufa*, which is quite common in Türkiye, for the first time in terms of the presence of entomopathogenic protists. Within the scope of the study, a total of 109 *L. rufa* adults, 67 living and 42 dead samples, collected from *Crataegus monogyna* in city center of Isparta, were dissected and examined under a light microscope. As a result of dissection, a coccidian pathogen was identified from this pest for the first time. The identified coccidian pathogen was observed in the fatty tissue of adults. Polysporocystic oocysts of the pathogen are evidence of infection, and the pathogen's oocysts have been measured at 30-38 µm in diameter. Additionally, the infection rate was found to be 2.8%. Thus, with this study, *L. rufa* was examined for the presence of entomopathogenic protists for the first time in Türkiye. Since entomopathogenic protists play an important role as natural suppressive factors in pest populations, the present study is a preliminary study and is of great importance in terms of preventing or minimizing the damage caused by possible *L. rufa* outbreaks that may occur in agricultural and forest areas in the future.

Keywords: *Crataegus monogyna*, *Labidostomis rufa*, Entomopatohogen, Coccidia, Isparta, Türkiye

INTRODUCTION

The Chrysomelidae family is one of the most species-rich families of the Coleoptera order, which is very important in terms of species number and species diversity (Şen and Gök, 2009). Chrysomelids are mostly known as oligophagous. However, some species are polyphagous. Almost all species of the Chrysomelidae family feed on plants, some are crop pests, and some are even quarantine species, making them an important subject of agricultural entomology and agroecosystems. Both adults and larvae feed on cultivated plants as well as some useful wild plants or shrubs. Chrysomelids cause significant damage, especially by feeding on the leaves of plants (Hsiao, 1988). Many species cause significant crop losses when they reach high populations. Furthermore, species belonging to this family are known to cause damage not only directly but also by transmitting diseases such as bacteria, viruses, and fungi (Borror and DeLong 1966; Kısmalı, 1973; Jolivet et al., 1988; Booth et al., 1990; Özdikmen and Coral Şahin

2021). *Labidostomis rufa*, a species of the Chrysomelidae family, is quite common in Türkiye. It is also known to affect fruit formation by feeding on flowers and young leaves, causing blossom drop (Coral Şahin et al., 2016; Özdikmen and Coral Şahin, 2021). *L. rufa* generally causes damage to buds and young leaves of *Crataegus monogyna* (Gök, 2003). This could become an undesirable situation in agricultural and forest areas, and Chrysomelidae species could become common agricultural pests (Yaman et al. 2024). Therefore, protecting agricultural products is crucial for both ensuring food security and contributing to the national economy. Today, agricultural pests are generally controlled with chemical pesticides. Chemical pesticides cause pesticide resistance, environmental pollution, and toxicity to mammals and other non-target animals (Isman, 2006). The widespread use of chemical pesticides is leading to increased interest in natural products. Alternative control methods are needed for effective and efficient control of plant pests (Freitas et al., 2020; Yaman et al. 2024). Interest in the use of entomopathogens (EPO) as an alternative to chemical insecticides in plant pest control strategies is increasing day by day (Yaman, 2012). Protistan pathogens, in particular, can play an important role as natural suppressive factors in pest populations under natural conditions. Therefore, examining the presence of protistan entomopathogens in pest populations can provide useful information for identifying natural factors in pest populations (Yaman et al. 2024). So, in this study, the presence of entomopathogenic protists in *Labidostomis rufa*, which is quite common in Türkiye, was examined for the first time.

MATERIAL AND METHOD

Insect Samples

Within the scope of the study, a total of 109 *L. rufa* adults were collected, 67 living and 42 dead samples, from *Crataegus monogyna* in the center of Isparta.

Microscopic Examination

Firstly, the samples were taken for macroscopic examination. *Labidostomis rufa* adult samples were dissected in Ringer's solution and then prepared wet smears including host fat body, malpighian tubules, gut epithelium, and hemolymph were examined for the presence of the entomopathogen infection under a light microscope at a magnification of 400–1000X. When infection was found, the slides were air-dried and fixed with methanol, then stained with freshly prepared 5% solution of Giemsa stain. They were then washed in running tap water, air-dried, and examined under a microscope (Yaman et al. 2019; Yaman and Sağlam Güvendik 2024).

RESULTS AND DISCUSSION

The current study identified a coccidian pathogen from *Labidostomis rufa* for the first time. The identified coccidian pathogen was observed in the fatty tissues of adults. The pathogens polysporocystic oocysts are evidence of infection, and the oocysts measured 30-38 µm in diameter (Figure 1). Furthermore, the infection rate was found to be 2.8% (Table 1).

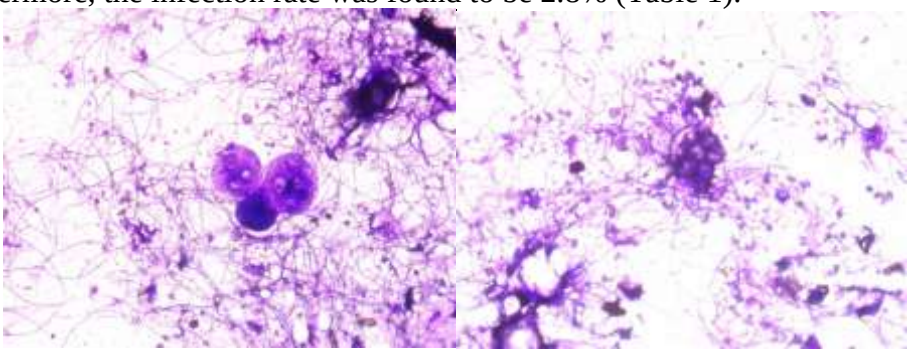

Figure 1. Coccidian pathogen detected in *Labidostomis rufa*

Table 1. Infection of coccidia in *Labidostomis rufa* populations in Isparta region

Number of examined live beetles	**Number of infected beetles**	**Infection rate (%)**	**Number of examined deed beetles**	**Number of infected beetles**	**Infection rate (%)**	**Total number of insects examined**	**Total Infection rate (%)**
67	1	%1,5	42	2	%4,8	109	%2,8

Coccidians occurring naturally in insect pest populations are highly pathogenic for them, therefore they have been considered as potential natural suppressing factor in insect pest populations (Yaman et al. 2024). Coccidia as a group of protistan entomopathogens are all intracellular parasites and multiply extensively in the host insect. Most species are host specific. Coccidia carry out extensive and more multiplication cycles in the insect host, so the pathogenic effects on the host are more pronounced (Solter et al. 2012; Yaman et al. 2023).

The Chrysomelidae family is commonly infected with protist entomopathogens. Therefore, protist entomopathogens are considered a potential factor that can suppress Cyrysomelid populations (Yaman et al. 2024). No study has been conducted on the presence *of Labidostomis rufa* entomopathogenic protists, which are quite common in Türkiye (İstanbul, İzmir (Lefèvre, 1872); Amasya (Weise, 1884); Afyon (Bodemeyer, 1906); Bilecik (Weise, 1900); İzmir (Sahlberg, 1913); Amasya (Medvedev, 1970; Tomov and Gruev, 1975); Ankara (Gruev and Tomov, 1979); Ankara, Konya, Nevşehir (Kasap, 1987); Denizli, İzmir, Manisa (Aydın and Kısmalı, 1990); Erzincan, Erzurum (Aslan and Özbek, 1998) (Gök, 2003). In the present study, entomopathogenic protists of *L. rufa* were examined for the first time in the world and in Türkiye.

The genus Labidostomis is known to have a diversity of more than 70 species distributed in North Africa, Europe and Asia (Warchalowski, 1985). Mimoun et al. (2025) reported that *Labidostomis taxicornis* preferred *Rumex* L. (Polygonaceae) as its food source in Algeria and that it was harmful. Coral Şahin et al. (2016) identified Labidostomis species causing damage to hazelnut orchards in Bartın province. In this context, they identified *Labidostomis beckeri*. The following year, they identified *Labidostomis rufa*, which damages almond, cherry, and apple trees in Karaman province, for the first time. In recent years, *Labidostomis longimana*, which damages pistachio trees by feeding on their young leaves and veins, has been reported to cause serious yield losses in pistachio production in Türkiye (Özgen and Tok 2008). While collecting *L. rufa* in the current study, it was observed that it damaged newly opening *C. monogyna* leaves (Figure 2).

Şekil 2. *Labidostomis rufa* feeding on *Crataegus monogyna* leaves

CONCLUSIONS

The present study includes the first records on the occurrence and prevalence of coccidian pathogens in the populations of *Labidostomis rufa* pests in Türkiye. Since entomopathogenic protists play an important role as natural suppressive factors in pest populations, the present study is a preliminary study and is of great importance in terms of preventing or minimizing the damage caused by possible *L. rufa* outbreaks that may occur in agricultural and forest areas in the future.

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VIABILITY AND VIGOUR OF CARROT (DAUCUS CAROTA L.) SEEDS IN DIFFERENT FERTILIZATION REGIMES

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ABSTRACT

The main goal of the present study is to establish the vital indicators and vigor of carrot seeds depending on the application of different fertilization regimes during seed production. Three levels of NPK were studied, as follows: N – 0, 50, 70, 90 kg.ha^{-1}, P – 0, 90, 140, 190 kg.ha^{-1} and K – 0, 100, 150, 200 kg.ha^{-1}, applied once and twice. The germination energy, laboratory and field germination, the growth vigor index of the seeds, as well as the onset of germination phases and deviations from normal seedling development, were determined. The highest germination energy, laboratory and field germination were found with a once fertilization with $N_{90}P_{90}K_{200}$, as well as with $N_{90}P_{90}K_{200}$, $N_{50}P_{190}K_{100}$ applied twice. Once fertilization with $N_{50}P_{90}K_{100}$ and $N_{90}P_{190}K_{200}$ increases the seeds vigor, while at the other term of the application, this was observed with $N_{90}P_{90}K_{100}$ and $N_{90}P_{90}K_{200}$. It has been established that different fertilization regimes have an impact on the beginning of the phases of seed germination, as well as on deviations from the normal development of seedlings.

Key words: vigour, germination, seedlings, phases of sprouting, sprout deviation

INTRODUCTION

Carrots (*Daucus carota* L.) are a major root vegetable crop. Their widespread use in the food industry requires obtaining high and stable yields. This, together with the increasing demands of vegetable growers on the quality of seed material, necessitates the need to search for different ways to optimize the technology of seed production in order to increase the quantity, but above all the quality of the obtained seed production. Chilingirov et al. (2018) and Haytova and Ivanova (2016) highlight the great importance of the vital status of seeds for the normal development of plants. Panchev (2019) also points out that for optimal germination and sustainable growth in seed-propagated plants, high seed quality is of particular importance.

The nutritional regime is of essential importance in the technology of growing carrot seed stalks, as the correct determination of the necessary amounts of macroelements nitrogen, phosphorus and potassium is a prerequisite for the normal development of seed plants and the production of high-quality seeds (Panayotov, 2005). Hooda et al. (2014) indicate that fertilization with higher rates of nitrogen and phosphorus improves the productivity of the seed stalks and increases the quality of the obtained seeds. A similar statement is made by Ravinder and Kanwar (2002). Panayotov (2015) and Ilyas et al. (2013) indicate that phosphorus is essential for better plant insemination and seed quality. Iqbal et al. (2023) also emphasize the importance of the nutritional regime in the carrot seed production system, claiming that fertilization with $N_{150}P_{125}K_{65}$ kg.ha^{-1} results in an increase in seed yield and quality. According to Kushwaha (2009), increasing the nitrogen fertilizer rate to 100 kg.ha^{-1} improves the viability of carrot seeds. On the other hand, Panayotov et al. (2021) indicated that carrot seed viability significantly increased after application of higher levels of phosphorus and potassium. Similar conclusions have been formulated by Stepuro (2008).

The control of the carrot fly (*Psila rosae* Fabr.) is essential for the successful seed production of carrots. This pest is widespread in Europe, North America and New Zealand (Collier and Finch, 1996; Ramert and Ekbom, 1996; Sivasubramaniam, 1996). Initially, organochlorine compounds were used to control the carrot fly, but in the 1950s, resistance to this group of insecticides developed (Wright and Coaker, 1968). In modern plant protection, insecticides from the group of synthetic pyrethroids are widely used to control the pest.

The main objective of the present study was to assess the impact of different fertilization regimes in carrot seed production on their viability and vigour.

MATERIAL AND METHOD

The experiments were carried out during the period 2017-2019 in the Experimental Field and Laboratory of the Department of "Horticulture" at the Agricultural University - Plovdiv, Bulgaria with carrots, variety Tushon. The following variants with different fertilization levels in kg.ha^{-1} and application term were studied:: once fertilization: 1.$N_0P_0K_0$ – control; 2.$N_{70}P_{140}K_{150}$ (recomended); 3.$N_{50}P_{90}K_{100}$; 4.$N_{50}P_{90}K_{200}$; 5.$N_{50}P_{190}K_{100}$; 6.$N_{50}P_{190}K_{200}$; 7.$N_{90}P_{90}K_{100}$; 8.$N_{90}P_{90}K_{200}$; 9.$N_{90}P_{190}K_{100}$; 10.$N_{90}P_{190}K_{200}$ and twice fertilization: 11.$N_{50}P_{90}K_{100}$; 12.$N_{50}P_{90}K_{200}$; 13.$N_{50}P_{190}K_{100}$; 14.$N_{50}P_{190}K_{200}$; 15.$N_{90}P_{90}K_{100}$; 16.$N_{90}P_{90}K_{200}$; 17.$N_{90}P_{190}K_{100}$; 18.$N_{90}P_{190}K_{200}$. The different fertilizer rates are determined based on the recommended fertilization for carrot seed production in Bulgaria $N_{70}P_{140}K_{150}$ (Madzhirova, 1968; Kolev et al. 1977). In the once fertilization, the entire amount of phosphorus and potassium was applied during the autumn deep plowing, and the nitrogen - before planting the stecklings, while in the twice fertilization - half of the amount of phosphorus and potassium was applied during the autumn deep plowing, the other half - before planting the stecklings, and the nitrogen - half when planting the stecklings and the rest, when the first inflorescences appear.

Ammonium nitrate (N 34%), triple superphosphate (P_2O_5 46%) and potassium sulfate (K_2O 50%) were used. Soil preparation was carried out by deep plowing in the autumn and furrowing in the spring at a distance of 80 cm. Seed production was carried out using the technology adopted in Bulgaria by preliminary production of stecklings (Murtazov et al., 1984).

The stecklings were produced by sowing at the end of June and harvesting in mid-November, after which they were placed for storage during the winter period in a pit. The stecklings were planted in mid-March on a furrow surface according to a scheme of 80x30 cm. Each variant was presented in four replicates with a experimental plot size of 7 m^2 and a explored area of 6 m^2 (Barov, 1982).

During the vegetation of the seed plants, all necessary cultural practices were carried out for their normal development. The seed production was harvested when 60-70% of the seeds were fully mature, and the rest were waxy ripe. The seed stalks were placed for 10 days for post-harvest ripening of the seeds, after which seed extraction was implemented. The seeds were stored in paper bags under laboratory conditions.

Germination energy and germination were determined according to the requirements of ISTA (2016). Field germination was determined by sowing 100 seeds in soil in four replicates, at a temperature of 23^{0}C and a humidity of 80% water field capacity and counting the germinated seeds on the 25th day after sowing. The vigor index was calculated using the "Initial vegetative productivity of seeds" method (Panayotov, 2013).

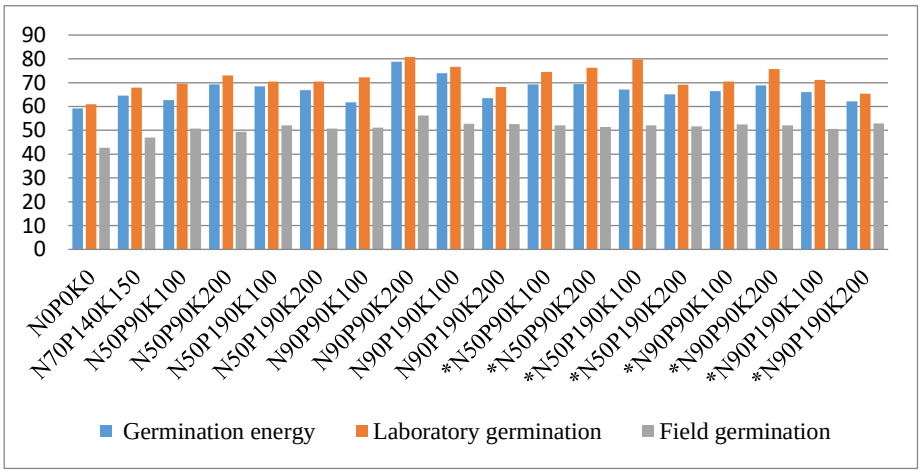
The mass occurrence of the individual phases of seed germination was monitored chronometrically in four replicates of 50 seeds: water absorption (WA), imbibition (I), development of the embryo root (ER) and development of the embryo tip (EA) according to Minkov et al. (1972), The water absorption was deteriminated – when the seeds absorbed of 40% water of the seed's own weight; imbibition - when the seed absorbing water more than 70% of its own weight; development of the germinal root - when it is displayed outside the seed coat; development of the embryo tip - when the embryo tip and cotyledons are released from the seed coat. Deviations from the normal development of the seedlings were established according to the description of ISTA (2016) and according to the classification of Wellington (1972).

Yellow sticky traps were placed to detect the presence of the carrot fly (*Psila rosae* Fabr.) during the period of steckling production and the cultivation of seed plants. Various insecticides from the group of synthetic pyrethroids will be used for control if the presence of this pest is observed. The data from the study were subjected to analysis of variance (Fowel and Cohen, 1992), and the least significant differences between the individual variants were calculated using the Fisher test at p = 0.05, according to the ANOVA method. Due to the similarity in trends over the three years of the experiment, the results shown are the average three-year data.

RESULTS AND DISCUSSION

The viability potential of vegetable seeds is determined by various indicators, of which germination energy and germination are some of the most important (Copeland and McDonalds, 2001; Black et al., 2006; Panayotov, 2015). Seed quality depends on many environmental and agrotechnological factors. The correct implementation of plant protection and especially the contriol of pests is also of great importance for the normal development of seed plants. During the vegetation period, when field experiments were carried out, no pests with an economic threshold of harmfulness were observed in steckling growing as well as of seed production.

The application of a different nutritional regime in the technology of carrot seed production has a significant impact on the vital status of the obtained seed production (Figure 1). In all tested variants, a higher germination energy of the seeds was recorded, compared to the unfertilized control. This indicator has a maximum value after a once fertilization with $N_{90}P_{90}K_{200}$ – 78.88%. This represents an increase of 14.33% compared to the fertilized control, and to the unfertilized control this difference is even greater and reaches 19.66%. High results were also established in the variants $N_{90}P_{190}K_{100}$ – 73.99% (once) and $N_{50}P_{90}K_{200}$ – 69.44% and $N_{90}P_{90}K_{200}$ – 68.88%, applied twice.



* twice fertilization

Figure 1. Viability of carrot seeds depends on fertilization regime (%)

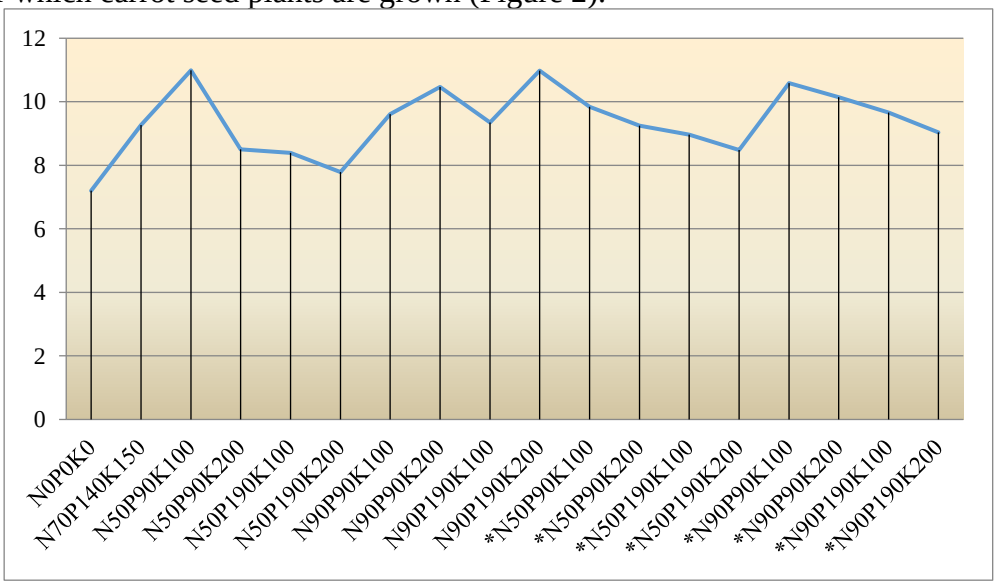
A slight decrease in the energy with which the seeds germinate, compared to the fertilizer rate used until now in carrot seed production in Bulgaria, is observed with the combination $N_{90}P_{190}K_{200}$ in both fertilization regimes, as well as with a once fertilization with $N_{50}P_{90}K_{100}$ and $N_{90}P_{90}K_{100}$.

All studied fertilization levels and regimes led to higher seed germination compared to the unfertilized and fertilized controls. This is especially strongly observed after a once application of $N_{90}P_{90}K_{200}$ (80.88%). A very close result was also reported for the fertilizer rate $N_{50}P_{190}K_{100}$ applied twice – 79.77%. A slight decrease compared to the fertilized control was found with the twice application of the maximum amounts of the three fertilizers ($N_{90}P_{190}K_{200}$–65.44%). Statistically significant differences were found between the unfertilized control and the tested variants, except $N_{90}P_{190}K_{200}$ (twice).

Under field conditions, optimal temperature and humidity for normal germination and emergence of seeds cannot be provided, as they are achieved in a laboratory setting. This necessitates monitoring the behavior of seeds in a production environment. Field germination values, expressing the percentage of seedlings from a given sample or batch, are always lower than those established for laboratory germination (Cholakov, 2009). Field germination is an important indicator that, together with germination energy and laboratory germination, contributes to a better characterization of the vital state of seeds (Panayotov, 2015).

The use of different fertilizer rates and term term of application of mineral fertilizers affect the germination of carrot seeds under field conditions. The results obtained showed that all tested fertilizer combinations cause better field germination compared to the two controls – unfertilized and fertilized with the recommended amounts of the three fertilizers. The differences between the individual variants are small, but nevertheless, it can be noted that the highest germination percentage is distinguished by seeds from plants fertilized once with $N_{90}P_{90}K_{200}$ – 56.25%. This represents an increase of 9.23% compared to the fertilized control and 13.59% compared to growing seed plants without fertilization.

Seed vigor is a very important indicator that characterizes not only the ability of seeds to germinate, but also to form normal seedlings and subsequently plants under different environmental conditions (Marcos-Filho, 2015). Therefore, determining the seed vigor is of great significance in assessing the seed quality (Panayotov, 2013). The seed vigor index is influenced by the nutritional regime under which carrot seed plants are grown (Figure 2).



* twice fertilization

Figure 2. Index of carrot seeds vigor under application of different fertilization regime

The monitored indicator reaches maximum values after a once fertilization with $N_{50}P_{90}K_{100}$ and $N_{90}P_{190}K_{200}$ - 10.99 and 10.98, respectively. Close to these results, at the same time of application of mineral fertilizers, was also established for the $N_{90}P_{90}K200$ variant (10.47). When applying the different fertilizer combinations twice, the seeds obtained after fertilization with $N_{90}P_{90}K_{100}$ (10.59) and $N_{90}P_{90}K_{200}$ (10.14) were characterized by higher vigor. No significant differences were found between the two periods of application of the mineral fertilizers.

It can be noted that carrot seed viability improves after fertilization with higher levels of phosphorus and potassium. Similar conclusions were reached by Iqbal et al. (2023) and Panayotov et al. (2021).

The different levels and terms of mineral fertilizer application affect the duration of the individual germination phases (Table 1).

Carrot seeds absorb water within about 1 hour, as was found for most of the tested variants. The second phase “imbibition” begins 1 to 2 hours later, and significantly earlier, already in the first hour, this is observed for the fertilizer rates $N_{50}P_{90}K_{100}$ and $N_{90}P_{90}K_{100}$ applied once, as well as for the twice application of $N_{50}P_{190}K_{200}$.

Table 1. Occurrence of germination phases in carrot seeds depending on the applied fertilization regime (hours)

Variants	Water absorption	Imbition	Embryo root	Embryo tip
Once fertilization				
$N_0P_0K_0$	2	3	56	184
$N_{70}P_{140}K_{150}$	2	3	48	176
$N_{50}P_{90}K_{100}$	1	1	48	184
$N_{50}P_{90}K_{200}$	1	2	48	144
$N_{50}P_{190}K_{100}$	2	3	56	176
$N_{50}P_{190}K_{200}$	1	3	48	184
$N_{90}P_{90}K_{100}$	1	1	48	192
$N_{90}P_{90}K_{200}$	1	3	48	168
$N_{90}P_{190}K_{100}$	1	2	48	192
$N_{90}P_{190}K_{200}$	2	3	56	176
Twice fertilization				
$N_{50}P_{90}K_{100}$	2	3	48	144
$N_{50}P_{90}K_{200}$	2	3	48	176
$N_{50}P_{190}K_{100}$	1	2	48	200
$N_{50}P_{190}K_{200}$	1	1	48	160
$N_{90}P_{90}K_{100}$	2	2	48	176
$N_{90}P_{90}K_{200}$	1	2	48	136
$N_{90}P_{190}K_{100}$	1	3	48	184
$N_{90}P_{190}K_{200}$	1	3	48	184
LSD p=0.05%	0.62	0.99	8.80	39.75

The development of the embryo root occurs at 48 hours for almost all fertilizer combinations except for $N_{50}P_{190}K_{100}$ and $N_{90}P_{190}K_{200}$ applied once, in which a slight delay is observed – at 56 hours, respectively. The shortest time for the development of the embryo tip was established after two fertilizations with $N_{90}P_{90}K_{200}$ (136 hours), in contrast to the fertilizer rate recommended so far for carrot seed production, in which this phase occurs at 176 hours, and when growing the seed plants without fertilization, this time increases to 184 hours. It can be noted that this phase also occurs relatively quickly in the variants $N_{50}P_{90}K_{200}$ (once) and $N_{50}P_{90}K_{100}$ (twice) – at 144 hours. A significantly greater delay in the onset of the last phase among the tested variants was observed with the twice application of $N_{50}P_{190}K_{100}$ – at 200 hours. The results obtained indicate that the influence of the different nutritional regimes is observed more strongly as germination progresses, when enzyme activity increases and physiological processes leading to the release of accumulated nutrients in the seeds are intensified (Copeland and McDonald, 2001; Panayotov, 2015).

It can be assumed that optimizing the nutritional regime leads to the accumulation of a greater amount of nutrients in the seeds, which helps more quickly passing the individual phases of germination, especially strongly manifested in the last two - development of the embryo root and development of the embryo tip.

The results for the deviation from normal seedling development are of significant importance for the qualification of seed production, as they show to what extent the seed can develop a normal seedling and subsequently a normal plant (Table 2). At both fertilization periods, the $N_{90}P_{90}K_{200}$ combination leads to the lowest percentage of deviation in seedlings, 5.88% (once) and 4.55% (twice), respectively. A decrease was also recorded in the variant fertilized twice with $N_{90}P_{90}K_{100}$ (5.99%). Compared to these results, for the fertilizer rate used so far and the unfertilized control, the deviations established are 6.55% and 7.66%, respectively.

Table 2. Deviations from normal seedling development depending on the fertilization regime

Variants	%	Type of deviation in percentage to total number of deviations
Once fertilization		
$N_0P_0K_0$	7.66	E - 9.27; K - 42.22; R - 41.60; C - 44.66;
$N_{70}P_{140}K_{150}$	6.55	E - 5.34; K - 37.45; R - 46.66; C - 41.77;
$N_{50}P_{90}K_{100}$	7.22	E - 6.32; K - 35.92; R - 50.00; C - 44.44;
$N_{50}P_{90}K_{200}$	6.99	E - 4.77; K - 35.54; R - 43.33; C - 38.21;
$N_{50}P_{190}K_{100}$	6.66	E - 4.75; K - 37.50; R - 50.00; C - 34.33;
$N_{50}P_{190}K_{200}$	6.10	E - 3.77; K - 19.84; R - 42.84; C - 40.66;
$N_{90}P_{90}K_{100}$	6.22	E - 4.82; K - 44.16; R - 43.33; C - 35.77;
$N_{90}P_{90}K_{200}$	5.88	E - 8.66; K - 35.11; R - 48.14; C - 46.88;
$N_{90}P_{190}K_{100}$	6.66	E - 2.55; K - 31.40; R - 42.83; C - 33.55;
$N_{90}P_{190}K_{200}$	7.00	E - 4.90; K - 33.33; R - 45.83; C - 42.32;
Twice fertilization		
$N_{50}P_{90}K_{100}$	7.32	E - 4.10; K - 35.88; R - 44.96; C - 40.53;
$N_{50}P_{90}K_{200}$	7.00	E - 4.66; K - 27.70; R - 43.99; C - 42.66;
$N_{50}P_{190}K_{100}$	6.99	E - 4.16; K - 29.49; R - 35.54; C - 37.50;
$N_{50}P_{190}K_{200}$	6.66	E - 2.00; K - 24.38; R - 46.05; C - 41.62;
$N_{90}P_{90}K_{100}$	5.99	E - 1.88; K - 35.77; R - 32.29; C - 50.00;
$N_{90}P_{90}K_{200}$	4.55	E - 3.77; K - 33.33; R - 50.00; C - 36.40;
$N_{90}P_{190}K_{100}$	7.00	K - 31.42; R - 44.18; C - 45.32;
$N_{90}P_{190}K_{200}$	7.99	E - 4.16; K - 37.72; R - 45.22; C - 35.94;
LSD p=0.05%	0.95	

E – short embryo root ; K – lack of hairs on embryo root;
R – lack of branches of embryo root; C – unopened cotyledons

The main types of abnormalities that occur are a short embryo root, lack of embryo root hairs, lack of embryo root branches and unopened cotyledons. The highest percentage of manifestation is characterized by abnormalities of type R (lack of branches) and C (unopened cotyledons), followed by those of type K (lack of embryo root hairs) and E (short embryo root).

The results obtained do not differ significantly between the individual variants, but the effect of the different fertilizer levels applied is noticeable. This is especially well observed when applying mineral fertilizers twice, where the percentage of the individual deviations decreases, very clearly manifested in the first two types - short embryo root and lack of hairs on the embryo root, and in the variant $N_{90}P_{190}K_{100}$, the presence of sprouts with a short embryo root is not even detected.

CONCLUSIONS

Different nutritional regimes have a significant impact on the vital status of carrot seeds. The highest germination energy was recorded for $N_{90}P_{90}K_{200}$ – 78.88% with a once fertilization, laboratory germination of approximately 80% – for $N_{90}P_{90}K_{200}$ (once) and $N_{50}P_{190}K_{100}$ (twice). Field germination is improved most strongly by a once application of $N_{90}P_{90}K_{200}$ – 56.25%. A stronger impact is observed from higher levels of phosphorus and potassium.

Once fertilization with $N_{50}P_{90}K_{100}$ and $N_{90}P_{190}K_{200}$, as well as twice fertilization with $N_{90}P_{90}K_{100}$ and $N_{90}P_{90}K_{200}$, increased seed vigor by about 20% compared to the unfertilized and fertilized controls.

The time for the occurance of the individual phases of seed germination is influenced by the different rates and terms of mineral fertilizer application, more pronounced in the last phase -

development of the embryo tip, as the period is significantly reduced, especially after fertilizing twice with $N_{90}P_{90}K_{200}$.

Applying the combination $N_{90}P_{90}K_{200}$ at both terms contributes to the lowest percentage of deviations from normal seedling development in carrot seeds.

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MORPHOLOGICAL AND DECORATIVE EVALUATION OF NEW, PROMISING GLADIOLUS VARIETIES

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ABSTRACT

The aim of the study was to establish the decorative value of different gladiolus varieties under Bulgarian conditions, grown outdoors. The experiments were carried out in the Experimental Field of the Department of Horticulture at the Agricultural University-Plovdiv. The study was conducted with the gladiolus varieties Fado, Oasis and Amsterdam. The corms were planted in in period between 5-10 April according to the 15 x 15 cm scheme. All agrotechnological practices for normal plant development were applied. The mass, height and diameter of the corms of the individual varieties were recorded. The phenophases of sprouting, appearance of the flower stalks, beginning and end of flowering were monitored. The height and diameter of the stem, the number of leaves and the leaf area, the number of developed and undeveloped flowers, the length, diameter and color of the flower were studied. The number of developed branches/corm and productivity of cut flowers was recorded. It was found that the Amsterdam variety has the earliest sprouting, the phenophase of the beginning of flowering begins later, but it has the longest duration of flowering and forms the largest corm. The leaf area and the number of leaves, as well as the height of the stem, were the highest values in this variety. At the same time, the

number of undeveloped flowers in it is the lowest, and the diameter of the flower occupies the middle position between the studied varieties, reaching 10,7 cm. It is also characterized by the highest productivity, 357 016 flower stalks per ha. approximately 10% more than Oasis varieties. For the conditions of Bulgaria when growing gladiolus in open areas, the application of the Amsterdam variety is recommended.

Keywords: Peduncle, Flower Diameter, Corms, Leaf area

INTRODUCTION

The main line of flower cultivation is the production of cut flowers. A significant number of species are used for cut flowers. Gladiolus is in second place among the four main crops used for cut flowers. Bai et al. (2009) pointed out that it is widespread in many countries and is grown both in field and greenhouse conditions. The species is characterized by high decorative value of the cut flower and its valuable economic qualities make it preferred and sought after on the domestic and foreign markets during the spring-autumn period (Bose et al., 2003). It is used both for bouquets and for creating various arrangements. The diversification of varieties of this crop is significant. Varieties with a huge diversity of colors have been created - from snow-white, all the colors of the rainbow, to dark purple and almost black. They can be pure tones or with two colors. In this regard, it is important to carry out research to identify suitable varieties, depending on the specific climatic and soil conditions. For the creation of new varieties, one of the main methods of selection in gladiolus is intervarietal and interspecific hybridization (Denisa et al., 2012). As a result of applying this method, breeders from the Cluj-Napoca region, Romania, have created over ten new established hybrids suitable for outdoor cultivation.

Along with morphological studies, a modern method for assessing the genetic relationships between individual gladiolus hybrids is by applying DNA analysis. In this regard, Pragya et al. (2010) investigated an extremely large collection of 54 gladiolus genotypes. Based on nine main morphological characteristics and the application of 225 random amplified polymorphic DNA (RAPD) markers, amplified with 25 random primers, they revealed the genetic relationships between the selected hybrids.

The identification of varietal differences and the completion of the characteristics of individual varieties were achieved by Kamble et. al. (2004) through phenological observations. In experiments conducted with nine varieties of gladiolus, they found that the earliest development and flowering were exhibited by the Snow-Princes and American Beauty varieties, with sprouting occurring in 58,20 days and flowering in 66,70 days. The cultivar Magic variety developed less well.

A large part of the varieties distributed in Bulgaria are foreign, with the planting material imported from the Netherlands. In Bulgaria, through hybridization of the Oasis x Blues varieties, a new gladiolus variety Iva was obtained with very valuable decorative qualities, with a purple color, a medium-high and very strong light green stem and very early flowering and is suitable for greenhouse and field production, as well as for landscaping gardens and parks (Ivanova et al., 2019).

The manifestation of the characteristics specific to each genotype is very much dependent on the specific climatic conditions, above all, the availability of optimal light, especially in the 4-6 leaf phase. This requires conducting targeted scientific testing of individual varieties and establishing their behaviors under the relevant conditions (Shillo and Halevy, 1996). In this regard, Kaninski et al. (2012), in order to establish the impact of the specific conditions of Bulgaria, studied a large set of varieties with different planting dates and found that plant development and flowering were accelerated at later dates.

The aim of the study was to establish the decorative value of different gladiolus varieties, grown outdoors under Bulgarian conditions.

MATERIAL AND METHOD

The experiments were carried out in the Experimental Field of the Department of Horticulture at the Agricultural University-Plovdiv, during the period 2022-2024. The gladiolus varieties Fado,

Amsterdam and Oasis were studied. The soil on which the experiments were conducted is alluvial-meadow (Fluvi soil) with a humus content of 4,04% and pH 8,06. The content of mobile nitrogen is 47,53 mg/kg soil, of mobile phosphorus – 52,6 mg/100g soil and of mobile potassium – 60,45 mg/100 g soil (Trayanov, 2021).

The preparation of soil began with the application of 15 $kg.da^{-1}$ phosphorus as a triple superphosphate and 20 $kg.da^{-1}$ potassium, as potassium sulfate. After that, the deep autumn plowing was done and continued with the rough formation of a flat raised bed. Its fine preparation was carried out in the spring. Twenty days before planting, the nitrogen fertilizer, as ammonium nitrate at a dose of 24 $kg.da^{-1}$ nitrogen, was applied.

Before planting, the diameter, height and weight of the corms were investigated. In the period 5-10 April, the corms were planted by the scheme 15 x 15 cm in four replicates, with 100 plants in the experimental plot or 440,444 corms per hectare. Each agrotechnical practice in the vegetation that is necessary for the normal growth of plants was applied.

Phenological observations of the beginning of sprouting, the beginning of the flower stalk appearance, the beginning and end of flowering were registered when 10% of the plants were in the respective phase (Dimova et al., 2012).

The biometric measurements of the height and diameter of the flower stalk and the number of leaves and leaf area (measured by an electronic leaf-area meter WinDias) per plant in the mass flowering stage were studied. The number of developed and undeveloped flowers was recorded. The colors, diameter and length of the flower were established. The measurements were carried out on 10 plants from each replicate. The number of flower stalks, developed from one corm was determined. The yield of cut gladiolus flower in the stage of mass flowering of the stalk was established.

The data were processed by analysis of variance, performed using the Fisher test at $p = 0,05$. The ANOVA method is described in Fowel and Cohen (1992). The trend in the experimental years was similar, therefore, the presented results are average values from three-year experiments.

RESULTS AND DISCUSSION

There is a wide diversity of gladiolus varieties, and therefore conducting research to identify suitable varieties depending on specific climatic and soil conditions is extremely important (Hort et al., 2012). Genotypic differences between the studied gladiolus varieties are also observed in the planting material according to the morphological features of the corm (Table 1). The diameter of the corms of individual gladiolus varieties varies within narrow limits from 3,5 cm for the Amsterdam variety to 3,8 cm for the Fado variety. The height of the corm is the largest in the Oasis – 1,40 cm, and the smallest in the Fado – 1,0 cm. The Amsterdam variety occupies an average position with a height of 1,23 cm. Greater variation was found in the corm weight trait. It was the largest in the Amsterdam variety, with an average of 69,26 g. In the Fado and Oasis varieties, it was significantly smaller, 38,03 g and 45,60 g, respectively.

Table 1. Morphological behaviors of gladiolus corm

Varieties	Diameter (cm)	Height (cm)	Weight (g)
Fado	3,8	1,0	38,03
Oasis	3,6	1,4	45,60
Amsterdam	3,5	1,23	69,26
LSD p=5,0%	0,24	0,93	4,30

The difference in the sprouting period between the tested varieties is relatively small – from 25 to 30 days after planting the corms (Table 2). The earliest sprouting was recorded for the Amsterdam variety – on day 25, followed by the Fado– on day 26. It was the most delayed for Oasis – on day 30. Slight variation was observed in the next phenophase, the emergence of the flower stalk. The earliest

onset of this phase was recorded for the Fado variety – 16 days after sprouting. The differences between the studied genotypes increase as the vegetation progresses. This is observed very clearly in the phase of the beginning of flowering. The earlier appearance of the flower stalk in the Fado also causes earlier flowering – already on day 90 after sprouting.

The latest flowering was observed in the Amsterdam variety, with a difference of 11 days compared to the earliest variety. A positive characteristic of Amsterdam, compared to other varieties, is the later end of flowering. The shortest flowering period, of 62 days, was observed in the Fado variety, which ended 152 days after planting. The longest flowering period in Amsterdam lasted up to 167 days, or a period of 66 days after the onset of flowering. Ivanova et al. (2019) point out that for the conditions of Bulgaria, the average duration of flowering of gladiolus is within 50-60 days. This shows that the varieties tested in this study have longer flowering, which also defines them as more promising for outdoor cultivation. Statistical significance of the data was established.

Table 2. Phenological observation of different gladiolus varieties (days after sprouting)

Varieties	Sprouting*	Appearance of flower stalk	Begging of flowering	End of flowering
Fado	26	42	90	152
Oasis	30	48	95	160
Amsterdam	25	46	101	167
LSD p=5,0%	1,89	1,03	2,26	5,22

*The days after planting

The most important morphological indicator is the height of the flower stalk (Table 3). The importance of this feature as a decorative value is emphasized by Kumar et al. (2011) and by Ivanova et al. (2019). The highest plants were measured in the Amsterdam variety – 127,1 cm. The Fado variety is characterized by the lowest height - 96 cm. The height of the plants of the Oasis variety is smaller than that of Amsterdam, with 22,1 cm. Another vegetative feature describing gladiolus plants is the diameter of the flower stalk. Lepcha B et. al. (2007) emphasize that in addition to the height of the flower stalk, its diameter is also important for determining the decorative qualities of the gladiolus, especially in the case of cut flowers and their placement in vases. This characteristic varies from 2,1 cm for Oasis, through 2,3 cm for the Fado variety, to 2,5 cm for the Amsterdam variety. A difference between the individual varieties is also observed in the characteristic of the number of leaves. The plants of the Amsterdam variety have the largest number - 9, followed by the Oasis and Fado varieties, which have the same number - 8, of each. An important factor is also the leaf area. The Fado variety has the smallest leaf area – 960,59 cm^2. Next is the Oasis – 1022,13 cm^2. The largest leaf area is recorded for the Amsterdam variety -1238,42 cm^2. The data obtained are mathematically proven.

Table 3. Morphological characteristics of the gladiolus plant

Varieties	Heigh of flower stalk (cm)	Diameter of flower stalk (cm)	Number of leaves	Leaves area (cm^2)
Fado	96,3	2,3	8,0	960,59
Oasis	105,0	2,1	8,5	1022,13
Amsterdam	127,1	2,5	9,2	1238,42
LSD p=5,0%	4,70	0,34	0,62	10,12

The decorative value of flowering ornamental species is determined primarily by the number of developed flowers (Table 4). In terms of this characteristic, the lowest decorative value was found in the Oasis variety, with the smallest number of developed flowers – 8,2 and the largest number of

undeveloped flowers (4,5). The other two studied varieties, Fado and Amsterdam, have better decorative behavior, with the number of developed flowers being 10,5 and 12,3, respectively, and the undeveloped flowers being 3,2 and 2,1. The results are mathematically proven. This is confirmed by the proportion of developed flowers to the total number of flowers on a plant. This percentage is highest in Amsterdam – 85,41%, which indicates that this variety has the best decorative value.

Table 4. Development of gladiolus flower of one flower stalk

Varieties	Number of developed flowers	Number of undeveloped flowers	% developed flowers of the total numbers
Fado	10,5	3,2	76,64
Oasis	8,2	4,5	64,56
Amsterdam	12,3	2,1	85,41
LSD p=5,0%	1,09	0,53	

The next important indicator is the diameter of the flower (Table 5), with the highest value found in the Oasis variety – 11,8 cm, followed by Amsterdam (10,7 cm). The smallest flower diameter is characterized by the Fado variety (10,4 cm). For the decorative value of gladiolus flowers, their length is also of particular importance. This characteristic follows the same trend as for the flower diameter. The longest flowers are those of Oasis, reaching 10,4 cm, in second place are those of Amsterdam, and again the smallest size is that of Fado. The color of the flower is decisive in assessing the decorative qualities. The tested varieties are of different colors: white for Amsterdam, cyclamen for Fado and pink for Oasis.

Table 5. Morphological behavior of gladiolus flower

Varieties	Diameter of flower (cm)	Length of flower (cm)	Color of flower
Fado	10,4	9,6	Cyclamen
Oasis	11,8	10,4	Pink
Amsterdam	10,7	9,7	White
LSD p=5,0%	0,62	0,34	

In addition to the decorative value when growing cut flower crops, their productivity is also assessed. Shillo and Halevy (1996) emphasize that the development and productivity of gladiolus are particularly influenced by the specific conditions under which it is grown. Under the conditions of conducting the experiments (city of Plovdiv, Bulgaria), the yield of cut flowers was determined (Table 6). In this case, it was established in relation to the number of cut flowers obtained. The highest cut flower yield was obtained from the Amsterdam variety – 357 016 nr.ha^{-1}, or this represents 80,32% of the planted corms. Relatively high cut flower productivity is also shown by the Fado plants – 349 010 nr.ha^{-1}, which is 78,52% and in last place is Oasis with 70,20% of formed flower stalks related to all planted corms. In the tested varieties, it can be noted that they develop an average of one flower stalk per corm, with Amsterdam developing slightly more than one. The data are statistically significant.

Table 6 Productivity behaviors of gladiolus plant

Varieties	Number of flower stalks/corm	Yield form cutting flower stalk (nr.ha^{-1})	Percentage of yield to total planted corms

Fado	1,1	349 010	78,52
Oasis	1,0	312 082	70,20
Amsterdam	1,2	357 016	80,32
LSD p=5,0%	1,70	138,34	2,62

CONCLUSIONS

Individual gladiolus varieties differ slightly in terms of their morphological characteristics, more significant differences are observed in terms of their weight.

The differences in the duration of individual phenophases increase as the vegetation progresses, most clearly evident in the stage of the beginning of flowering. The earliest flowering characterizes the Fado variety, but the period of flowering is the longest in the Amsterdam variety.

The best developed plants are characterized by the Amsterdam variety, which develops flower stalks with the greatest height and diameter, as well as the largest leaves. This variety also has the biggest percentage of normally developed flowers. The flowers are the largest in the pink variety Oasis.

The highest productivity was found in the Amsterdam variety with 357 016 nr.ha^{-1} flower stalks, reaching 80,32% of normally developed flower stalks compared to the total planted corms. Next in line is the Fado variety, with 78,52% and lastly Oasis.

For the conditions of Bulgaria, the Amsterdam variety can be recommended as the most suitable for cultivation in field conditions.

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MONITORING OF THE GREENHOUSE WHITEFLY (TRIALEURODES VAPORARIORUM) IN THE LUSHNJA DISTRICT

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ABSTRACT

The Whitefly is a highly polyphagous pest capable of surviving on over 250 plant species belonging to 84 different families. This pest primarily affects vegetable crops, particularly tomato, eggplant, pepper, bean, potato, and zucchini, but also ornamental plants. Adult whiteflies are typically found on the tips of the shoots and on the undersides of young leaves, where they lay their eggs. The study was conducted in 2024 in the village of Hysgjokaj, Lushnje, in a 1-dynym greenhouse cultivated with tomatoes, cultivar Lidia 1 & 2. Yellow sticky traps were used to monitor the pest, placed both inside the greenhouse and in its surrounding perimeter. The trap was placed at a height of 20 cm above the vegetative canopy of the plant and was adjusted accordingly as the plant increased in height. Population dynamics recorded two peaks: the first on April 30 with 102 whiteflies, and the second on May 28 with 124 whiteflies. These population peaks corresponded with rising temperatures and the emergence of new generations of the greenhouse whitefly. Furthermore, the monitoring revealed the presence and spread of *Trialeurodes vaporariorum*, posing a threat to vegetable crops. The implementation of integrated pest management (IPM) measures is necessary to reduce whitefly infestations and prevent further spread.

Keywords: The greenhouse whitefly, *Trialeurodes vaporariorum*, monitoring, maximum population density, cultivar Lidia.

INTRODUCTION

The greenhouse whitefly (*Trialeurodes vaporariorum*) is a polyphagous pest capable of living on over 250 plant species belonging to 84 different families. It is one of the key pests in protected environments (J. Tedeschini 2010). The greenhouse whitefly (*Trialeurodes vaporariorum*) originally comes from tropical and subtropical America, probably Brazil or Mexico. It occurs on many crops, including aubergine, beans, cucumber, sweet pepper, tomato, roses, gerbera and poinsettia (Koppert 2025).

Older plants can become reservoirs for pests and pathogens. (Jeremy Jubenville 2024).
The greenhouse whitefly (GHWF) (*Trialeurodes vaporariorum* Westwood) has been a problem in greenhouses for many years. Within the last ten years this whitefly pest has been emerging as a serious threat to vegetable and fruit production in the field as well **(**William M. Wintermantel, 2004). *T. vaporariorum* has also demonstrated a remarkable ability to develop resistance to many synthetic insecticides used for control (Adam Pym et al., 2019). Compared to other pests of ornamentals, whiteflies have a long life cycle, ranging from two-and-a-half to three weeks under warm conditions to two months under cooler conditions (UF IFAS Extension 2025).

An experiment at the Peter the Great Botanical Garden showed that mixtures of essential oils and Neem oil had an insecticidal and repellent effect on *Trialeurodes vaporariorum*, reducing the pest's fertility and providing long-term protection for plants. (Kirill G Tkachenko et al., 2023). In the battle against the greenhouse whitefly (*Trialeurodes vaporariorum*), HortiPro introduces the pheromone Pherodes as an innovative solution for the signalling and monitoring of this persistent pest. (HortiPro

2024). According to J Jumardi et al. (2020) have emphasized that: is very important in monitoring the pest on potato because we now know that for monitoring eggs and adults. Based on Colead (2024).

A follow-up in the field where the whitefly infested produce was harvested should also be done and appropriate management measures taken to control the pest. Only produce inspected to be free from whiteflies and other quarantine pests should be granted for their export.

MATERIALS AND METHODS

Yellow sticky traps were used to monitor the pest, these traps were placed both inside and outside the greenhouse, where a regular weekly program was implemented.

The trap inside the greenhouse was positioned at a height of 20 cm above the vegetative tips of the plants and was moved upwards as the plants grew. Thanks to this trap, the population dynamics were tracked by counting the number of adult insects caught each week. After counting, the adult insects were removed with a small pair of tweezers and recorded in the workbook. Additional traps were placed near the door and windows. The traps were replaced once a week. For pest identification on tomato plants, besides regular weekly monitoring, visual inspections were carried out using a magnifying loupe.

The study was conducted in a tomato greenhouse in the village of Hysgjokaj, Lushnja, from January to mid-June 2024. The greenhouse is conventional with an area of 0.1 Ha, solar-heated polyethylene greenhouse, where the tomato cultivar Lidia 1 and 2 with were planted. The greenhouse has a suitable soil for vegetable cultivation.

According to some authors, the threshold critical for chemical intervention in tomato crops is 10 larvae per cm² of leaf area, while others emphasize 0.5–1 adult insect per plant or 10 adult insects per trap per week.

Figure 1. Greenhouse in Hysgjokaj, Lushnja (Google Maps)

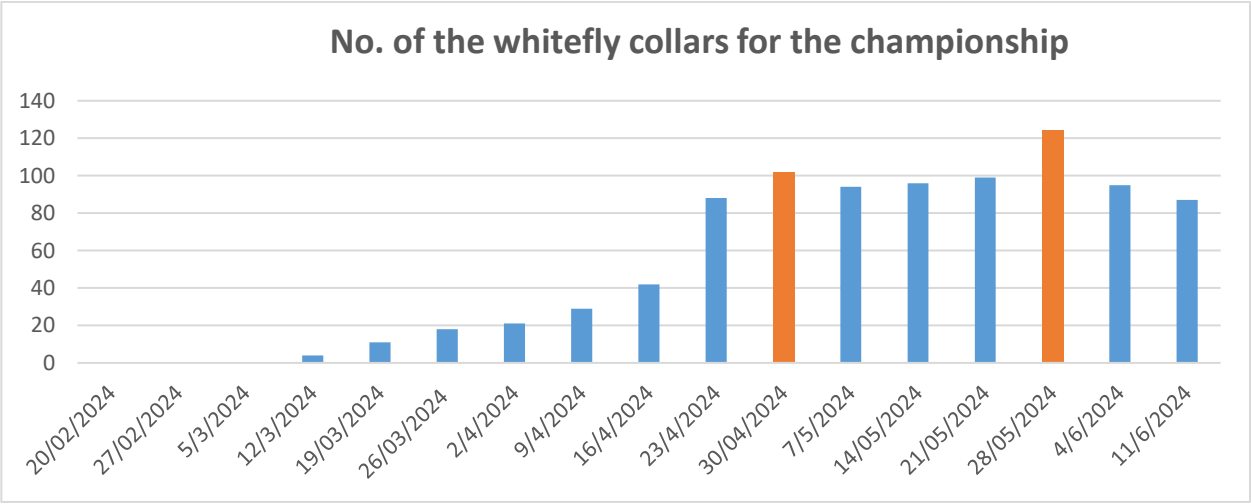
Figure 2. Greenhouse in Hysgjokaj, Lushnja

RESULTS AND DISCUSSION

From all the work carried out in the greenhouse using pheromone traps to capture whiteflies, it was observed that their capture began on March 12, followed by an increase on April 30, which we identify as the first peak with a count of 102 whiteflies. In the days that followed, the number of whiteflies caught by the pheromone remained high, but on May 28, a second peak was recorded with 124 whiteflies. On June 4 and 11, a considerable number of whiteflies were also caught by the pheromone traps. Both peak periods corresponded with rising temperatures and the emergence of new generations of the greenhouse whitefly. Below, we have prepared a table showing the number of whiteflies monitored during the period from February to June.

Table 1. Number of whitefly terns caught during 2024 for each analyzed date

Date	No. of white collars
February 20	0
February 27	0
March 05	0
March 12	4
March 19	11
March 26	18
April 02	21
April 09	29
April 16	42
April 23	88
April 30	102
May 07	94
May 14	96
May 21	99
May 28	124
June 04	95
June 11	87



Charts 1. Population dynamics by sampling dates.

The graph shows two population peaks: the first on April 30 with 102 whiteflies and the scond on May 28 with 124 whiteflies.

This study, was conducted in greenhouses covered with plastic in the village of Hysgjokaj, Lushnje district. The following conclusions were drawn:

- The greenhouse whitefly (*Trialeurodes vaporariorum)*, remains the most widespread pest affecting tomato crops in greenhouses.
- Adult whiteflies prefer the youngest and uppermost parts of the plants, while pupae are more commonly found on older leaves.
- Yellow sticky traps are highly effective for monitoring the population dynamics of this pest.
- In addition to their efficientcy, these traps are also cost – effective.

DISCUSSION

As shown in the graph, the whitefly population exhibited a gradual increase from the end of February,marking the beginning of the early insect activity,likely influenced by rising temperatures in the protected environments of the greenhouse.

Two main population peaks were recorded on April 30 and May 28, 2024, representing the periods of maximum activities for both generations.

Following the second peak, a gradual decrease in trap catches was observed likely due to pest management and control measures.

The population dynamics observed in this study align with findings from international literature, wich report that *Trialeurodes vaporarium* exhibits continuous activity and multiple generations during the cultivation period in protected enviroments such as plastic greenhouses.

Similar studies in tomato greenhouses in Spain, Italy and Turkey have reported two to three population peaks during the season, with maximum activity in the months of April - May and July.These findings for the peaks at the end of April and the end of May. (Sanchez et al., 2001; Oztemiz et al.; 2019).

This agreement with the international literature shows that the method used for monitoring - yellow sticky boxes - is reliable for the estimation of the population of *Trialeurodes vaporariorum*, and that our results are comparable to those of other countries in similar agroclimatic conditions.

CONCLUSIONS

- Monitoring and implementing integrated control measures against *Trialeurodes vaporariorum* presents an effective strategy to reduce damage, improve agroecological sustainability, and decrease dependence on chemical pesticides.
- The greenhouse whitefly (*Trialeurodes vaporariorum*) remains the most widespread pest in this area and causes significant damage to tomato crops in greenhouses.
- Yellow sticky traps are very effective for tracking the population dynamics of this pest.
- Besides being efficient, these traps are also relatively low-cost.
- Additionally, integrated measures help preserve biological control agents and improve the biological balance in protected production systems, increasing economic efficiency and the safety of agricultural production.

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INTEGRATED CONTROL OF THE CODLING MOTH (*CARPOCAPSA POMONELLA*) IN THE KORCA REGION

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ABSTRACT

Carpocapsa pomonella is the most dangerous and frequent pest of apple crops. The demange is caused by worms that feed on fruit. This pest is present and causes damage in other crops as well as pears, quince, and walnuts. In our country, it has found favorable conditions for the development of its life cycle, giving two to three generations per season. This study aims to monitor the codling moth population in Pirg, Korca during 2024. The monitoring was carried out in an apple orchard of the Starking variety with a surface area of 0.1ha, where traps with sexual pheromones were used for both generations. The traps was placed inside the tree crown. The progress of catching codling moth butterflies for the first generation was carried out for the period 15 May - 5 June, where the maximum point was on 25 May with 8 butterflies. While the progress of capturing butterflies for the second generation was realized in the period 2 July - 9 September, and the maximum capture point was on 19 July with 9 butterflies. The results helped in the early detection of this pest and determining the optimal period for intervention with protective measures, contributing to the more sustainable management of this pest.

Key words: Codling moth, *Carpocapsa pomonella*, monitoring, traps, management.

INTRODUCTION

Codling moth is the most dangerous and frequent pest of apple, pear, quince and walnut. (F. Musa 2014). In apple, it is considered as a key pest, from all the countries of the world where the apple plant grows (E. Isufi 2022). The codling moth, Cydia pomonella, is a globally distributed pest, primarily affecting apple and pear orchards. It has been introduced to many regions where apples are grown, causing significant economic losses to fruit production. In 2022, it continued to be a major pest in apple orchards (EPPO Global 2022). Generations/years vary depending on the soil and climate zones (BioFruitNet 2022). In apple and pear, the larvae often enter through the calyx (eye) or the ripening cheek of maturing fruits, although entry may occur anywhere on the fruit surface. They then bore down to the core of the fruit, leaving a prominent entry hole which has a red coloration around its rim. This hole becomes blocked with brown excreta (frass) as the larvae continue to feed on the flesh and seeds of the fruit. A smaller, unblocked exit hole can be seen on the fruit later, once the larva has escaped. Attacked fruit also tends to drop prematurely, often during the June. A single larva will usually attack only one fruit, and fruits are rarely attacked by more than one larva. Damage is generally greater in areas where there are two or more generations per year. (CABI, 2021). The impact on the quantity and quality of the fruit makes it a challenge for producers and agricultural technicians. Furthermore, its effects not only impact the harvests, but also the export capacity and access to new markets, due to its international phytosanitary implications (Mónica Sánchez 2025). Monitoring and control is very important since this insect hibernates in the plot, and it is necessary to reduce its population by using several control strategies simultaneously: Delta traps with a female pheromone diffuser for attracting males. Sexual confusion: A cloud of female pheromone specific to *Carpocapsa* is created in the whole plot, preventing males from finding females and thus avoiding both mating and, therefore, laying. Control of very high populations is very difficult, and control of the first generation is key (GreenNets2023) The pest exhibits a strong resistance capacity to both synthetic and natural insecticides, while shifting seasonal temperatures disrupt the reliability of phenology-temperature models for predicting targeted control strategies (E. Charles Whitfield * et.al., 2024). Effective management of *Codling Moth* populations requires a holistic approach integrating multiple control tactics. IPM strategy involves cultural, biological, and chemical control methods. Cultural practices, such as orchard sanitation, pheromone-based mating disruption, and targeted pesticide applications, help reduce *Codling Moth* populations. Biological control agents, including parasitoids and predators, can provide valuable natural suppression of this pest. Furthermore, judicious use of insecticides and selective formulations can be employed as part of an IPM program to minimize the impact on beneficial organisms and the environment (C Maggi et al., 2023). According to the author (B. Skenderasi et al., 2023), In addition, the use of sex pheromones can be used as part of an integrated past management approach that combines different control methods to sustainably manage past populations.

MATERIAL AND METHODS

The study was conducted in the village of Pirg in the Korca region, in an apple orchard with an area of 0.1 Ha and the Starking variety. The study was followed during the months of May to September 2024. The climate of Korca is a continental mediterranean climate with large changes in temperature. The relief is mainly mountainous and hilly. The average altitude is 1145 meters above sea level.

Figure 1. The orchard in Pirg, Korca

Figure 2. The orchard from (google earth)

Sex pheromone traps were used to monitor the pest. The trap was placed inside the crown of the apples in production. Trap monitoring to detect the biological progress of the codling moth was carried out at regular intervals and the data were recorded in a field notebook. Ferrotraps are essential for monitoring the flight dynamics of the codling moth. For early detection of the pest and to assess the effectiveness of the management strategy, a regular monitoring program is needed.

To monitor the flight dynamics of the codling moth *C. pomonella*, sex pheromone traps were used. For the first generation, at the beginning of May, a sex pheromone trap was placed in the orchard It was placed inside the crown of the apples in production, at an eye height of about 160 - 170 cm from the ground.

After June 20, the trap was set for the second generation and its monitoring began. The monitoring procedure for this generation was the same as for the first generation. The sex pheromone capsule was changed once/generation.

Figure 3. Monitoring pheromone

Figure 4. Monitoring the orchard

RESULTS AND DISCUSSION

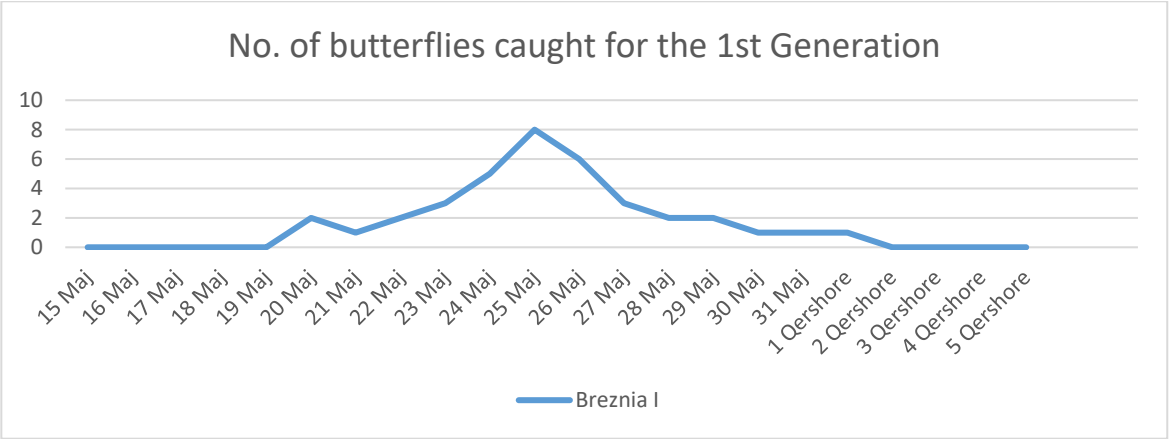
The trap was checked every morning until the first butterflies were caught, and then monitoring became weekly and very strict. The butterflies were removed with the help of tweezers and the data was recorded in a special notebook.

Monitoring of butterflies for the first generation began on May 15, where it was regularly followed every day and the maximum butterfly capture was reached on May 25 with 8 butterflies. Monitoring of the first generation continued until June 5 and during this period from May 20 to June 1, butterflies captured by pheromone were recorded.

For the first generation, the critical treatment threshold was reached on June 5. The treatments were applied out 10 days after the peak butterfly capture (recorded on May 25, with 8 butterflies), as this coincided with the emergence of the first larvae of the *Codlin moth*.

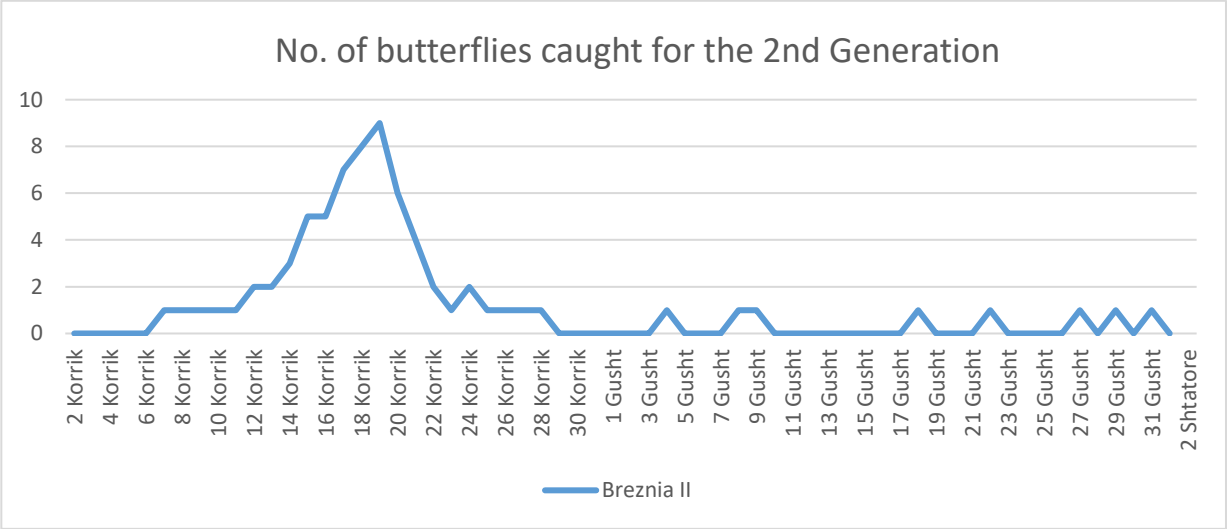
The progress of catching the first generation of codling moth butterflies in the Pirg of Korca area, for the period May 15 - June 5, and the maximum catch was on May 25 with 8 butterflies.

Monitoring of butterflies for the second generation began on July 2, where it was regularly monitored every day and the maximum butterfly capture was reached on July 19 with 9 butterflies. Monitoring of the second generation continued until September 9 and during this period from July 7 to August 31 butterflies captured by pheromone were recorded.



Charts 1. Number of butterflies caught for first generation.

Meanwhile, during the second generation, two treatments were applied. The first treatment was carried out on July 29, following the peak batterfly capture recorded on July 19 (9 butterflies). A second treatment was applied one week after the first.



Charts 2. Number of butterflies caught for the second generation.

The progress of catching the second generation of codling moth butterflies in the Pirg of Korca area, for the period July 2 - September 9, and the maximum catch was on July 19 with 9 butterflies.

According to the literature Ecomjedisi Durres (2014), the critical treatment threshold is 10 days after maximum flight or when 1-2 eggs or larvae per 100 fruits.

According to Ecomjedisi Durres (2014), this threshold is set limit at 10 days after the peak flight or the detection of 1-2 eggs or larvae per 100 fruits.

The peak capture of codlin moth from the first generation coincides with period of optimal environmental conditions- (temperature and humidity) wich are highly favorable for the mass emergence of male moths from pupae located in the soil. This increase in moth numbers typically occurs 7-14 days after a stustained rise in temperature and corresponds tu period when moth are most active in mating. behavior. At this stage, pheromone traps operate at maximum efficiency in attracting males.

Similary the peak capture of second- generation moths coincides with the moment when optimal conditions (temperature and humidity) are very favorable for the massive emergence of male moth from the pupae of the first generation located in the ground.

CONCLUSIONS

Monitoring the population of *Carpocapsa pomonella* using pheromones is essential for:

- Early detection of the presence of the insect
- Determining the optimal time for intervention
- For environmental safety and does not harm beneficial insects
- Assessing the effectiveness of the protective measures implemented
- Reducing unnecessary pesticides

Also, to have the best possible effectiveness against this key pest of this crop, all other IPM measures must be combined, such as:

- Agrotechnical measures
- Physical and mechanical measures.
- Biological measures.
- Chemical measures

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RADIOGRAPHIC EVALUATION OF THE ROLE OF TITANIUM SCAFFOLD POROSITY ON BONE REGENERATION AND IMPLANT STABILITY IN TTA

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ABSTRACT

Advances in Tibial Tuberosity Advancement (TTA) techniques for cranial cruciate ligament rupture (CrCLR) in dogs rely on the selection of different implant material designs that balance strength, biocompatibility, and osseointegration. Porous titanium scaffolds have emerged as a transformative option, offering a unique combination of structural stability and improved bone regeneration. This study evaluates the impact of scaffold porosity on bone healing and implant stability, focusing on their radiographic performance over an eight-month postoperative period. The study included 15 cases, treated with TTA using porous titanium scaffolds. Radiographic evaluations were performed immediately postoperatively and at months 3, 6, and 8 to assess bone remodeling and scaffold integration. Radiographic evaluations revealed progressive osseointegration, with complete osseointegration achieved in all cases by eight months. Smaller scaffolds promoted faster callus formation and higher trabecular density, while weight-based variations highlighted the importance of tailoring implant designs to individual patient profiles. These radiographic findings confirm the critical role of porous titanium scaffolds in osseointegration in advancing TTA procedures, enhancing short-term outcomes.

Keywords: Tibial Tuberosity Advancement, Titanium Scaffold Porosity, Bone Regeneration, Implant Stability.

INTRODUCTION

Tibial Tuberosity Advancement (TTA) is a widely adopted surgical technique for addressing cranial cruciate ligament (CCL) ruptures in canines. This procedure aims to stabilize the stifle joint by altering the biomechanical dynamics of the patellar tendon (Kim et al., 2008; Krotscheck et al., 2016). A critical aspect of TTA involves the use of implants to maintain the advanced position of the tibial tuberosity,

with their design and material properties significantly influencing integration with bone tissue and overall stability (Eltlhawy et al., 2023; Shah et al., 2019; Spoerke et al., 2005).Titanium and its alloys are among the most commonly used materials for orthopedic implants, owing to their excellent biocompatibility, corrosion resistance, and favorable mechanical properties (Khan et al., 1999; Long & Rack, 1998). One key factor determining the performance of titanium implants is their porosity, which can mimic the extracellular matrix of bone to enhance osseointegration—the process by which bone tissue adheres to the implant surface (Chen et al., 2017; Liang et al., 2022; Van Der Stok et al., 2013). Successful osseointegration ensures implant stability and optimal load transfer between the implant and bone, both of which are essential for long-term clinical success (Agarwal & García, 2015; Elias et al., 2012).The porosity of titanium scaffolds also plays a pivotal role in bone regeneration. Scaffolds with interconnected pores facilitate vascularization and new bone ingrowth, critical for healing large bone defects (Agarwal & García, 2015; Wen et al., n.d.). Studies have demonstrated that porous titanium scaffolds loaded with osteoinductive factors, such as Bone Morphogenetic Protein-2 (BMP-2), can achieve full regeneration of segmental bone defects (Van Der Stok et al., 2013). However, achieving the correct porosity is essential, as excessive porosity may compromise mechanical strength, while insufficient porosity limits biological integration (Abbasi et al., 2020; Ryan et al., 2006). Therefore, an optimal balance between porosity and mechanical integrity is critical for successful orthopedic applications (Van Grunsven et al., 2014).

In the context of TTA, implant stability is of paramount importance, as micromotion at the bone-implant interface can hinder osseointegration and lead to implant failure (Brunski, 1999; Szmukler-Moncler et al., 1998). Factors such as implant porosity and surface characteristics directly influence primary stability and subsequent biological responses. Thus, understanding the interplay between scaffold design, bone regeneration, and implant stability is crucial for improving surgical outcomes in TTA procedures (Karageorgiou & Kaplan, 2005; Raffa et al., 2021; Rousseau et al., 2021).

This study aims to evaluate the influence of titanium scaffold porosity on bone regeneration and implant stability in TTA. By analyzing radiographic images , we seek to determine the optimal porosity that balances mechanical strength with biological integration, thereby enhancing the efficacy of TTA implants.

MATERIAL AND METHOD

Population

Radiographs of thirty-eight cases of dogs that underwent porous TTA according to the technique described in a previous study (Trisciuzzi et al., 2019) were retrospectively reviewed between January 2023 and January 2024. Inclusion criteria consisted of dogs that had postoperative X-rays (T0) and were 3 months (T3), 6 months (T6), and 8 months postoperatively (T8), showing no implant failure. Cases of previous surgical re-intervention of CCL tear repair, other reported complications, and X-rays that were not available 8 months postoperatively were excluded.

Radiographic Evaluation

Mediolateral and craniocaudal radiographic images of the treated limb were obtained at four time points: immediately after surgery (T0), 3 months (T3), 6 months (T6), and 8 months (T8) to monitor bone regeneration at the osteotomy site, osseointegration of the scaffold, and implant stability. Radiographs were independently analyzed by three board-certified radiologists, blinded to study details to ensure unbiased evaluation. Radiographic assessment focused on key parameters, including:

- Bone-implant interface: Evaluation of osseointegration and radiolucency.

- Bone regeneration (Tibia tuberosity osteotomy): Evaluation of callus formation and trabecular continuity.

The healing of the osteotomy site was assessed and scored on a scale of 0 to 4, according to a previously published study (Barnhart et al., 2016) which was adapted for our technique. The sites were defined as the proximal osteotomy region in the cage, the cage region, and the distal osteotomy region in the cage. It is a 0–4 scale, with 0 indicating no bone healing; 1 indicating early bone union without a bridge between the tibial tuberosity and the tibial shaft; 2 indicating bone union at one site; 3 indicating bone union at two sites; 4 indicating union at all three sites.

- Implant stability: Detection of movement or displacement.

Grouping

The following groups were used for analysis: Cage sizes: Grouping based on cage sizes: small (4.5 mm to 6 mm), medium (7.5 mm to 9 mm), and large (10.5 mm to 12 mm). Weight-based groups: small dogs (10-25 kg), medium dogs (26-50 kg), and large dogs (51-75 kg). Time-based comparisons: Time-based analysis of radiographic parameters at T0, T1, T2, and T3 to assess healing progress.

Data collection and analysis

Quantitative data were extracted using radiographic software for bone density, callus volume, and implant displacement. The porosity of the titanium scaffold was correlated with these parameters to determine its effect on bone healing and implant stability. Descriptive statistics summarized the data, while inferential analyses assessed group differences and changes over time:

• Within-group comparisons: Repeated-measures ANOVA and mixed-effects models assessed changes over time.

• Between-group comparisons: ANOVA tests assessed differences between porosity and weight groups.

• Correlation analysis: Regression models analysed relationships between radiographic results and porosity.

Statistical significance was set at $p < 0.05$

RESULTS AND DISCUSSION

Population

Fifteen cases met the inclusion criteria, with suffocation nodes examined. Thirteen cases did not reach the 8-month postoperative follow-up, did not return for radiographic review, or did not have complete X-ray examinations. The mean age was 83.6 ± 36.6 months, the mean weight was 28.9 ± 11.24 kg. This population included seven female and eight male dogs consisting of: one (n = 1) was an American Pit Bull Terrier, one (n = 1) was a German Shepherd, one (n = 1) was a Dogue de Bordeaux, one (n = 1) was a Boxer, two (n = 2) were Beagles, one (n = 1) was an American Staffordshire Terrier, one (n = 1) was a Corsican, one (n = 1) was a Labrador Retriever, two (n = 2) were Rottweilers, and three (n = 3) were mixed-breed dogs and one (n = 1) was a Neapolitan Mastiff.

Radiographic Evaluation

Interobserver consistency analysis revealed no significant differences, indicating a uniform assessment among different observers.

Careful examination demonstrated a consistent bone-implant interface. Plain radiograph evaluation revealed that bone growth occurred in all animals. In the immediate vicinity of the implant, remodeling of the bone structure could be found in both the proximal and distal regions of the tibial tuberosity osteotomy.

Radiographic evaluations revealed progressive osseointegration at the bone-implant interface in all dogs. At the tibial tuberosity, bone trabeculae extended toward the porous network of the implant with a densification around the implant. Within the porous network, bone growth was primarily along the implant posts, with more bone at the periphery than in the center of the porous network. Within the porous network at the cortical and endosteal levels, dense bone filled almost the entire porous region.

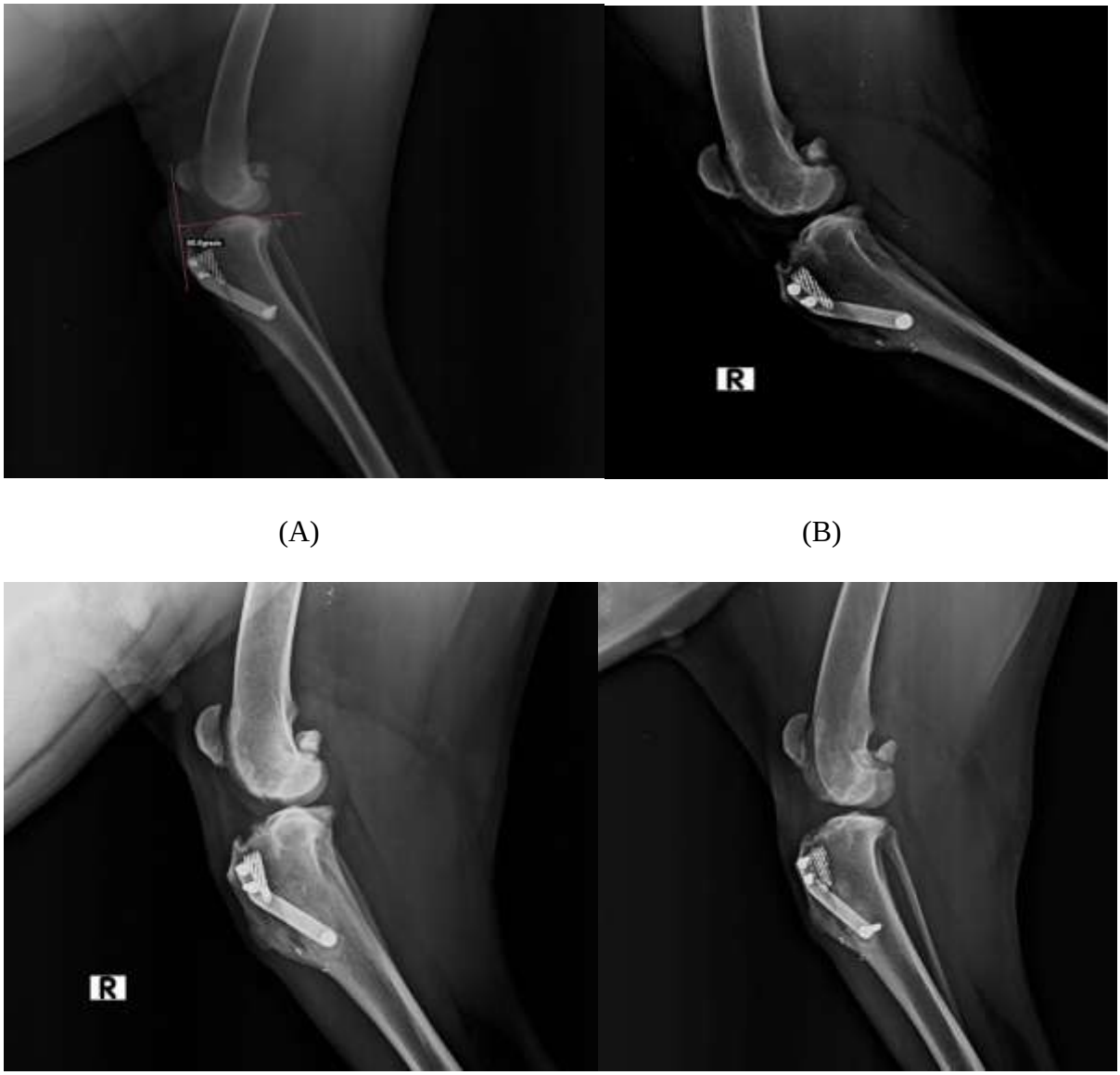
At T1, radiolucent areas surrounded the implant in 88 % of cases, indicating minimal initial osseointegration. At T2, partial continuity at the bone-implant interface was observed in 67% of cases, with significantly reduced radiolucency ($p < 0.05$). At T3, 100% of cases showed complete osseointegration, indicating that there were no small radiolucent areas without clinical instability.

Evaluation of mediolateral radiographs revealed bone callus formation (new periosteal bone formation) which was evident along the entire length of the implant, particularly at the proximal and distal tibial tuberosity. Complete bone healing was defined as the presence of a mineralized bridging callus in the osteotomy region between the implant and the Maquet hole, or in the osteotomy region adjacent to the implant. Bone callus formation was evident by T1 in all cases, with variations based on cage size and weight groups. Small cages showed faster and denser callus formation, with a 40% increase in mean callus volume from T0 to T1, stabilizing by T2. Medium and large cages demonstrated comparable rates of callus formation but slower stabilization. By T3, all groups showed trabecular continuity and complete remodeling.

Analysis of trabecular bone density revealed significant improvements at all time points ($p < 0.01$). Smaller cages correlated with higher trabecular bone density at T3, especially in medium-weight dogs. Larger dogs showed slightly slower trabecular remodeling, regardless of cage size. Radiographs taken at any time point showed no movement or displacement of the implant and there was also no negative reaction around the scaffold or screws.

This study investigates the impact of titanium scaffold porosity on bone regeneration and implant stability in canines undergoing Tibial Tuberosity Advancement (TTA) for cranial cruciate ligament rupture. Our findings indicate that the use of porous titanium scaffolds facilitates progressive osseointegration, with complete integration observed in all cases by eight months postoperatively. Additionally, variations in callus formation and trabecular bone density were noted across different cage sizes and weight groups, suggesting that implant design and patient characteristics significantly influence healing outcomes.

The role of implant porosity in promoting osseointegration has been well-documented. Porous titanium scaffolds are designed to mimic the extracellular matrix of bone, promoting cell attachment, proliferation, and differentiation (Doi et al., 2021; Shao et al., 2023; Zuo et al., 2021). This structural similarity enhances vascularization and new bone ingrowth, which are critical for the healing of large bone defects. Studies have shown that a macropore size ranging from 200 to 500 μm is optimal for the ingrowth of new bone tissues and the transportation of body fluids (Jeyachandran & Cerruti, 2023; Mukasheva et al., 2024). In our study, the titanium scaffolds utilized had a pore size of approximately 1 mm, which may have contributed to the effective osseointegration observed (Hong et al., 2020).



(A) (B)

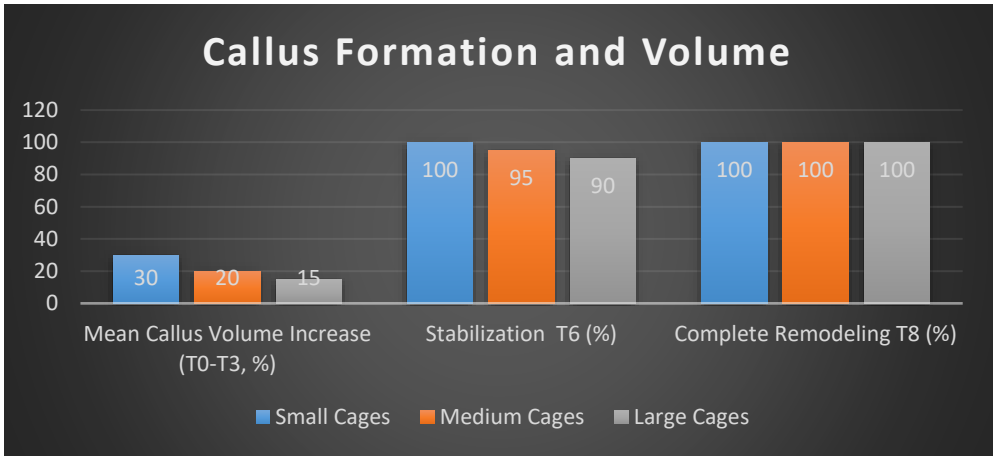
(C) (D)

Figure 1. (A)The radiographic mediolateral projection of a dog who had undergone Porous TTA after surgery and post-operative measurement.

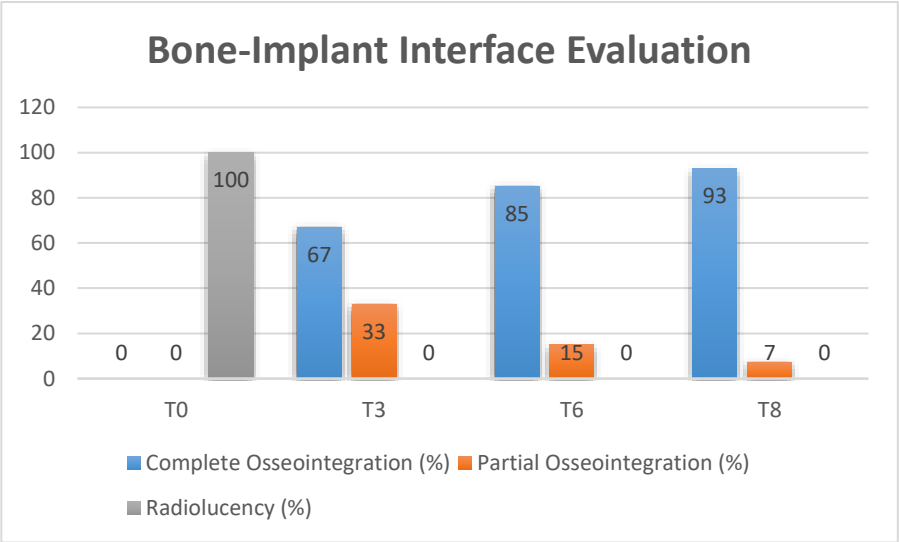
(B) Osseointegration of the bone and porous titanium cage 3 months after the intervention.

(C) Osseointegration of the bone and porous titanium cage 6 months after the intervention.

(D) Osseointegration of the bone and porous titanium cage 8 months after the intervention.



(A)



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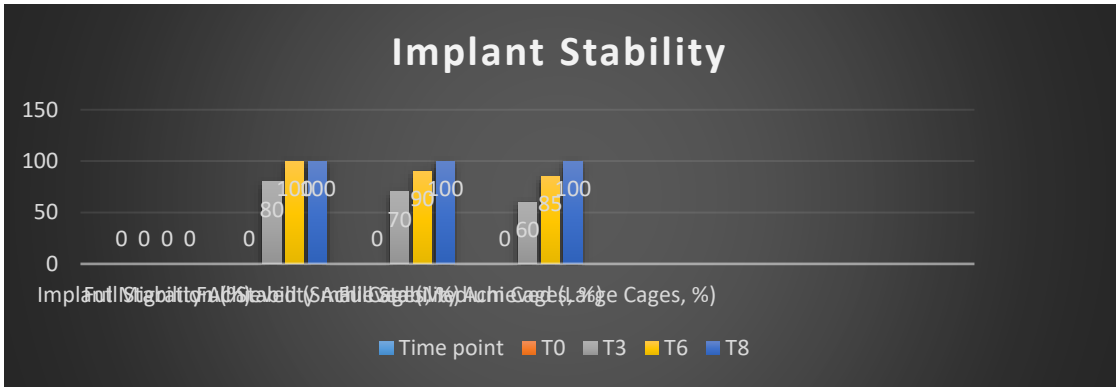

(C)

Figure 2. (A). A graphical representation of bone callus formation and its volume from T0 to T8.

(B) A graphical representation of the bone-implant interface assessment from T0 to T8.

(C) A graphical representation of the Implant Stability assessment from T0 to T8

The mechanical properties of titanium implants, including their elastic modulus, are also influenced by porosity (Bartolomeu et al., 2019). Human cancellous bone possesses a stiffness ranging from 12 to 23 GPa; careful control and modification of manufacturing parameters to achieve similar strengths is

imperative for practicality of integration (Kang et al., 1998; Morgan et al., 2018). Correctly predicting the Young's modulus for foams is imperative for actual biomedical integration; a mismatch of Young's moduli between the implant and the bone can result in stress-shielding effects from a disproportional handling of stress. The implant which typically exhibits a higher Young's modulus than the bone will absorb most of the load. As a result of this imbalance, the starting bone density will be reduced, there will be tissue death and, eventually, implant failure (Baba et al., 2023; Kashef et al., 2008).

In our study, smaller cages (4.5 mm to 6 mm) demonstrated faster and denser callus formation compared to medium and large cages. This observation aligns with previous research suggesting that implant size and design can influence the rate and quality of bone healing. Smaller implants may provide a more favorable surface-area-to-volume ratio, enhancing cellular interactions and nutrient diffusion, thereby accelerating the healing process (Alkentar et al., 2023; Bandyopadhyay et al., 2019; Elias et al., 2012; Hernandez & Woodrow, 2022; Wang et al., 2022)

Weight-based differences in bone regeneration were also observed. Smaller dogs (10-25 kg) exhibited more rapid trabecular remodeling compared to larger dogs (51-75 kg), regardless of cage size. This finding suggests that patient-specific factors, such as body weight, play a crucial role in the healing process. Heavier dogs may experience increased mechanical stress at the implant site, potentially slowing the rate of bone regeneration. These insights underscore the importance of considering individual patient characteristics when selecting implant designs and materials. Radiographically, the first signs of osseointegration confirmed the results of the in vitro study, allowing the first signs of bone deposition inside the osteotomy to be appreciated as early as 60 days after surgery. This study is a practical example of how the precise design of porous material can strongly change the total performance of the system even without changing the external shape of the same (Trisciuzzi et al., 2019).

The absence of implant migration or displacement in our study indicates that the porous titanium scaffolds provided adequate primary stability throughout the healing period. Implant stability is essential for successful osseointegration, as micromotion at the bone-implant interface can hinder bone ingrowth and lead to fibrous tissue formation instead of the desired bony integration (Bernardi-Villavicencio et al., 2020; Hong et al., 2020; Li et al., 2021). The interconnected porous structure of the titanium scaffolds likely contributed to this stability by allowing bone tissue to infiltrate and anchor the implant securely (Shum et al., 2023).

While our findings are promising, it is important to acknowledge the limitations of this study. The retrospective design and relatively small sample size may limit the generalizability of the results. Additionally, the exclusion of cases with complications or incomplete data could introduce selection bias. Future prospective studies with larger cohorts are warranted to validate these findings and to explore the long-term outcomes of porous titanium implants in canine TTA procedures.

CONCLUSIONS

This study demonstrates that porous titanium scaffolds significantly enhance osseointegration and implant stability, in Tibial Tuberosity Advancement (TTA) for dogs with cranial cruciate ligament rupture (CrCLR). Radiographic evidence confirmed bone healing . These findings emphasize the critical role of scaffold porosity in advancing veterinary orthopedic practices and highlight its potential relevance in human medicine. Future innovations in scaffold design, including bioactive coatings and graded porosity, could further optimize clinical outcomes and broaden the applicability of these implants.

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OXIDANT STATUS OF SPIRULINA EXTRACTS

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ABSTRACT

Arthrospiraplatensis Spirulina is a cyanobacteria species known for its rich nutritional content and biologically active phycocyanin components. It is a dietary protein that is used safely and beneficially as a supplementary food additive. Spirulina contains two types of biliproteins: C-phycocyanin and allophycocyanin. Phycocyanin is a water-soluble, blue photosynthetic pigment with high antioxidant and strong fluorescent properties. In this study, oxidative stress values and DNA protective activities of Spirulina extracts with 35% phycocyanin content were evaluated. Total Oxidant Level (TOL) and Total Antioxidant Level (TAL) were determined using Rel Assay Diagnostics kits. pBR322 plasmid DNA and UV-C method were used for DNA protective activity. It was determined that spirulina extracts at different concentrations showed good antioxidant properties and oxidative stress values of all samples were low. All Spirulina extracts showed good DNA protective activity.

Key words: Spirulina, DNA, TOL, TAL

INTRODUCTION

Spirulina is a type of blue-green algae with significant protein quality and amino acid composition, with high potential to be used as a sustainable protein source. Phycocyanin, which forms the blue part, is a water-soluble, non-toxic, photosynthetic pigment substance with high antioxidant and strong fluorescence properties. It is the most economically important phycobiliprotein type found in high amounts in cyanobacteria. Spirulina has been used as a food supplement for many years as a plankton alga or cyanobacteria (Pierro and et al., 2001, Dillon and et al 1995). Spirulina has been widely investigated in the pharmacological field due to its anti-inflammatory, antioxidant and anticancer effects (Bhat and Madyastha 2000, Subhashini and et al., 2004). The antitumoral activity of marine algae was first studied by Nakazawa in aqueous extracts. It was stated that the polysaccharide content of aqueous extracts was related to antitumour activity (Taşkın and et al., 2010). In vivo application of phycocyanin purified from spirulina (5.0 mg kg-1 body weight) was found to suppress tumour transparency and increase the mean survival time of tumour-bearing hamsters (Yogianti et al 2014). Phycocyanin, a natural product purified from Spirulina, has been found to effectively inhibit pancreatic cancer cell proliferation and xenograft tumor growth in vitro and in vivo. Data reported in the literature indicate that phycocyanin has a strong anticancer effect on various cancer cell types, such as lung cancer colon cancer, breast cancer (Cai and et al 1995), and bone marrow cancer, both in vitro and in vivo. *Arthrospira platensis* DNA protective activity was evaluated in previous studies. Bhat & Madyastha (2001) found

that phycocyanin and its chromophore, phycocyanobilin, effectively scavenge ONOO-, a potent physiological inorganic toxin. Hamed, M. et al (2025) found protective effects of Spirulina platensis against ultraviolet radiation-A (UV-A)-induced oxidative stress, immunotoxicity and histological damage on African catfish (*Clarias gariepinus*). Pleonsil & Suwanwong (2013) stated that c-phycoyanin can reduce the percentages of DNA damage caused by the Fenton reaction in a dose-dependent manner. There are studies showing that spirulina cultivated in the Marmara region also has antioxidant capacity and DNA-protective activity (Sibel BO, 2018 and Güroy B, 2022). In another study evaluating the in vivo antitumoral effect of phycocyanin administered to Balb-c mice carrying Ehrlich Ascites Tumor (EAT) by the gavage method, an inhibitory effect on tumor formation was observed (Sibel BO and etc 2023).

Considering the existence of these studies in the literature, we evaluated the protection of spirulina with 35% phycocyanin content, which is sold in the market as a food product, against oxidants and DNA at different concentrations in this study.

MATERIALS AND METHODS

Arthrospira platensis (containing 35% phycocyanin) purchased from Double Wood LLC (Sharon Hill, PA 19079 USA). Total Antioxidant Level (TAL) and Total Oxidation Level (TOL) Assay Kits were purchased from Real Assay Diagnostics (Gaziantep, Turkey). The DNA of the pBR322 plasmid demonstrates two bands in the agarose gel electrophoresis. These are the fast walking supercoiled DNA (scDNA; super-stranded DNA; without fracture) which are the natural form of the plasmid and the slow walking open circular DNA (ocDNA; DNA containing a single fracture in the chain). The exposure of the DNA to UV radiation in the presence of H_2O_2 causes fracture in the open-circular DNA and the emergence of the linear DNA (linDNA; includes one or more fractures in the two chains (Tepe et al. 2011).

RESULTS AND DISCUSSION

The OSI values of these four different extracts were determined by measuring the total antioxidant levels (TAL) and total oxidant levels (TOL). Smaller OSI values indicate less oxidative stress. The OSI values of extracts were detected at very low levels in our study. This shows that the oxidant activity at low levels of OSI is quite low, and that both different samples are quite good antioxidants. As it was given in Table 1, the levels of the OSI values showed that the oxidant activity was very low level all of the samples.

Table 1: Phycocyanin-rich *Arthrospira platensis ex*tract solutions' antioxidant, oxidant, and OSI values.

***Arthrospira platensis* Extract**	**TAL (mmol/L)**	**TOL (mmol/L)**	**OSI**
F1	4.333	0.00605	0.1396
F2	3.921	0.07436	0.1896
F3	1.618	0.00347	0.2145
References	>2 Very good	<5 Very good	<1 Good

As shown in 40 mg and 20 mg total antioxidant capacities of extract containing samples in different concentrations show very good antioxidant properties (>2), while 10 mg, which has a lower concentration, still had a good antioxidant value. All samples were found to have low oxidant levels, with the order of F3<F1<F2.

As seen in the DNA bands in Figure 1, it was determined that F1 and F2 samples, evaluated at phycocyanin-rich *Arthrospira platensis* extracts concentrations of 40 mg and 20 mg showed showed better DNA protective activity with various bands, even at the lowest concentration compared to the controls.

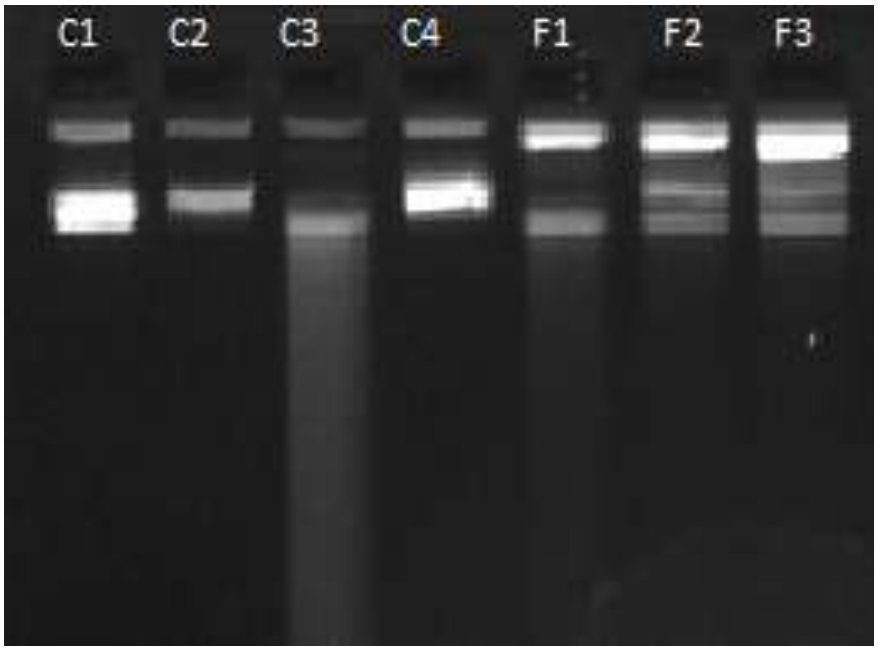

Fig. 1. Phycocyanin-rich *Arthrospira platensis* extract DNA protective bands. C1: Plasmid DNA (3 µl) + dH_2O (6 µl). C2: Plasmid DNA (3 µl) + dH_2O (6 µl)+UV. C3: Plasmid DNA (3 µl) +dH_2O (6 µl)+H_2O_2 (1 µl). C4: Plasmid DNA (3 µl) + dH_2O (6 µl)+UV+H_2O_2 (1 µl). F1 (40 mg): Plasmid DNA (3 µl)+Extract (5 µl)+UV+H_2O_2 (1 µl). F2 (20 mg): Plasmid DNA (3 µl)+Extract (5 µl)+UV+H_2O_2 (1 µl). F3 (10 mg): Plasmid DNA (3 µl)+Extract (5 µl)+UV+H_2O_2 (1 µl).

CONCLUSION

In conclusion, the oxidant and DNA protective activity of spirulina used as a food additive with 35% phycocyanin content was determined in this study. It was determined that high doses of phycocyanin in the extracts had higher antioxidant capacity and had good protective activity on DNA even at low concentrations.

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RESEARCHING COGNITIVE TECHNOLOGIES IN BROADCAST OPERATIONS

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ABSTRACT

Technologies such as artificial intelligence, virtual studios, augmented reality (AR) and **tracking** systems have significantly changed the television industry. These technologies have not only increased people's enjoyment but also made TV broadcasting easier, faster and more interactive. Virtual studios reduce costs and offer entertaining and educational shows in news, sports and entertainment programs using augmented reality. Tracking systems enable virtual materials to be combined with real images to create a single content. This article examines the basic concepts, technologies and results in television broadcasting. This study also covers the tasks of cognitive technologies. It is stated that applications such as artificial intelligence servers, holographic broadcasts and blockchain-based content distribution will be widely used in the future.

Keywords: Cognitive Technologies, Virtual Studio, Augmented Reality, Tracking Systems, Digital Broadcasting

INTRODUCTION

Cognitive technologies are systems that imitate or improve the way people think and make decisions. Examples include artificial intelligence (AI), machine learning, augmented reality (AR), and virtual reality (VR). These technologies are causing big changes in television broadcasting and are transforming traditional broadcasting methods.

Thanks to AI-powered systems, virtual studios, AR applications, and tracking systems have become more advanced. For example, subtitles can be created automatically, video content can be categorized

by topic, and personalized video suggestions can be offered to viewers. This helps videos get prepared faster and presented with higher quality. For the younger generation—children of the internet—virtual studios make video content more interesting. Therefore, cognitive technologies are becoming a main part of the TV world. This article will examine technologies such as virtual studios, tracking devices, augmented reality (AR), and virtual reality (VR) that affect television broadcasting.

VIRTUAL STUDIOS

Virtual spaces are digital environments used in television broadcasting that replace old physical decorations. In these types of broadcasts, people usually stand in front of a solid- colored background—most often a green screen. Then, this background is replaced with a virtual image in real time using image processing techniques. This creates the feeling of being in different locations without needing a real physical space.

Recently, LED-based virtual studios have started to appear as an alternative to green screen (green box) technology. In these studios, large LED screens are used to show the background image directly, and both the camera and the people on stage can see it in real time. This makes it much easier to match lighting, get the correct camera angle, and make the scene look more realistic.

Figure 1: Green Box

Visual content made with game engines is commonly used in virtual studios. Powerful graphics engines like Unreal Engine and Unity help create realistic environments instantly, which increases their use in the media world. Adding virtual studio tools to television broadcasts brings great benefits both financially and technically. The main advantages of this technology are:

Cost Reduction: Virtual studio setups remove the need for physical sets and decorations, which creates a big cost advantage for in-studio broadcasting. Many virtual sets can exist in the same digital space, making equipment use more efficient and removing the need for building and moving decorations.
Flexibility and Time Saving: Decor changes in digital environments can be done faster and more easily than in real locations. This saves time during content production and allows for quick creation of shows with different ideas.
Improved Visuals and Realism: Thanks to high-resolution textures, lighting techniques that simulate real light behavior, and real-time shading from graphic engines, a visual experience close to physical reality can be provided. This advanced visual atmosphere increases the realism of the content and strengthens viewer engagement.
People in Different Locations Together: With virtual studios, people in different places can appear together as if they are in the same room. This tool allows people to speak as if they are physically together. It makes it possible to produce shows where speakers and audiences from different cities meet on the same screen, removing location problems almost completely.

TRACKING SYSTEMS

To use virtual studios and augmented reality (AR) applications effectively and realistically, it is necessary to set up highly accurate tracking systems.

Figure 2: Tracking Systems

These systems track the position and movement of cameras, objects, and people in real time during television production. Their main purpose is to align digitally created virtual content with physical images in a spatially and temporally synchronized way.
In broadcasts with moving cameras or presenters constantly changing positions on stage, presenting virtual backgrounds or AR graphic elements in a consistent way with the real image is only possible with precise and low-latency tracking systems. Otherwise, the line between virtual and real elements will shift, creating a strange look for the viewer and reducing the effect of the AR experience.
In this sense, the consistency of tracking methods is not only a technical matter, but also very important for maintaining the audience's feeling of realism. Currently, these tracking devices are believed to be built on different ideas such as vision-based, sensor-supported, and hybrid systems.

HOW DOES IT WORK?

Tracking systems form the technological foundation that enables physical camera movements to be reproduced in digital environments with high precision and minimal latency. These systems rely on three principal approaches: optical tracking, sensor-based tracking, and hybrid tracking.
Optical tracking systems operate by monitoring visual reference points in the studio— typically reflective markers, coded patterns, or natural textures. High-speed tracking cameras capture these references, and image-processing algorithms analyze how their positions shift between frames. Through triangulation and 3D reconstruction techniques, the system determines the camera's position (X, Y, Z) and orientation (pan, tilt, roll). Although highly accurate, optical methods may be influenced by lighting conditions, reflections, or temporary loss of visual markers.
Sensor-based tracking systems rely on IMUs, accelerometers, gyroscopes, and encoder units attached to the camera or PTZ mechanisms. These sensors continuously measure angular velocity, acceleration, lens

focus, and zoom states. Their main advantage is delivering fast, low- latency data independent of lighting conditions. However, because sensor measurements can accumulate error over time, they are prone to drift unless corrected by an external reference.

For this reason, modern broadcast systems predominantly use hybrid tracking, which merges optical and sensor data. In hybrid solutions, the high-frequency but drift-sensitive sensor readings are corrected with the highly accurate but lower-frequency optical references. This is achieved using sensor fusion algorithms such as Kalman Filters, Extended Kalman Filters (EKF), or Visual–Inertial Odometry (VIO). The result is a tracking output that combines low latency with long-term stability and millimetric accuracy.

The tracking data—including camera position, orientation, zoom, focus, and lens distortion parameters—is transmitted to real-time graphics engines using protocols such as FreeD, MOS, NDI metadata, or SMPTE ST 2110. The rendering engine recalculates the virtual scene for each frame based on this data, ensuring accurate alignment between virtual and real imagery. Even millisecond-level mismatches can cause visible drift or misalignment, making frame-accurate synchronization essential. Through this combination of sensing hardware, data fusion methods, and synchronized data delivery, tracking systems provide the technical infrastructure required to consistently merge physical and virtual elements in virtual studio and AR productions.

VISION-BASED TRACKING SYSTEMS

Among tracking technologies, vision-based systems stand out due to their powerful software and hardware compatibility. In this method, computer vision techniques are used to analyze surrounding objects, shapes, and natural visual elements. Features such as markers, edges, or surface patterns in the camera's view are detected, and the camera's position in space is continuously calculated based on these elements.

Figure 3: Marker in a Vision-Based Tracking System

The biggest advantage of vision-based tracking systems is that they work using only a camera and good software, without needing special sensors or difficult setups. This is especially useful for portable or moving equipment. Recently, as phone chips have become faster and cameras better, the use of these visual tracking systems has increased a lot. In TV shoots, shaped markers are placed on the ceiling and tracked by cameras. This allows precise calculation of the camera's position and angle. Virtual graphics

that match this position information are instantly added behind the presenter in the studio, changing perspective as the camera moves. This technique helps deliver a real-time image to the audience.

SENSOR/MODULE-BASED TRACKING SYSTEMS

In virtual studio and augmented reality (AR) systems, it is very important to accurately know where the camera is and how it moves. To do this, special devices and sensors are used. These sensors track the camera's direction, speed, and movement in real time. For example, a sensor (IMU) placed inside the camera calculates which way the camera is turning and how fast it is speeding up or slowing down. Other sensors attached to the tripod or rail systems that move the camera measure the camera's left-right or up-down movements. With this information, the camera and virtual images on the screen move smoothly together.

Figure 4: Sensor and Module-Based Tracking Method

In some advanced systems, infrared (IR) emitting devices are installed on the studio ceiling. Cameras are equipped with receivers that detect these IR signals. This setup allows for very precise determination of the camera's position. Thanks to this technology, virtual images and real scenes can be perfectly synchronized.

These tracking systems are broadly divided into two categories: vision-based systems and sensor-based systems. However, nowadays, hybrid systems that combine both approaches are becoming more common. For example, sensors inside the camera measure rough movements, while computer algorithms process this data for more accurate results. This combination makes the tracking system faster and more reliable.

APPLICATIONS AND EXAMPLES

Tracking systems are not only useful inside studios but also play a crucial role in outdoor shoots and sports broadcasts. For instance, in a football match, adding virtual offside lines or player running paths on the screen requires extremely accurate tracking of the camera's movements. This ensures that the added graphics appear as natural parts of the field, providing viewers with a more immersive and realistic experience.

One of the most commonly used tracking systems in broadcasting involves optical sensor setups that use special markers (reflective markers) installed on the ceiling. Sensors attached to the cameras track these markers to determine the camera's exact position in real- time. Systems like Mo-Sys StarTracker and Stype Kit are widely popular in this field due to their ease of use and minimal infrastructure requirements. Some systems combine mechanical and optical components—for example, cameras

mounted on rails or cranes use potentiometers to measure movement. The sensors attached to the cameras also take into account zoom and focus adjustments. All this information is combined to ensure that virtual graphics on the screen move correctly in sync with the camera.
Thus, the quality of virtual studios and realistic visual effects strongly depends on the accuracy and reliability of these tracking devices.

Figure 5: Use in Football Matches

AUGMENTED REALITY (AR)

Augmented Reality (AR) is a technology that overlays digital objects onto real-world images. Through AR, computer-generated graphics and objects are added to the real environment. This way, the user perceives both the real world and virtual elements as a unified whole.
Therefore, augmented reality is not only used in games. In television broadcasting, AR technology is employed to offer viewers more engaging programs. Its usage is increasing day by day in news, sports broadcasts, entertainment, and game shows.

AR IN NEWS BROADCASTING

In news programs, augmented reality enriches information delivery through 3D graphics, data tables, or map visuals that appear beside the presenter. For example, on election night broadcasts, a 3D map of Turkey projected onto the studio floor or the graphical representation of fault lines during an earthquake news segment are among the practical uses of AR in television production.

Figure 6: Augmented Reality (AR) in News Broadcasting

AR IN SPORTS BROADCASTING

Sports broadcasting is one of the most intensive users of augmented reality. Applications include displaying player statistics on screen at the presenter's cue during live match commentary, showing digital arrows and drawings on the field for tactical analysis, or replacing advertising boards on the sidelines with digitally targeted content based on geography.

Figure 7: Augmented Reality (AR) in Sports Broadcasting

Augmented reality redefines visual storytelling and increases interaction with the audience. In this respect, AR holds a critical place among innovative technologies shaping the future of television broadcasting.

BROADCASTING SOFTWARE AND IMPLEMENTATION METHODS USED IN AUGMENTED REALITY

Using AR graphics in TV broadcasts is made possible not only by powerful computers but also by specialized software. Programs such as Vizrt, Avid/Brainstorm, ChyronHego, Ross Video, and Ventuz are highly preferred by users in this field. These software solutions enable real-time 3D graphics, background removal (keying), and camera motion tracking with ease.
The Vizrt infrastructure has become an industry standard in international broadcasting organizations for virtual studio and AR graphics production.
In these systems, pre-modeled 3D objects and animations are integrated into live physical images synchronized with motion tracking systems. When this process is done by digital overlay directly on the camera footage instead of LED screens, it is called video-based augmented reality.

GREENBOX TECHNOLOGY AND APPLICATIONS

Augmented reality in TV broadcasting is generally used together with greenbox technology. The studio background is designed to be a single color (green or blue). The floor is removed using chroma keying, and virtual backgrounds or graphic layers are placed instead. This makes the presenter appear as if they are in a real environment to the viewer.
Greenbox technology is commonly used in weather bulletins, election broadcasts, and news programs. When integrated with augmented reality, virtual maps, graphic animations, or objects appearing behind the presenter provide a more understandable experience for the audience.

Figure 8: Greenbox Technology

AR WITHOUT GREENBOX

AR applications that do not require greenbox technology have also become possible with advanced broadcasting infrastructure. In these setups, the presenter is placed within a real studio set, while digital objects are directly placed into the physical environment in 3D. For example, a digitally projected laptop on the presenter's desk or a digital logo placed on the studio floor are examples of such applications.

Figure 9: AR Without Greenbox (TRT WORLD)

One of the biggest technical challenges is ensuring that real and virtual objects appear naturally together. When the presenter must pass in front of a virtual object, the system needs to detect this in real time. To achieve this, some advanced broadcast systems automatically extract the presenter's silhouette (matte extraction) from the camera feed and layer digital objects accordingly to maintain visual integrity. Studios running these systems are equipped with special technologies that seamlessly integrate physical environments with virtual graphics.

EXAMPLE APPLICATIONS

Augmented reality technology is used by numerous news and sports broadcasting organizations worldwide. During elections, this technology is frequently used. Media companies such as TRT, BBC, and CNN display 3D images and animated shapes in the studio to show results on election nights. In sports broadcasting, AR is also commonly used. Examples include showing the first down line in American football in yellow and displaying players' names on 3D digital panels during football matches.

FUTURE OUTLOOK

The development of cognitive technologies in television broadcasting is increasing day by day. In the near future, it is expected that experiences in the field of Extended Reality (XR)— which combines Augmented Reality (AR) and Virtual Reality (VR) applications—will grow significantly. Accordingly, hologram technology and volumetric videotape systems will make it possible for a news anchor or guest who is not physically present in the studio to appear in front of the viewer as a life-sized, three-dimensional projection.

Indeed, the concept of "holographic news presentations" is no longer just a futuristic idea but has become a concrete broadcasting technology currently under development.

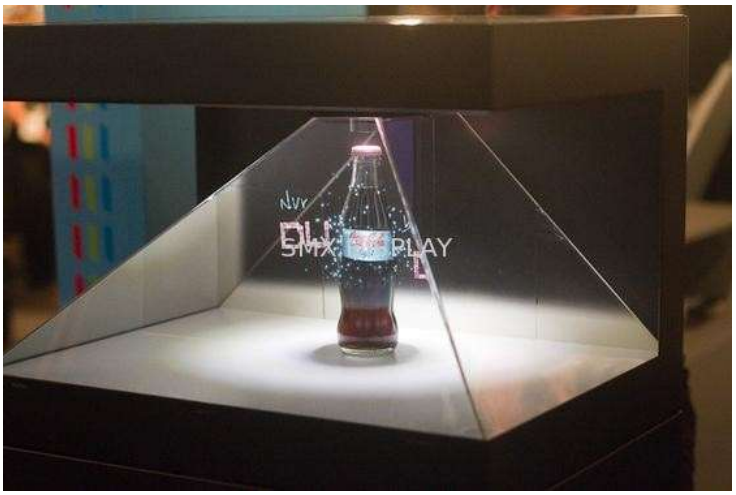

Figure 10: Holographic Presentation

With the advancement of these technologies, during news programs or conferences, hosts can appear in viewers' living rooms or public spaces as three-dimensional holograms, as if they were physically present. Such applications have the potential to free television broadcasting completely from spatial limitations.

AR/VR TRENDS

Wearable technology developments, such as AR glasses, have necessitated adapting television broadcasting to new platforms. Thanks to these technologies, viewers can experience sporting events with 3D graphics or watch TV series in immersive 360° cinematic environments via VR headsets.

With the growing popularity of the metaverse concept, broadcasting organizations will increase efforts to create content in virtual worlds. For example, a news channel may establish a virtual news center within the metaverse. Broadcasts could be conducted using avatar reporters. These developments will transform viewers from passive consumers into active, interactive participants in the broadcasting experience.

In the future, viewers will be able to participate in polls during broadcasts through AR extensions and obtain information about presented products. Thus, the viewing experience will become more personalized.

Figure 11: VR & AR Glasses

DIGITAL ANCHORS AND ARTIFICIAL INTELLIGENCE

The use of artificial intelligence (AI) technologies in the media sector is becoming increasingly diverse. One of these areas is the active role of digital anchors in news broadcasting. In 2018, China's official news agency Xinhua introduced an AI-powered digital news anchor, marking a world first. These systems are created by modeling the voice and face of a real news presenter. AI allows these anchors to broadcast continuously throughout the day without breaks.

Figure 12: AI Presenter

Similar AI presenter have started to be used in countries like South Korea. These "virtual twins" can broadcast in multiple languages. However, the use of AI anchors raises some ethical and transparency concerns, particularly because viewers may not know whether they are watching a real person or an AI. AI presenter are mostly used for repetitive broadcasts such as weather or economic news, but it is expected they will play larger roles in entertainment shows and advertisements in the future.

BLOCKCHAIN-BASED BROADCASTING

Blockchain technology has recently attracted growing interest in the media and broadcasting sector. Thanks to its decentralized nature, blockchain creates a direct link between content creators and viewers. Traditionally, content producers have often depended heavily on platforms, relinquishing a significant

portion of their revenue to intermediaries. However, blockchain-based systems allow producers to deliver their content directly to the target audience.
For instance, when an episode of a series is uploaded encrypted on a blockchain network, viewers can access this content directly, and payments are transferred straight to the producer. This method reduces costs and strengthens content ownership rights.
Additionally, the licensing status and authenticity of digital content can be tracked via blockchain. Digital assets similar to NFTs make it easy to verify whether content is original or not. This advancement is considered a significant step towards combating piracy.
Recent studies have demonstrated that live video broadcasts can be conducted through blockchain-based peer-to-peer (P2P) systems, promising more secure and efficient streaming solutions.

CONCLUSIONS

Television broadcasting has undergone significant changes in recent years. At the core of this transformation are cognitive technologies. These technologies impact not only the technical aspects but also everything from content production to the connection established with the audience. For example, virtual studios both reduce costs and offer more visual options. Augmented reality makes news or sports broadcasts more engaging.
Of course, behind the scenes, certain technologies ensure these systems work smoothly. Tracking systems continuously monitor the position of cameras and objects in real-time. This prevents any misalignment or disruption in the displayed images. Additionally, AI-powered automation speeds up content creation and enables unconventional broadcast formats.
Interestingly, this transformation is not just a topic for today. The broadcasting world is rapidly advancing towards innovations like AR, VR, holograms, and the metaverse. However, one important point to keep in mind is that these technologies should not completely replace traditional broadcasting. For instance, digital anchors should not substitute human anchors but rather assist them. Similarly, systems like blockchain must be used carefully, with attention to copyright and security issues.
In summary, cognitive technologies are transforming both the content and presentation style of television. When used correctly and balanced, these technologies make it possible for television to remain a powerful medium in the future.

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THE DEVELOPING NEW BREAD WHEAT VARIETIES WITH EVALUATING YIELD AND QUALITY TRAITS IN EDIRNE

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ABSTRACT

Bread wheat (*Triticum aestivum* L.) is a major cereal crop in Turkey and also in Edirne province in Trakya, where favorable soil and climatic conditions support high yield potential and grain quality. The region is also an important center for multi-environment testing of bread wheat genotypes, contributing to the development of high-yielding, stable cultivars adapted to rainfed conditions. Bread wheat variety development focuses on improving both grain yield and end-use quality traits such as protein content, gluten strength, and baking performance. In the Edirne–Trakya region of Turkey, Tragen R & D Co. located in Trakya Technopark breeding programs emphasize high-yielding, stable cultivars with good quality performance under rainfed conditions and variable environments. Among candidate lines, higher seed yields were obtained from CAN 39 and YK 6 candidate lines belonged to Tragen Co. exhibited better performance than control varieties both for seed yield and also quality. As result, CAN 30 candidate line was selected and sent to registration trials in August 2025.

Key words: Bread Wheat, Trakya Region, Seed yield, Adaptation, Yield traits

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the most planted cereal and mostly growing in dry areas and in as well as in different environments of world and in many regions of Turkey mostly planted crop and mostly growing in dry conditions in Turkey (Baser, 2022). Wheat production in Turkey reached 21 million tons in 2024 growing season but in 2025, compared to the previous year, wheat production decreased by 13.7% to 17.9 million tons. The highest production provinces are Konya, Sanliurfa and Diyarbakir and in mostly in Middle Anatolia and Marmara Region and Southeast Anatolia Region.

Trakya region is also one of the critical areas in Turkey where bread wheat production is widespread, and it is known to contribute an average of 2.5 million tons to the country's production. Trakya Region provinces Tekirdag, Edirne and Kirklareli exist in the top ten producing provinces (Öztürk et al., 2024; TEPGE, 2025). Although the average rainfall in the region varies from year to year, the average long-term total rainfall is known to be 718.8 mm. However, in recent a couple years, there were very dry growing seasons and wheat production suffered a lot from these drought conditions. Furthermore, long-term climate data shows that there is a high variation in monthly rainfall and temperature values during the autumn, winter, and spring months, which cover the physiological periods that determine yield in bread wheat cultivation, such as germination-emergence, tillering, stem elongation, and flowering. A trend of increasing average and maximum temperatures and decreasing rainfall has been observed. Due to changes in abiotic stress factors, epidemics of fungal diseases such as Septoria leaf spot and rust occur year after year, negatively impacting yields. Therefore, selecting and cultivating stable varieties that can adapt to changing

environmental conditions is crucial in Trakya region (Beser et al., 2019 and 2021, Baser et al., 2021; Baser, 2022).

Edirne province and Trakya region is a significant region for cereal production in Turkey. Trakya Agricultural Research Institute (TARI) in Edirne, in this region is leading institution by the focusing on developing high-yield, disease-resistant, and high-quality bread wheat cultivars suitable for the region's humid, temperate climate. Very high yielding varieties were developed such as *Pehlivan, Selimiye, Aldane, Gelibolu,* and most recently *Değirmen* (Ozturk, 2023, 2024).

Çalışkan and Balkan, (2024). evaluated ten advanced lines against standards like *Selimiye* and *Rumeli*. Some candidate lines were identified as promising candidates for future registration due to superior gluten index and protein ratios.

Quantitative traits in wheat, such as grain yield and quality characteristics, are controlled by numerous genes and are highly influenced by environmental factors. Therefore, the performance of different varieties can vary in different environments. Identifying these differences in performance, defined as genotype x environment interaction, and selecting high-value genotypes is crucial, as is selecting stable genotypes that minimize these performance differences (Gökdere et al, 2023). Quality of bread wheat is also considering as so significant criteria in Turkish market and influencing preference of industry with assessing of gluten amount and index, protein content, zeleny sedimentation, alveograph value, flour yield, thousand seed weight, hectoliter weight and grain hardness respectively (Öztürk, 2021; Baser, 2022). Besides, fungal diseases especially wheat leaf rust, stripe rust and stem rust caused by *Puccinia recondita f. sp. tritici*, *Puccinia striiformis* f. sp. *tritici*, and *Puccinia graminis* f. sp. *tritici*, respectively are the most significant biotic stress resulting vital yield loses and seed quality in Trakya Region. (Baser et al., 2021; Baser, 2022). The study conducted in Edirne in 2024 and in 2025 growing seasons.

MATERIAL AND METHOD

The yield trials were conducted in Edirne in 2023-2024 and 2024-2025 growing seasons to determine yield performances of bread wheat candidate lines. The trials planted on time with planting machine in dry conditions and germination of plants were well in the experiments. The trials conducted based on the Seed Registration and Certification Center's Wheat Technical Instructions. In both growing seasons, climatic conditions were characterized by rainfall below the long-term average and temperatures above the long-term average. Fertilization and weed control were carried out on time, and harvesting was done as early as possible after maturation was completed.

There were 11 lines in the trials with two controls which planted in Trakya region which is European part of Turkey (Tragen 103 and Nemere). Tragen R & D company candidate lines existed in the experiment. The previous crop was sunflower in the rotation in the fields and there was no irrigation in in both locations. The experimental design was a Randomized Completely Block Design (RCBD) with four replications in the study. The four rows' plots were 6-m long with the six rows as 17 cm plant spacing and 500 grains per m^2, density, so total plot area was 6.12 m^2 and plot area was at harvest 5.10 m^2.

Trials were planted by planter in 28 November in 2023 and in 17 November in 2024 in Edirne. The experiments were harvested by harvest combine in 20 July in 2024 and 15 July 2025 in next year. After harvesting the plots, they were treshed and weighed and plot results were adapted 10% humidity then quality analysis were performed from each plot of varieties based on requested by government rules.

The compose fertilizers (20-20-0, Zn) applied 250 kg/ha dose at planting. At tillering stage in February, 150 kg/ha Urea (%46), in March in April 250 kg/ha Ammonium Nitrate (%26). Weed control was applied in 15 March 2025 with 350 ml/da (chlorotoluran + 40g/l diflufenican) in the study. Weed control was in 27 March 2025 with same herbicide and same dose.

RESULTS AND DISCUSSION

No adverse conditions affecting the measurement of variety performance were observed during the trials. The trials provided opportunities to identify drought-tolerant varieties. Two-year and combined yield results and replicated yield results per decare by year is given in Table 1. Field observations from the trials are presented in Tables 2 and 3, technological analysis results in Tables 4 and 5. Quality analyses related to the trials were conducted at the Edirne Commodity Exchange. The trial observations from emergence to harvest conducted in all varieties existed in the experiments (Figure 1, 2, 3 and 4).

Based on study results; some Tragen R & D company candidate lines observed promising results and had higher performances than almost all control cultivars in Edirne location. There were lower yields were obtained in the lines in 2024 comparing with 2025 season. Among candidate lines, higher seed yields were obtained from CAN 39 candidate line as well as YK 6, YK 14 over standard averages in 2024. In 2025, CAN 39 performed well than controls and YK 6 existed in same group. In the combined two-year values, CAN 39 and YK 6 exhibited better performance than control varieties (Table 1). Based on experiment results, CAN 39 and TRAGEN YK-6 was existed in top rank compared with control cultivars-based seed and flour quality results and TRAGEN Ltd wheat cultivars had favorable results based this study.

Figure 1. Wheat experiments by Tragen Company in 2024.

Almost all varieties existed in the study, these candidates and controls were awned varieties except YK 6 and YK 15. Besides, almost all varieties are earlier spiking dates except number 5 and 7 lines. Due to dry periods in both years during the vegetation season both in winter and spring, there were no diseases observed in all varieties as well as no lodging (Table 2 and 3). Plant heights of varieties changed between 81 to 96 cm. The promising candidate line CAN 39 had higher plant heights than controls but YK 6 exhibited similar.

All varieties had red color grains in the experiments. Based on quality analysis in the study, the candidate line CAN 39 showed favorable and acceptable performance in many desired quality traits in both years (Table 4 and 5). Gluten content, and protein content are the main criteria for quality

evaluations while marketing in wheat by buyers and industry. Based on protein values, the protein contents of varieties are changed between % 13,6 and 18,4. The gluten content (%) of the varieties changed between 31 and 49,2 %. However, the gluten values noticeably lower in 2024 comparing with 2025 season as well as lower protein values too (Table 4 and 5).

Table 1. 2024-2025 wheat seed yield trial results and the averages (kg/da).

Cultivars	2023-2024 Season	2024-2025 Season	Average
1-Tragen-103 (Std)	486.1 BCD	580.5 CD	533.3 CD
2-YK-6	545.9 A	662.4 AB	604.2 AB
3-Nemere (Std)	531.4 AB	654.6 B	592.9 B
4-MV-Felleg (2023-24 VD-4)	523.7 ABC	493.0 E	508.4 D
5-MV-Seuso (2023-24 VD-7)	391.9 E	546.3 DE	469.1 E
6-YK-10/19 (2023-24 VD-8)	482.7 CD	533.5 DE	508.1 D
7-YK-13 (2023-24 VD-9)	323.7 F	508.3 E	415.9 F
8-YK-14/22 (2023-24 VD-10)	551.9 A	581.9 CD	566.9 BC
9-YK-15/22 (2023-24 VD-11)	439.0 DE	494.4 E	466.7E
10 - CAN 39 (Candidate)	**555.3 A**	**720.3 A**	**637.8 A**
11-2022-2023 K-GB-1 (2023-24 VD-14)	476.8 CD	607.5 BC	542.2CD
F CV (%) LSD (kg/da)	0.0001** 6.94 48.35	0.0001** 7.1 59.57	0.0001** 7.08 38.35
Average (kg/da	482.6	580.2 Kg	531.4

Figure 1. Wheat experiments by Tragen Company in 2024.

Table 2. Edirne 2023-2024 Season Bread Wheat yield trials field observations

#	Spike Type	Plant Height (cm)	Spiking date (day /month)	Cold damage (1-9)	Lodging level %	Yellow Rust	Stem Rust	Brown Rust	Powdery Mildew (0-99)	Other Diseases (0-99)	Yield (kg/da)
1	Awn	96	02.05.	1	%0/%0	-	-	-	-	-	486.1 BCD
2	No Awn	86	02.05	1	%0/%0	-	-	-	-	-	545.9 A
3	Awn	87	05.05	1	%0/%0	-	-	-	-	-	531.4 AB
4	Awn	86	05.05	1	%0/%0	-	-	-	-	-	523.7 ABC
5	Awn	87	10.05	1	%0/%0	-	-	-	-	-	391.9 E
6	Awn	89	02.05	1	%0/%0	-	-	-	-	-	482.7 CD
7	Awn	87	12.05	1	%0/%0	-	-	-	-	-	323.7 F
8	Awn	95	05.05	1	%0/%0	-	-	-	-	-	551.9 A
9	No Awn	79	02.05.	1	%0/%0	-	-	-	-	-	439.0 DE
10	Awn	**95**	**03.05**	**1**	**%0/%0**	-	-	-	-	-	**555.3 A**
11	Awn	85	03.05	1	%0/%0	-	-	-	-	-	476.8 CD

Table 3. Edirne 2024-2025 Season Bread Wheat yield trials field observations

#	Spike Type	Plant Height (cm)	Spiking date (day /month)	Cold damage (1-9)	Lodging level %	Yellow Rust	Stem Rust	Brown Rust	Powdery Mildew (0-99)	Other Diseases (0-99)	Yield (kg/da)
1	Awn	88	01.05	1	%70/%10	-	-	-	-	-	580.5 CD
2	No Awn	85	02.05	1	%0/%0	-	-	-	-	-	662.4 AB
3	Awn	86	02.05	1	%0/%0	-	-	-	-	-	654.6 B
4	Awn	82	03.05	1	%0/%0	-	-	-	-	-	493.0 E
5	Awn	87	06.05	1	%0/%0	-	-	-	-	-	546.3 DE
6	Awn	91	02.05	1	%0/%0	-	-	-	-	-	533.5 DE
7	Awn	86	10.05	1	%0/%0	-	-	-	-	-	508.3 E
8	Awn	90	03.05	1	%0/%0	-	-	-	-	-	581.9 CD
9	No Awn	77	02.05.	1	%0/%0	-	-	-	-	-	494.4 E
10	Awn	**90**	**04.05**	**1**	**%0/%0**	-	-	-	-	-	**720.3 A**
11	Awn	81	02.05	1	%0/%0	-	-	-	-	-	607.5 BC

Table 4. Edirne 2023-2024 Season Bread Wheat quality results

Cultivars	Grain Color	Grain Hardiness	1000 Seed Weght (g)	Hectoliter Weight (kg)	Protein (%)	Sedim. (Zel) ml	Water Absorption Farinograph %)	Energy Alveograph W	Softening Degree (Farinograph) (BU)	Gluten (%)	Gluten Index (%)	Flour Yield (%)
1-Tragen-103 (Std)	Red	98,5	30.2	77.1	17.1	51	-	549	-	43.8	64	-
2-YK-6	Red	-	29.7	72.3	16.1	49	-	-	-	34.5	78	-
3-Nemere (Std)	Red	110	30.7	73	17.1	35	-	507	-	45.0	28	-
4-MV-Felleg (2023-24 VD-4)	Red	-	32.2	73.2	17.6	40	-	-	-	46.7	55	
5-MV-Seuso (2023-24 VD-7)	Red	-	28.4	68.8	17.8	50	-	-	-	48.5	58	-
6-YK-10/19 (2023-24 VD-8)	Red	-	32.1	74.6	16.4	42	-	-	-	51.6	55	-
7-YK-13 (2023-24 VD-9)	Red	-	28.2	78.0	17.5	41	-	-	-	42.5	60	-
8-YK-14/22 (2023-24 VD-10)	Red	-	32.2	79.5	18.3	37	-	-	-	49.2	53	-
9-YK-15/22 (2023-24 VD-11)	Red	-	31.0	71.5	15.7	37	-	-	-	38.9	50	-
10- CAN 39 (Candidate)	Red	**115,1**	**29.1**	**72.6**	**15.8**	**53**	-	**455**	-	**35.9**	**73**	-
11-22-23 K-GB-1(23-24VD14)	Red	-	29.1	72.6	18.0	57	-	-	-	47.8	53	29.1

Table 5. Edirne 2024-2025 Season Bread Wheat quality results

Cultivars	Grain Color	Grain Hardiness	1000 Seed Weght (g)	Hectoliter Weight (kg)	Protein (%)	Sedim. (Zel) ml	Water Absorption Farinograph %)	Energy Alveograph W	Softening Degree (Farinograph) (BU)	Gluten (%)	Gluten Index (%)	Flour Yield (%)
1-Tragen-103 (Std)	Red	70.2	30.5	75	15.3	44	-	454	-	33.0	83	-
2-YK-6	Red	-	31.8	70.2	15.4	45	-	-	-	35.8	67	-
3-Nemere (Std)	Red	82.4	32.2	70.3	15.6	35	-	448	-	39.1	41	-
4-MV-Felleg (2023-24 VD-4)	Red	-	35.8	67.8	14.6	41	-	-	-	34.2	54	-
5-MV-Seuso (2023-24 VD-7)	Red	-	28.4	64.5	15.5	40	-	-	-	35.9	67	-
6-YK-10/19 (2023-24 VD-8)	Red	-	30.8	73.1	14.5	38	-	-	-	34.7	53	-
7-YK-13 (2023-24 VD-9)	Red	-	274	79.6	18.4	38	-	-	-	47.4	59	-
8-YK-14/22 (2023-24 VD-10)	Red	-	34.6	69.5	15.2	44	-	-	-	31.2	81	-
9-YK-15/22 (2023-24 VD-11)	Red	-	28.4	65.5	13.6	30	-	-	-	31.5	39	-
10- CAN 39 (Candidate)	Red	**73,5**	**34.6**	**67.2**	**15.0**	**42**	-	**472**	-	**30.9**	**89**	-
11-22-23 K-GB-1(23-24VD14)	Red	-	31.975	70.2	15.8	36	-	-	-	39.3	53	-

Figure 2. Candidate Wheat Line CAN-39 in the yield trials in 2025

Figure 2. Candidate Wheat Line CAN-39 in the yield trials in 2025

CONCLUSIONS

As results, Tragen wheat candidate lines exhibited favorable and promising results such as CAN 39 and YK 6 based on quality and yield traits observed in this study. Among promising these candidates, CAN 30 was selected and sent to registration trials in August 2025. The research studies continue to develop higher yielding bread wheat varieties with testing in future trials then selected ones send to registration trials to register.

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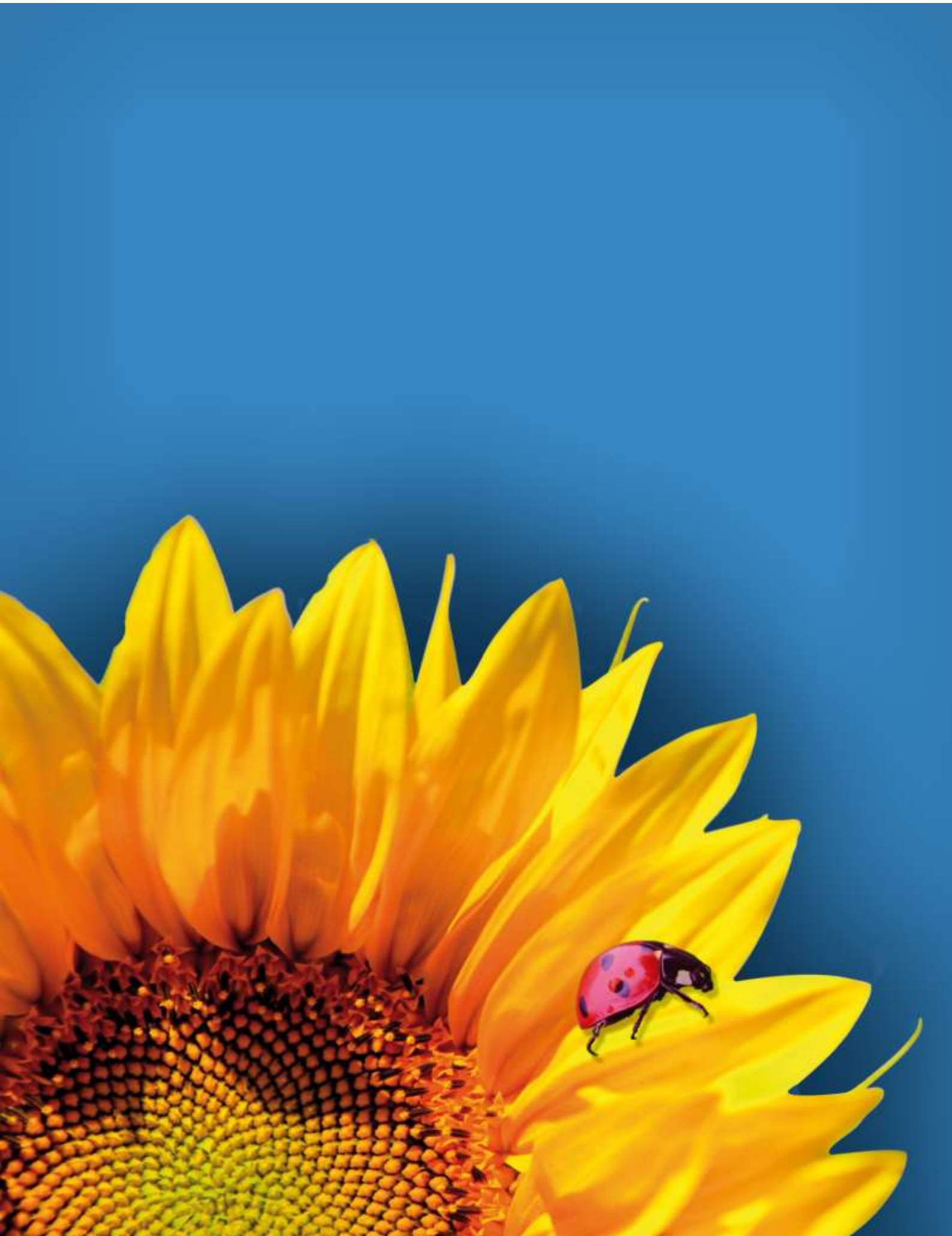
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