PROCEEEDINGS OF INTERNATIONAL AGRICULTURAL, BIOLOGICAL & LIFE SCIENCE CONFERENCE

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PROCEEEDINGS OF INTERNATIONAL AGRICULTURAL, BIOLOGICAL & LIFE SCIENCE CONFERENCE

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WELCOME NOTES

You are welcome to our AGBIOL Conference that is organized by Trakya University. 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 is 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 benefit from the interaction with each other.

In September 2018, it will be held the first edition of the AGBIOL Conference, with ambition of the organizers to make it a periodical event. We are proud to announce that in the AGBIOL 2018 will take part more than 700 scientists and researchers from all over the world. There were submitted 823 scientific papers, of which 363 will be presented as oral talks and 460 as poster presentations. The full author list of all submitted papers comprises 2091.

Our conference is a premier international science, technology and business forum focusing on Agriculture, Biology and Life Science. The technical sessions highlight invited and volunteer speakers. Three student posters will be selected to receive 1st, 2nd and 3rd place monetary awards and a certificate during the conference.

We love our nature and care about the environment. We wanted to make our conference as much greener as possible, using less paper. The participants' posters were submitted via conference web page and will be presented on electronic poster screens, developed particularly for this purpose. Abstract book is published in electronic version, and copy of it on flash memory stick, will be provided on each participant.

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Edirne is not only a very nice, lovely and historical city at the edge of Europe, but located just at the heart of Balkan region and history endowed with monuments reminding imperial past. We are much pleased to host all of you in Edirne and Turkey.

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

We wish you nice stay in Edirne!

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ENTOMOPATHOGENS IN THE MANAGEMENT OF STORED PRODUCT PESTS

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ABSTRACT

Fumigation is mainly applied to control of stored product pests in the world and in Turkey. The ruining of natural balance among living organisms, the resistance of pests for pesticides, and residues on crops are produced by application of pesticides widely. Nowadays, incremental necessity has been occurred to find out alternatives to chemicals. Biological control is a novel method to replace chemicals. Insect pathogens which kill insects causing disease are agents such as bacteria, fungi, nematodes, viruses, protozoa. The mass of entomopathogenic bacteria are in genera Coccobacillus and Bacillus. *Coccobacillus acridiorum* produced disease in grasshoppers. *Bacillus thuringiensis* and *Bacillus popillia* are important disease causing agents against lepidopteran pests. Entomopathogenic fungi *Beauveria bassiana, Metarhizium anisopliae*, and *Verticillium lecanii* cause disease on larger insects than other pathogens. These are rather prevalent on the insects in orders *Lepidoptera, Homoptera, Hymenoptera, Coleoptera*, and *Diptera.* Insects, especially living in soil at one of its life cycle such as larva, pupa or adult are highly susceptible against entomopathogenic nematodes. Neosteinernematidae, Steinernematidae and Heterorhabditidae are the families containing these nematodes. They are obligatory insect pathogens in nature. Entomopathogenic nematodes impacts many insect species with a broad host range. Entomopathogenic viruses at least 16 families are very important in biological control to affect insects pests. Baculoviruses are produced commercially and applied as a biological control agent to manage significant agricultural and forestry insects, especially in Orders Lepidoptera and Hymenoptera. Entomopathogenic protozoans such as Nosema locusta are a substantial role in the ecologically management of populations of insect pests. In this review, application and potentials of entomopathogens as biological control agents of harmful insect species on stored products has been abstracted.

Keywords*:* *Entomopathogens, Insect pests, Stored products*

INTRODUCTION

Infestation of stored bulk grain and processed commodities by insects causes big economic loss (Hagstrum and Flinn, 1995). These insects damage the product by physical yield and quality loss, inducing mould growth, contamination of products with insect bodies. They can shelter inaccessible places and survive on even little bit food. They reproduce and increase their population quickly. Then move from cracks and crevices, perforated floors, and inside machinery into stored bulk products to infest them (Campbell et al., 2004). The order Coleoptera includes about 250.000 species. Fourty families of this order contain insects harmful on stored products world-wide. Bostrichidae, Bruchidae, Cucujidae, Curculionidae, Dermestidae, Silvanidae and, Tenebrionidae are some of these families (Rees, 1996).

Government regulations, environmental and human health concerns, resistances of insects to insecticides, pesticide residues on crops, changing consumer demands limit the presence and use of chemical insecticides against these pests (Durmuşoğlu et al. 2010). Entomopathogens are biological control agents causing diseases in insect populations. These are organisms such as bacteria, fungi, nematodes, viruses, and protozoa. They can be a safe alternative for stored-product pests in unreachable places, because some biological control agents actively search out pests in these cryptic habitats (Schöller and Flinn 2000). In this paper, the research and applications of entomopathogens against stored products are summarized.

Entomopathogenic Bacteria

Entomopathogenic bacteria are most commonly used microorganisms against insect pests present. The most widely used ones, spore-forming facultative bacteria producing crystals. The bacteria enter the insect body through the mouth with food. They form endospor and protein crystals. These crystals contain toxins. The insects are killed because the toxins or bacteria wrap the body of insect.

The majority of entomopathogenic bacteria take place in *Coccobacillus* and *Bacillus* species. *Coccobacillus acridiorum* is a type of grasshopper pathogen. *Bacillus thuringiensis* and *Bacillus popillia* are the other two important species. In our country, bacteria are recommended and used against harmful species in order Lepidoptera some of them below.

*Archips*sp. (http://www.agroziraat.com/mmeyve/Archipsspp.html)

Heliothis armigera pityocampa (http://www.inra.fr/hyppz/RAVAGEUR/6helarm.htm)

Thaumetopoea (https://en.wikipedia.org.wiki Thaumetopoeidae

Entomopathogenic Fungi

Fungal diseases in insects, the lighting in the Italian Agostino Bassi's parasitic nature of white muscardine disease of silkworm were known since the 1834-1835 year. Entomopathogenic fungi, capable of infecting insects in Order Lepidoptera, Homoptera, Hymenoptera, Coleoptera and Diptera included, are quite common. The fungi encounters host with chance in environmental conditions. Population density depends on the amount of surrounding fungal spores and insect pests. The host entrance into cuticle is realized by both with lytic enzymes and by means of mechanical formation of appressorium (Figure 1). After penetrating the cuticle and epidermis, spores germinate and multiply in the insect body. The

metabolites formed by fungi cause physiological and biochemical changes in the host and results in insect death.

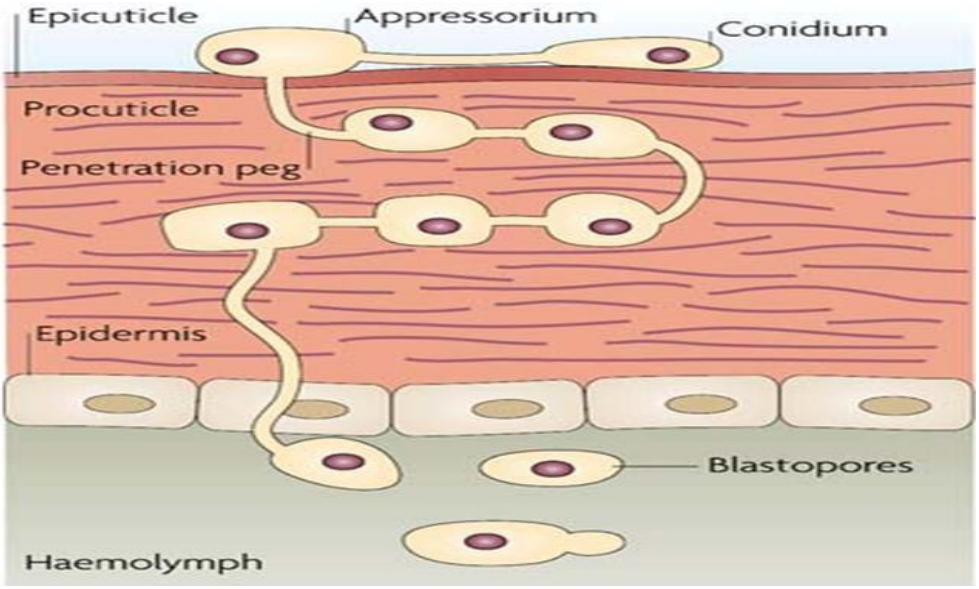

Figure 1. Penetrating of fungus into the cuticle and epidermis, and appressorium formation (http://www.nature.com/nrmicro/journal/v5/n5/fig_tab/nrmicro1638_ft.html)

Lord (2011) used *Beauvaria bassiana against Dermestes maculatus* and it was successful with 82% in 75% humidity. Khashaveh (2011) observed 68-92% mortality in the population of *Sitophilus granarius* and *Tribolium castaneum* adults against which *Beauvaria bassiana* applied. Ahmad (2010) used *Lecanicili lecanii, Isaria fumosarose* and *Metarhizium anisoplia* against *Sitophilus zeamays* adults and determined 100% insect mortality. Lord (2009) obtained 10% more mature death on *Rhyzopertha dominica, Oryzaephilus surinamensis, Cryptolestes ferrusineus* using *Beauvaria bassiana with* diatomaceous powder formulation. Batta (2008) used *Beauvaria bassiana* and *Metarhizium anisoplia* against *Rhyzopertha dominica, Sitophilus oryzae, Tribolium castaneum, Sitophilus oryzae* adults and got the highest mortality rate (85-96%) on *Sitophilus oryzae*. Hansen (2007) determined *Beauvaria bassiana* to be a successful control agent against *Sitophilus granarius with* rate of 83-98% mortality.

Entomopathogenic Nematodes

Entomopathogenic nematodes (EPNs) (*Rhabditida: Neosteinernematidae Steinernematidae and Heterorhabditidae*) do not cause contamination of ground water and are harmless to plants and animals. As biological control agents, EPNs attract attention increasingly in research area recently. Their ideal properties such as; the broad host spectrum, to be able to kill their hosts within 24-48 hours, to be producible commercially easily in vivo or in vitro, having ability to search actively their hosts, settling in application areas and staying effective for a long time, having easy applicability, being in compliance with many chemicals and being safe for the environment are important for their preferability.

They are soil dwelling, aquatic organism. They have motile bacteria (*Xenorhabdus* spp. and *Photorhabdus* spp.) living in their intestine. Nematode and bacterium are mutualistic symbionts and obligate, lethal parasites of insects. EPNs can be found everywhere on earth and infect many different insects (Smart, 1995). Infective juveniles of the nematodes living in the soil enter the host insect's haemocoel through mouth, anus, and respiratory openings or cuticle's

thin sections. Once in the insect, infective juveniles of the nematode release the bacteria that are carried in the intestine. Bacteria block the insect's immune system, multiply and kill the insect using many different toxins and causing septicemia in the hemolymph (Figure 2).

Bacterial cells reproduce rapidly. The nematodes eat the bacteria and some of body tissue of the insect, and reproduce for 3 or 4 generations depending on the food source. Over 100,000 nematodes exit the insect (Burnell and Stock, 2000) (Figure 2).

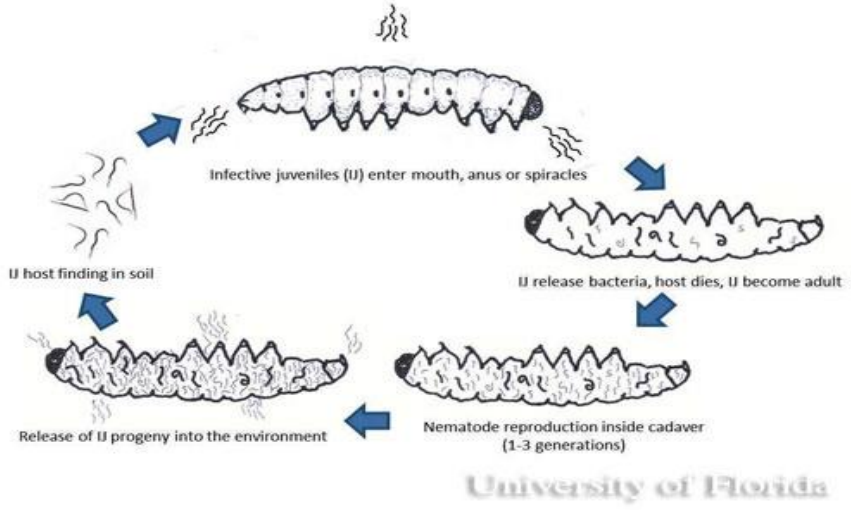

Figure 2. The simple life cycle of entomopathogenic nematodes

(http://entnemdept.ufl.edu/creatures/nematode/entomopathogenic_nematode.htm)

Tradan et al., (2006) in their study used four species of entomopathogenic nematodes (*Steinernema feltiae*, *S. carpocapsa*, *Heterorhabditis bacteriophora* and *H. megidis*) against storage pests, *Sitophilus granarius* and *Oryzaephilus surinamensis* in the laboratory to determine the activity of nematodes against adults. LC_{50} value was found as 803-1195 Ijs/adult on *S. granarius* and 921-1335 Ijs/adult on *O. surinamensis.* Shahina and Salma (2010) studied seven local (Pakistani) entomopathogenic nematodes (*Steinernema pakistanense, S. asiaticum, S. abbasi, S. siamkayai*, *S. feltiae, Heterorhabditis bacteriophora* and *H. indica* on *Sitophilus oryzae's* adults and pupae in the laboratory. Consequently, the LC_{50} value for pupae of *S. oryzae* was 42-169 Ijs/pupae and for adults, 55-370 Ijs/adult. Canhilal et al., (2013) determined the biological activity of nine endemic nematodes obtained from a survey conducted in the various districts of Kayseri Province against *Sitophilus oryzae* adults. The lowest LC_{50} value was 57.96 IJs/adult for *S. carpocapsae* 076 isolate, while the highest LC_{50} values was 922.95 Ijs/adults for *S. feltiae* OZV-5-S isolate.

Entomopathogenic Viruses

Baculovirus is important as biological control agents especially in the control of pests in agriculture and forestry. It is produced commercially and used against pests belonging to Lepidoptera and Hymenoptera. In Brazil, *Baculovirus anticarsi* is used successfully against *Anticarsi gemmatalis* causing substantial harm in soybeans. Nakai (2013) in Japan used granulovirüs (GV) as a biological agent against the tea leaf crimpers (*Adoxophyes honmai* and

Homona magnanima, Lepidoptera: Tortricidae) and there was no harmful infections during the four growing seasons.

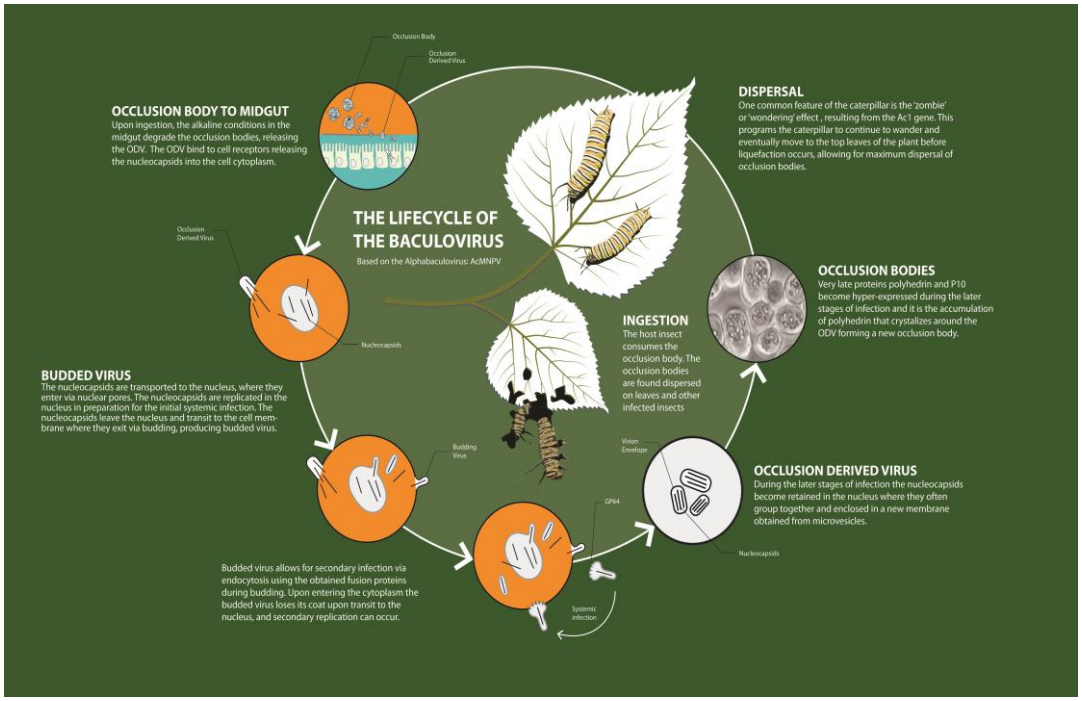

Figure 3. The life cycle of the baculovirus

(https://oetltd.wordpress.com/2014/07/17/growing-pains-the-life-cycle-of-the-baculovirus/)

Entomopathogenic Protozoa

Entomopathogenic protozoa are usually host-specific and slow effect is caused due to chronic infection. *Nosema locustae* has been developed as a commercial product for grasshoppers control (Henry and Oma, 1981). Entomopathogenic protozoa needs live hosts to be produced and shows quite slow effect. Therefore, it has limited application for biological control.

CONCLUSIONS

Chemical fumigation is the most commonly used method against of stored product pests in the world and in our country. Widely used insecticides against harmful organisms damage the natural balance existing among organisms, cause harmful organisms to acquire resistance to pesticides and residues in the crops. In recent years, biological control is emphasized as an alternative to chemical control. Entomopathogens have an important place in the biological control because they have a wide host range, are harmless to the environment and human, and could be applied with conventional sprayers. They can be used more against stored product pests with the development of new biotechnical methods such as collecting pests in some stations to meet them with entomopathogens.

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THE KEY ELEMENT IN THE CONTROLLING OF EURYGASTER INTEGRICEPS; ECONOMIC THRESHOLD

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Abstract

Wheat and barley have a significant insect pest, the sunn pest, Eurygaster integriceps Puton (Heteroptera: Scutelleridae) in Turkey. It affects the yield and the quality of flour of which bread is made. In this study; the yield loss due to white spike damage caused by overwintered adults and kernel damage by nymphs and new-generation adults in wheat fields were defined to set up an economic threshold (ET) for the sunn pest. To evaluate the relationship between overwintered adult density and white spike damage, and between percent kernel damage and sedimentation value of the flour, a regression analysis was performed. White spike damage comprised at low levels (0.1 – 1.7%) in the study fields and the relationship between overvintered adult density and white spike damage was not statistically important in bread and durum wheat. Average damaged kernels by E. integriceps were 4.2% in bread wheat and 5.4% in durum wheat. A positive relationship between nymph and new-generation adult density, and kernel damage in bread and durum wheat was found. We determined the sedimentation values of flour that was made of wheat kernels on which the pest fed. It was 7-89 in bread wheat, and 9-28 in durum wheat. There was no effect of sunn pest density on gluten strength up to 2.1% kernel damage in bread wheat or up to 0.9% kernel damage in durum wheat, but kernel damages above these levels restrained dough quality. We assessed these limit values in the regression formula and we found the economic thresholds as 8.1 and 9.2 nymphs/m² in bread and durum wheat, respectively. While the existing ET (10 nymphs/m²) may still be acceptable in durum wheat, it may be reduced to 7-8 nymph/m² for some wheat varieties and regions, especially for low yield levels (~2000 kg/ha) in bread wheat.

Keywords: *Economic threshold, wheat, sunn pest*.

INTRODUCTION

Wheat, *Triticum aestivum* L. is grown on about 9 million ha area annually with the production of approximately 20 million tons in Turkey (Anonymous 2008). It is important basic food crop consumed mostly as bread in the country. It provides a substantial component of the human diet; cereal (mostly wheat) products provide 53 and 66% of the per capita dietary supply of calories and protein, respectively (Anonymous 1980). It is also consumed as animal feed and used in industry to make various processsed foods. The country exports the about 10% of wheat production. The South Eastern Region of the country represents 13 and 8% of wheat acerage and production, respectively (Anonymous 2008).

The sunn pest, *Eurygaster integriceps* Put. (Hemiptera: Scutelleridae), is a very damaging insect pest of wheat and barley in Turkey (Lodos 1982). Overwintered adults of the sunn pest attack the leaves and stems of young, succulent wheat and barley plants, causing them to wither and die prior to spike formation. They also suck the base of the spike during the early growing period resulting in whitish spikes without kernels, producing white spikes. Yield losses by this pest are estimated at 50-90% in wheat and 20-30% in barley. Apart from the direct yield reduction, the insect injects digestive enzymes during feeding that reduce the baking quality of the dough. If as little as 2-3% of the grain has been fed on, the entire grain lot may be rendered unacceptable for baking purposes because of poor quality flour (Lodos 1982).

The sunn pest is univoltine. Adults rest under bushes and litter at high elevations around cereal fields during the hot and dry months of late summer and autumn. They hibernate during the cold and often severe winter months on hillsides of the mountains. In spring, when soil surface temperature reaches 15°C at overwintering sites, adults migrate to cereal fields. Migration typically continues for 7-10 d. Overwintered adults appear in the fields over a 1-4 week period. After feeding, females lay eggs on leaves, stems, and spikes. After five nymphal instars, a pupal stage occurs and new-generation adults are seen. These new-generation adults feed and return to higher elevations after barley and wheat harvest (Lodos 1982).

When migration to the fields ends, technical consultants survey fields and overwintered adults are counted in 0.25 m^2 frames to determine field densities. Fields are also monitored for egg parasitism by *Trissolcus* spp. (Hymenoptera, Scelionidae) when 20-30 % of the eggs are 10-12 day-old. Spraying is not conducted if the overwintered adult densities are at or below 0.8, 1.0 and 1.5 adult/m^2 and the parasitism rates are 40%, 50% and 70%, respectively, (Simsek & Sezer 1985). Finally, nymph density is determined in the same manner as for the overwintered adults. The most effective time to spray the sunn pest is during the first two nymphal instars. At the end of the survey, if nymph density reaches 10 nymphs/m^2, fields are sprayed, and this usually coincides with the milky stage of winter wheat (Lodos 1982).

This insect was first reported from the South Anatolia Region of Turkey in 1927 and there have been many outbreaks from the 1950s to the present. Detailed studies on the sunn pest were begun in the 1950s in Turkey (Simsek 1998). The government managed sunn pest control from 1927, until 2001 when an integrated pest management (IPM) approach was adopted. Sunn pest management was changed from aerial application to ground spraying, shifting responsibility to farmers. Currently, ground sprays for sunn pest control are conducted on 1-2 million ha area annually (Anonymous 2004). Government provides technical support and farmers are supposed to apply insecticide with their equipment, as determined by official technical consultants.

One of the key factors affecting the success of IPM programs is economic threshold (ET). The economic threshold used for sunn pest control was established about 50 years ago in the region and Country (Yuksel 1968). There is a need to revise the ET because of changes in climatic conditions, wheat varieties used, agronomical practices, and crop diversity. The purpose of this study was to determine plants (spikes), nymphs and new-generation adults (NGAs) density, and kernel damage caused by nymphs plus NGAs in wheat fields to redefine the ET for the sunn pest in the region.

MATERIALS AND METHODS

The study was conducted in 17 one-ha insecticide-free bread and durum wheat fields in Gaziantep, Kilis and Kahramanmaras provinces in southeastern of Turkey. There were 9 fields of bread wheat and 8 fields of durum wheat. Several varieties of bread and durum wheat were

used. Variety was not held constant over all fields. When the migration of adults from overwintered sites to cereal fields ended, weekly surveys to determine adult and nymph density were begun in each field by using a 0.25 m^2 frame. A total of 25 frames tossed at random in each field were sampled, and overwintered adults, nymphs, and new generation adults were counted in the each frame. The results of these counts were multiplied by 4 and presented.

During the surveys, at the beginning of the milky stage of wheat, all healthy and damaged spikes in each frame were recorded. Before harvest, all plants in each frame were cut and put in a paper bag, and brought to laboratory. In the laboratory, spikes were dried and threshed, and the kernels cleaned. The kernels from each frame were weighed to determine yield per field. The mean yield from 25-0.25 m^2 was used to estimate the yield per ha for each field. Then kernels from all 25 frames were combined and 1-kg kernels taken from this combined kernels for each field. From this sample, 100 kernels, up to 20 times, (total = 2000 kernels) were randomly selected. These sub-samples were checked under the dissecting microscope and damaged and undamaged kernels were separated (Dortbudak 1974), and percent kernel damage was regressed against nymphs and new-generation adult density at the final count in each field.

Sedimentation test

The kernels combined from 25 sampling frames were also used for sedimentation test. All milling was conducted at 23°C and 60% relative humidity. Wheat samples were cleaned and tempered overnight to optimum moisture, as described by Williams et al. (1988). Tempered wheat was milled using Buhler laboratory mill type MLU-202 (Uzwil, Switzerland), with break roll gaps adjusted to B_2 = 1.2/1000 cm, B_3 = 0.8/1000 cm, C_1 = 1.2/1000 cm and C_3 = 0.8/1000 cm. Medium hard soft-wheat clothing was used. Buhler Bran finisher MLU-302 (Uzwil, Switzerland) was used to extract "bran flour", which was combined with all six flour streams. The Modified Sodium Dodecyl Sulphate (SDS) Sedimentation Test (Cressey & McStay 1987) was used to evaluate wheat-insect damaged in wheat.

Statistical analysis

Regression analysis was used to predict kernel damage (%) based on final nymph and new-generation adult density ($P < 0.05$). A correlation analysis was applied to determine the relation between overvintered adult density and white spike damage, and between percent kernel damage and sedimentation value ($P < 0.05$). All statistical analysis was done using SPSS for windows (2003). Data from the two years was combined for regression and correlation analyses.

RESULTS AND DISCUSSIONS

Adult migration was completed during the last week of April and weekly survey studies were started. Sunn pest adults were present in field trials two to four weeks after migration was completed. Nymphs of the sunn pest were seen in the middle of May and reached the new-generation adult stage, which is the most damaging stage, in the first week of June.

Average overwintered adult density was 1.1 per m^2 in bread wheat (Table 1), and 1.4 per m^2 in durum wheat (Table 2). Adult populations in some study fields decreased or increased in consecutive sampling dates. This was likely because of sunn pest movement in or out of the fields.

The nymph population averaged 7.2/m² in bread wheat, and 15.5/m² in durum wheat, respectively. While bread wheat yield averaged 4798 kg/ha (Table 1), yields for durum wheat were 3820 kg/ha (Tables 6).

No leaf or stem damage was observed because when the sunn pest completed migration to the fields, wheat plants reached 10-15 cm in height, and it was late for the sunn pest to damage leaves and stems, as observed by Lodos (1961).

White spike damage (overwintered adult damage)

White spike damage occurred at low levels. It averaged 0.3% in bread wheat (Table 1), and 0.6% in durum wheat (Table 2). Correlation analysis indicated that there was no significant relationship between overwintered adult density and white spike damage caused by overwintered adults in bread ($r = 0.288$, $r^2 = 0.083$, $P = 0.226$) or durum wheat ($r = 0.568$, $r^2 = 0.322$, $P = 0.071$).

Canhilal et al. (2005) also found that the low level of white spike damage (<0.1-0.9%) occurred at various overwintered sunn pest adult densities (1, 2, 3, 5, 10 overwintered adults/m²) in large field cages (2 by 2 by 1.7 m) and was not statistically significant in bread or durum wheat.

On the other hand, Kılıç et al. (1973) found that 0.4, 1.0-1.5, 1.6-2.0, and 2.1-2.3 overwintered sunn pest adults/m^2 caused 1.1%, 3.6%, 4.2% and 6.6% white spike damage in wheat fields, respectively. Şimşek et al. (1997) stated that when overwintered adult density was one adult/m^2, 7% stem damage and 1.9% spike damage occurred. These high levels of white spike damage differ from our results, perhaps because of high levels of overwintered adult parasitism that might have occurred in the fields, reducing adult feeding and damage.

Kernel damage (nymph and new-generation adult damage) in bread wheat

Average kernel damage caused by nymphs and new-generation adults was 4.2% in bread wheat (Table 1). There was a positive relation between nymph and new-generation adult density, and percent kernel damage in regression analysis ($r = 0.947$, $r^2 = 0.898$, $P = 0.000$). The regression equation used to predict percent kernel damage, based on nymph and new-generation adult density per m^2, was $Y = -0.899+0.364X$, (SE a=1.041, SE b=0.046, $P = 0.000$). Sedimentation values ranged from 7 to 89. The relation between sedimentation values and percent damaged kernels was strongly negative ($r = -0.821$, $r^2 = 0.674$, $P = 0.003$). The sedimentation value dropped to 52 when percent kernel damage was 2.1 in sedimentation tests (Table 1). No effect of sunn pest density on gluten strength up to kernel damage of 2.1% was detected. Sedimentation value around 50 is generally accepted as the value at which dough quality is ruined (Fouad et al. 2005). When this value is entered in our equation, the nymph density that causes the kernel damage that ruins dough quality (the economic threshold) is 8.1nymphs/m². The practical tolerance for damaged kernels in industry, regardless of wheat type (bread or durum) or variety, is 2-3%. We found that the expected ET was 9.4 nymphs/m² when the 2.5 value, which is the average of 2-3% of tolerance for damaged kernels, is used in our equation.

The expected ET of 8.1 nymphs/m² obtained from the sedimentation value is different from the ET of 9.4 nymphs/m² calculated from the tolerance level for damaged kernels used in industry and ET (10 nymph/m²) regardless of wheat variety and region in Turkey. Thus, the ET (10 nymph/m²) may be lowered to 7-8 nymph/m² for wheat varieties and regions where there

are complaints and practical observations, and especially for low-yield levels (~ 2000 kg/ha) until more detailed research is conducted.

Table 1. Overwintered adult and nymph+new generation adult densities of the sunn pest, % kerneldamage, sedimentation values, % white spike damage, varieties, and yield in bread wheat field trials in Gaziantep and Kahramanmaras provinces

Place	Variety	Yield kg/ha	No. OW adults[a]/m²	% White spikes	No. nymphs + NGA[b]/ m²	% Kernel damage	Sedi-men-tation
T.Tigem	Golye	5210	0.5±0.1	0.1±0.1	1.9±0.2	0.6±0.2	77
I.Hanagzi	Golye	5210	0.5±0.1	0.1±0.1	2.9±0.2	0.4±0.1	64
I.Zincirli	Golye	6260	1.1±0.1	0.2±0.1	4.0±0.3	1.6±0.3	82
N.Ciftlik	Golye	6130	0.6±0.1	0.1±0.1	10.4±0.5	2.1±0.3	52
I. Sakcagozu	Özdemir Bey	4210	4.1±0.2	0.3±0.1	34.1±1.1	6.2±0.6	18
T.Tigem	Golye	4860	0.3±0.1	0.2±0.1	1.0±0.1	0.5±0.1	89
N.Ciftlik	Basribey	4200	0.3±0.1	0.9±0.2	6.2±0.4	1.2±0.2	69
I. Kozdere	Golye	3710	0.5±0.1	0.1±0.1	8.5±0.4	2.2±0.4	63
S. Degirmenonu	Golye	3392	2.4±0.1	0.9±0.2	55.8±1.2	22.7±1.2	7
Mean		4798	1.1	0.3	13.9	4.2	

[a]Overwintered adults, [b]New generation adults

Durum wheat

Average kernel damage was 5.4% in durum wheat (Table 2). A strong, positive relation was determined between nymph and new-generation adult density, and percent kernel damage in regression analysis ($r = 0.859$, $r^2 = 0.738$, $P = 0.003$). The regression equation obtained to predict percent kernel damage, based on nymph and new-generation adult density per m^2, was $Y = - 3.206+0.443X$, (SE a = 2.368, SE b = 0.108, $P = 0.006$).

Sedimentation values varied from 9 to 22. Most fields yielded low sedimentation values and were of poor quality. There was a strong negative relation between sedimentation values and percent damaged kernels ($r = -0.699$, $r^2 = 0.489$, $P = 0.027$). The sedimentation value was 28, which is around the limit that weakens gluten strength (Fouad et al. 2005), when the kernel damage was 0.9% (Table 2). When this level of kernel damage (0.9%) is placed in the equation, the nymph density that causes the kernel damage that spoils dough quality is 9.2 nymphs/m².

As in bread wheat, the expected ET is calculated as 12.9 nymphs/m² when 2.5, which is the average of 2-3% of tolerance for kernel damage, is used in our equation. This is much over the ET that is used (10 nymphs/m²) now in Turkey.

Table 2. Overwintered adult and nymph+new generation adult densities of the sunn pest, % kernel damage, sedimentation values, % white spike damage, varieties, and yield in durum wheat field trials in Gaziantep and Kilis provinces.

Place	Variety	Yield kg/ha	No. OW adults[a]/m²	% White spikes	No. nymphs + NGA[b]/ m²	% Kernel damage	Sedi-men-tation
O. Kutlar	Ege 88	4015	1.6±0.1	1.7±0.3	29.6±1.0	6.7±1.0	10
O. Havaalanı	Akcakale 2000	4680	2.4±0.1	1.0±0.2	38.7±0.8	19.0±1.6	9
O. Sanko	Ege 88	4660	2.6±0.2	0.6±0.2	14.9±0.5	3.5±0.7	22
E. Yavuzlu	Ege 88	5240	1.1±0.1	0.4±0.1	12.6±0.5	2.7±0.3	13
O. Kutlar	Ege 88	1692	0.2±0.1	0.1±0.1	5.3±0.2	0.9±0.2	28
O. Havaalanı	Akcakale 2000	3000	0.5±0.1	0.3±0.2	14.2±0.4	4.0±0.6	10
Y. Arpaci	Zenit	4776	1.9±0.1	0.4±0.2	26.9±1.0	4.2±0.5	9
E. Yavuzlu	Ege 88	2496	0.6±0.1	0.1±0.1	13.0±0.4	2.3±0.3	17
Mean		3820	1.4	0.6	19.4	5.4	

[a]Overwintered adults, [b]New generation adult

Although the level of kernel damage (0.9%) that weakens the gluten in our study differed from practical tolerance for kernel damage in industry (2-3%), the ET of 9.2 nymphs/m² calculated from the equation and the ET that is currently used (10 nymphs/m²) are similar. Hence, in durum wheat, the tolerance for kernel damage in industry should be lowered about 1%, but the ET which is 10 nymphs/m² appears to be still valid. However, almost no low-level kernel damage was recorded in the study plots; sedimentation was low and damage was high for all measured points. Thus the regression operated close to the lower limit of its valid range when it was used for gluten strength and ET calculations, or for 2.5% damage and ET. This should be considered when the results are used.

CONCLUSIONS

Differences between our results on the tolerance for kernel damage and ET, and the ones that are used in the country might have occurred because our studies and the previous studies were not conducted on the same varieties or in the same region, and there were some changes in climatic conditions, agronomical practices, and crop diversity over time. Therefore, future research should be done based on region, irrigated and rain-fed farming condition, wheat type and variety, and various yield levels.

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SUNN PEST MANAGEMENT POLICY CHANGE IN TURKEY; FROM CLASICAL APPROACH TO INTEGRATED PEST MANAGEMENT

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ABSTRACT

Wheat, *Triticum aestivum* L. and barley, *Hordeum vulgare* L are important crops in Turkey. They are grown in about 13,5 million ha area annually. The sunn pest, *Eurygaster* spp. (Hemiptera; Scutelleridae) is the most important insect pest of wheat and barley. These insects were first reported from the South Anatolia region of Turkey in 1927 and caused many outbreaks through the 1950s to the present. Detailed studies on the sunn pest were started in the 1950s. Government, only itself has managed the sunn pest control since 1927. Farmers had no responsibility until 2001. Then an IPM approach has been adopted in control of the sunn pest; the sunn pest control policy has been changed to switch from aerial application to ground spraying and leave the responsibilities to farmers gradually. Now the sunn pest control sprays have been done by ground equipments completely. Government provides only technical support and farmers are supposed to apply the insecticide by their equipment, as determined by official technical consultants. After switching ground spraying, insecticide treated area decreased dramatically from about 1.9 million ha in 2003 to 0.6 million ha in 2014. This clearly showed us how appropriate decision it was. But there is always a danger that farmer may apply insecticide more than necessary because there is no good control of pesticide application and residue in the country.

Keywords: *Eurygaster spp., wheat and barley, sunn pest, integrated pest management, policy*

INTRODUCTION

Wheat, *Triticum aestivum* L., and barley, *Hordeum vulgare* L., are important crops in Turkey. They are grown in about 13.5 million ha area annually; 9.5 million ha wheat and 4 million ha barley with the production of about 20 and 8 million tons, respectively (Anonymous 2002). The sunn pest, *Eurygaster integriceps* Put. (Hemiptera: Scutelleridae), is the most important insect pest of wheat and barley. Turkey is very good location for the sunn pest to spread over and make outbreaks because it is at the genetic origin of wheat and barley and the sunn pest is synchronized with wheat and barley well because of climatic condition, topographic structure, crop design and agricultural tradition. There are three important sunn pest species, *Eurygaster integriceps* Put., *E. maura* L., *E. ausriaca* Schrk. The dominant species is *E. integriceps* in the South and Southeast Anatolia and Thrace regions, while *E. maura* dominates in the Central Anatolia and Aegean Regions. Nymphs and adults of the sunn pest cause damage by feeding on leaves, stems, and grains and reduce yield dramatically. Yield loss caused is estimated at 50-90% in wheat and 20-30% in barley. Apart from the direct reduction in yield, the insect injects digestive enzymes during feeding that greatly reduce the baking quality of the dough. If as little as 2-3% of the grain has been fed on, the entire grain lot may be rendered unacceptable for baking purposes because of poor quality flour (Lodos 1982).

This insect was first reported from the South Anatolia Region of Turkey in 1927. The first outbreaks of the sunn pest were recorded in the South Anatolia in 1927-1929 and in the Southeast Anatolia in 1939-1941. Then the first sunn pest outbreaks occurred in Thrace in 1982, in the Aegean Region in 1987, in Central Anatolia in 1988, and in Marmara Region in 1990. These outbreaks have been going on to the present (Simsek 1997). Detailed studies on the sunn pest were begun in the 1950s. The government only itself has managed sunn pest control since 1927.

Sunn Pest Management in Turkey

Adults of the sunn pest rest under bushes and litter at the tops of mountains around cereal fields during aestivation in the hot and dry months of late summer and autumn. They hibernate during the cold and often severe winter months on hillsides of the mountains. In spring, when temperature on the soil surface reaches 15°C at overwintering sites, adults migrate down to cereal fields. Migration continues 7-10 days under normal weather conditions (Lodos 1982). Overwintered adults appear in the fields over a 6-8 week period. After feeding on leaves and stems, a reproductive phase in the annual cycle occurs; females lay eggs on leaves, stems, and spikes.

Official technical consultants begin surveys in certain overwintering areas in autumn and spring of each year to assess and predict the pest population for the following year by comparing the survey results with those of previous years. The beginning of migration from overwintering sites to fields is determined by observing the activity of the pest when the daily temperature reaches 15°C, usually in March and April. Successive counts are done from the beginning of migration until 90% of the overwintered adult population has left the overwintering site (Anonymous, 2000).

When migration ends, technical consultants start surveys in the field; adult sunn pests are counted in 0.25-square meter frames and densities are determined. Fields where the density is 0.5-0.8 adults/m² are monitored for egg parasitism when 20-30 % of the eggs are in the anchor-sign stage. If the egg parasitism rate is high enough, spraying is not conducted. Finally, nymph density is determined in the same manner as for the overwintered adult. At the end of the survey, if nymph density reaches 10 nymphs/m², fields are sprayed, coinciding with the milky stage of winter wheat (Lodos 1982). The most effective time to spray the sunn pest is during the first three nymphal instars. Hence chemical control begins when second-instars nymphs reach 40% in population. However, if chemical control can't be completed during this period, the insecticide applications are continued through the fourth or fifth instars (Anonymous, 2000).

Adoption of Sunn Pest IPM

Mechanical and physical control; collecting by hand or sweep nets and destroying, burning of bushes under which sunn pest overwinter on mountains were applied in early times because of insufficient materials, knowledge and organization. Even it is said that farmers had amulet made and hung on sticks in their fields to prevent the sunn pest damage. Mainly chemical control in the framework of technical instructions developed by excessive national researches was used in 1956-2001. The chemical applications were done by airplane mostly.

It was seen by time that only chemical application especially by airplane was not right solution. It was realized that the treated area (Figure 1) and chemical used (1,283 tons in 2003) were increasing with high cost (US$ 15 million in 2003) and environmental problems begun to be seen by years. It was because insecticide applications posed a risk to nature's balance, human health, water quality, wildlife, and the environment as a whole. It was worse by aerial application because of drifting of chemicals to non-target areas, worse effects on beneficial and less efficacy on the pest.

So the present insecticide-based strategies must have been replaced with multi-dimensional integrated pest management (IPM) approaches. Then an IPM approach has been adopted in control of the sunn pest in 2001. It has been decided that the elements other than chemicals of IPM such as biological and biotechnological control, cultural practices would be given priority.

Shifting from aerial to ground spray

The biggest challenge in implementing IPM for the sunn pest management was national agricultural strategies that rely on chemical control. The cost of insecticides was borne by government and the applications also were made by government. So faced with sunn pest infestation (mostly not at the economic threshold level), farmers applied pressure to their political representatives, who in turn requested the government to spray. Therefore the sunn pest was called political insect in Turkey. Thus a policy change was needed to devolve the insect control responsibility to farmers and remove the disincentives for adopting IPM as an alternative to agrochemicals.

Finally, after adoption of sunn pest IPM, as the first step, the sunn pest control policy was changed to switch from aerial application to ground spraying and leave the responsibilities to farmers gradually. It was not so easy to leave a 45-year habit. Public awareness and education at the level of political representatives, public institutions, farmer organizations, and farmers were crucial for the success of such policy change.

Several training sessions were arranged for farmers at every village where the sunn pest is problem to explain them why the policy change was crucial and teach the sunn pest; its biology and natural enemies, environmental protection, economic threshold (ET) and decision making to spray. Many public awareness meetings and panels in every city, sometimes in the districts with attendance of NGOs, local governors, cereal farmers, universities, media and all related parties were held to explain why we needed to change the sunn pest control policy. Separate meetings were also done with only political representatives of provinces previously the other meetings to get their support.

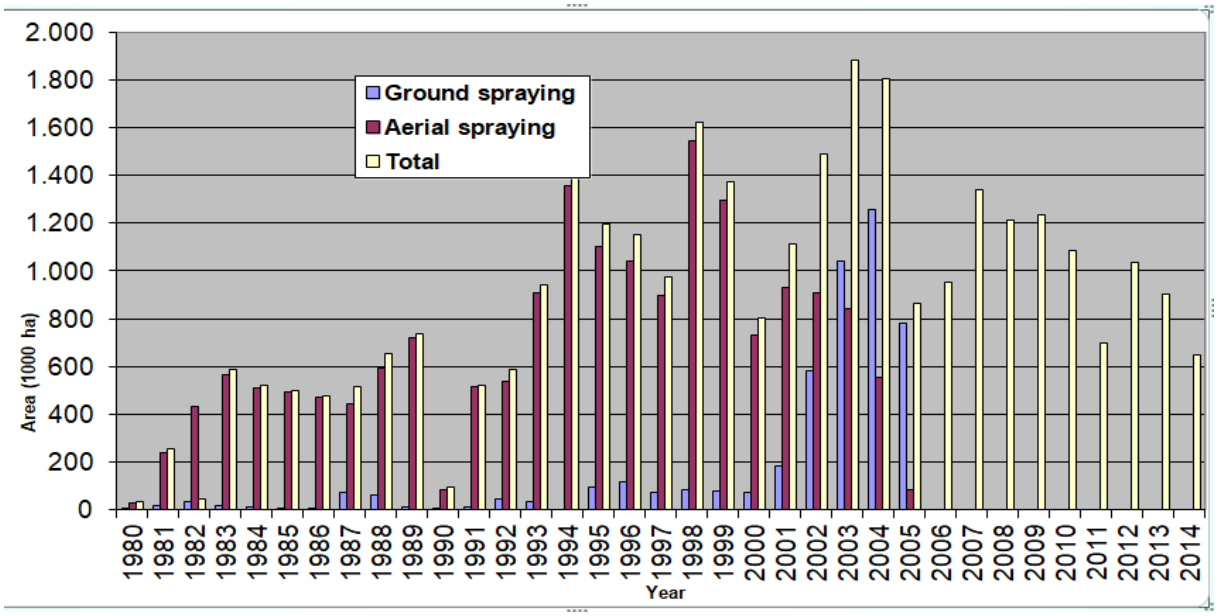

Figure 1. Ground spraying, aerial spraying and total area treated (ha) against sunn pest, *Eurygaster integriceps* in 1980-2014, Turkey

Separate meetings were also done with only political representatives of provinces previously the other meetings to get their support.

Tractor mounted sprayers were rented to farmers with free of charges where the farmers did not have enough equipments at the first year of shifting to ground spraying. These equipments were bought by government and some NGOs. There was gradually shifting. In the first year, 2001, some part of Trace region where the egg parasitoid was common and effective was chosen. Then, the remaining part of Trace region, Aegean and Marmara regions in 2002, Ankara, Konya, Eskişehir, Aksaray, Kırıkkale Provinces in Central Anatolia in 2003, everywhere except Diyarbakır, Mardin and Sanliurfa Provinces in 2004, everywhere except Siverek district in Sanliurfa Province in 2005 and all country in 2006 switched to ground spraying. The use of ground spraying as opposed to aerial application increased rapidly from 8% in 1997 to 56% in 2003. It was 69 and 90% in 2004 and 2005, respectively, and ground spraying was used completely in 2006. After switching ground spraying, insecticide treated area decreased dramatically from about 1.9 million ha in 2003 to 0.6 million ha in 2014 (Figure 1). This clearly showed us how appropriate decision it was. But there is always a danger that farmer may apply insecticide more than necessary because there is no good control of pesticide application and residue in the country.

Government provided insecticides with farmers for several years. Now there is no insecticide support and sunn pest control sprays are done by ground equipment completely. Farmers are supposed to apply the insecticide by their equipment, as determined by official technical consultants (Anonymous 2005-1). Technical support will be continued several years.

Research activities

Researches on the sunn pest's biology, bio-ecology, natural enemies and economic threshold, natural occurrence and effectiveness of egg parasitoids, chemical control and integration of all methods available have been conducted in Plant Protection Research Institutes since 1950s in the country.

In the framework of an international project, "Integrated Pest Management of Sunn Pest in West Asia", intensive research activities on economic threshold, host plant resistance, parasitoids, entomopathogenic fungi, semiochemicals in host and mate finding, and adaptive studies through the establishment of farmer field schools in pilot sites were carried out to develope and apply of appropriate, low-cost and environmentally acceptable IPM approaches in 2002-2005 in Turkey.

Over 2-year study in bread and durum wheat fields; adult parasitzm and egg parasitzm of sunn pest were 1-8% and 10-100%, respectively. No leaf or stem damage was observed because when the sunn pest completed migration to the fields, wheat plants reached 10-15 cm in height, and it was late for the sunn pest to damage leaves and stems. White spike damage was 0.03-1.67% at the density of 0.32-4.08 adults/m2, which was not significant (Canhilal et al., 2006)

The ET for sunn pest was redifined with the data generated from 2-year field research. The regression equations obtained to predict percent kernel damage for bred and durum wheat were $Y = -0.899+1.454X$ and $Y = -3.206+1.771X$, respectively. The threshold calculated were 8.12 and 9.16 nymphs/m² for bred and durum wheat, respectively. Present ET is 10 nymphs/m² regardless wheat type and variety and region. Thus the following sugesstions are made (Canhilal et al. 2006):

1-The ET may lowered to 8-9 nymph/m² for wheat varieties and regions where there are complaints and practical observations, and especially for low-yield levels (~ 2000-2500 kg/ha) until detailed research is conducted based on region, wheat variety, and yield level for bred wheat.

2-The ET 10 nymphs/m² appears still to be valid for durum wheat. But needs detailed research as for bred wheat.

A "National Integrated Sunn Pest Management Project" was also started in 2004. Establishing "green belts" to preserve the natural enemies of the sunn pest, studies on resistant varieties, parasitoids and economic threshold, and rearing and releasing its egg parasitoids (*Trisolcus spp*) and predatory birds such as partridge are some activities in the project. In the framework of the national project, about 3.8 million egg parasitoids in 2005 and 7.5 million in 2006 reared in the lab and released preferably in areas where there is reasonably high parasitzm (Anonymous 2005-2 and Anonymous 2006). About 200,000 partridges are planned to rear and released into overwintering sites in fall in 2006.

CONCLUSIONS

There was little sunn pest damage in some area where farmers were reluctant to enter their field with tractor at the beginning and then late for application in the first year of shifting. But overall there was a big success throughout the country because of ground spray. Percent kernel damage dropped to less than 1% in 2004. This meant an important quality increase. Thus, wheat import made previous years because of low quality wheat production was stopped.

After switching ground spraying, insecticide treated area decreased dramatically from about 1.9 million ha in 2003 to 0.6 million ha in 2014. This clearly showed us how appropriate decision it was. But there is always a danger that farmer may apply insecticide more than necessary because there is no good control of pesticide application and residue in the country.

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THE EFFICACY OF ENTOMOPATHOGENIC NEMATODES AGAINST *ZABRUS SPP.*

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ABSTRACT

Experiments were conducted to find out the susceptibility of the larvae of Zabrus spp. (Coleoptera: Carabidae), an important insect pest of wheat, against entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in the laboratory first time in the World. The entomopathogenic nematodes used in the trials were Steinernema feltiae-Commercial, S. feltiae-Endemic, S. carpocapsae, S. bicornutum, Heterorhabditis bacteriophora, and H. indica. Small plastic pots with a lid (8 cm in height, 6 cm in diameter) containing autoclaved soil have been utilized in trials. In the experiments, rates of 50, 100 and 200 infective juveniles (IJs)/cm^2 at 15, 20 and 25°C applied and they were repeated 2 times. Raising rate and temperature expanded the mortalities caused by nematodes. S. carpocapsae produced 75% mortality at the rate of 200 IJs/cm^2, which was the highest at 15°C. The lowest mortality with 5% at the rate of 50 IJs/cm^2 was caused by S. bicornutum 15°C. Steinernema carpocapsae at the rate of 200 IJs/cm^2, and S. feltiae-Endemic and H. indica at the rate of 50 IJs/cm^2 provided the highest (85%) and the lowest (20%) mortality, respectively at 20°C. At 25°C, S. carpocapsae at the rate of 200 IJs/cm^2 was the nematode caused the highest mortality with 95% while S. feltiae-Endemic at the rate of 50 IJs/cm^2 was producing 25% mortality which was the lowest. As a result, S. carpocapsae performed the best efficacy against Zabrus spp. and it was followed by H. bacteriophora and S. bicornutum.

Keywords: *Zabrus spp., entomopathogenic nematods, Steinernema, Heterorhabditis, biological control*

INTRODUCTION

Being an important crop in our country and in the world, wheat is an indispensable source of food for human nutrition. Annual wheat production in our country is approximately 18-20 million tons, wheat consumption however is approximately 16-17 million tons (Anonymous, 2008). Despite of an excessive wheat production, the losses in crop quality caused by pests and diseases makes a significant amount of wheat to be imported into our country. In addition to the diseases such as bunt, rust, smut, insect pests; sunn pest, cereal bugs, cereal spike beetles, and cereal ground beetles, *Zabrus* spp. are important and cause losses in wheat yield and quality (Lodos, 1989). Occurrence of high population densities of *Zabrus* spp. (Coleoptera: Carabidae) can cause yield losses of up to 100% in years and areas where none of control methods are applied. In our country, there are only seeds and surface chemical applications as control methods against this important pest.

In terms of plant protection, although there are many different control options against pests, the biological control is highly preferred over other methods, because of being the human, animal and environmental friendly method, maintaining the ecological balance and sustainability. As biological control agents, entomopathogenic nematodes (EPNs) (*Rhabditida:*

Steinernematidae and Heterorhabditidae) attract attention increasingly in research area recently. Their ideal properties such as; the broad host spectrum, to be able to kill their hosts within 24-48 hours, to be producible commercially easily in vivo or in vitro, having ability to search actively their hosts, settling in application areas and staying effective for a long time, having easy applicability, being in compliance with many chemicals and being safe for the environment are important for their preferability. They are soil dwelling, aquatic organism and have motile bacteria living their intestine. The bacteria in Steinernematidae are *Xenorhabdus* spp. and in Heterorhabditidae are *Photorhabdus* spp. Nematode and bacterium are mutualistic symbionts and obligate, lethal parasites of insects. EPNs can be found everywhere on earth and infect many different insects (Smart, 1995). Nematodes enter insect through natural openings. Once in the insect, the nematode releases the bacteria that are carried in the intestine. Bacterial cells reproduce rapidly and kill the insect within 24-48 hours using many different toxins. They also produce antibacterial and antifungal antibiotics not to allow any other other organisims in the host. The nematodes eat the bacteria and reproduce for 3 or 4 generations depending on the food source. Over 100,000 nematodes exit the insect (Burnell and Stock, 2000).

Entomopathogenic nematodes have been used in controlling insects since the 1930s (Smart 1995) in various climatic regions of the world. They are important biological control agents of soil-inhabiting insects (Gaugler 1981, Georgis and Poinar 1984, Klein 1990) such as Japanese beetles, mole crickets, and root weevils. They have also been used successfully against above-ground insects in cryptic habitats (Bedding & Miller 1981, Ralph 1981, Kaya 1988, Begley 1990, Kaya 1990, Vreditelyami et al. 1992), for example, navel orange worm, the codling moth and the artichoke plume moth, carpenter worms, and clearwing moths.

However, studies on EPN are very limited and some of them have just started in Turkey. Turkey having a diverse ecology shelters nine EPN species but the studies on the investigating of efficacy and usage of these species on pests of cultivated plants are very rare. In this study; we conducted experiments to find out the susceptibility of the larvae of *Zabrus* spp. against entomopathogenic nematodes in the laboratory first time in the World to produce basic data to use in the biological control of the insect.

MATERIAL AND METHOD

We studied entomopathogenic nematodes as an alternative to chemical control. The entomopathogenic nematodes used in the trials were *Steinernema feltiae-Commercial, S. feltiae*-Endemic, *S. carpocapsae, S. bicornutum, Heterorhabditis bacteriophora*, and *H. indica*.

Zabrus spp. larvae from wheat fields were collected digging into soil 25-30 cm at the end of March and beginning of April. They brought to lab in ice box. They let stay in plastic containers for 24 hours to differentiate the damaged ones during collecting and transportation. The trials have been conducted in small plastic pots with a lid (8 cm in height, 6 cm in diameter) containing autoclaved soil and repeated 2 times. The nematodes at 3 rates of 50, 100 and 200 infective juveniles (IJs)/cm^2 with 4 replicates applied evenly into plastic with pipet. Pots were placed in incubators at dark adjusted 15, 20 and 25 ^{o}C. They were checked after 7 and 10 days to count dead larvae. Efficacy was evaluated by comparing the treatments with untreated control.

RESULTS AND DISCUSSION

The mortalities caused by nematodes increased by increasing rate and temperature at the 10th day. The highest mortality with 75% at the rate of 200 IJs/cm^2 was caused by *S. carpocapsae* followed by *S. carpocapsae* at the dose of 100 and 50 IJs/cm^2 with 70% mortality and *H. bacteriophora* at dose of 200 IJs/cm^2 with 60% mortality. However they were statistically at

the same group. The lowest mortality with 5% mortality at the rate of 50 IJs/cm^2 was caused by *S. bicornutum* at 15°C (Table 1).

Table 1. The efficacy of entomopathogenic nematods against *Zabrus* spp. larvae at 15 °C in laboratory

Nematod	Dose (IJ/ cm^2)	Larva used 1st and 2nd year	7th day count			10th day count		
			Death (%) 1st year	Death (%) 2nd year	Death average (%)	Death (%) 1st year	Death (%) 2nd year	Death average (%)
S.feltiae Endemic	50	20+20	30	10	20bcde	30	30	30de
S.feltiae Endemic	100	20+20	35	15	25cde	35	35	35ef
S.feltiae Endemic	200	20+20	45	25	35def	50	50	50f
S.feltiae Commer	50	20+20	15	25	20bcdef	15	15	15abcd
S.feltiae Commer	100	20+20	30	25	27,5cde	30	30	30de
S.feltiae Commer	200	20+20	50	30	40ef	55	55	55gh
H. bacteriophora	50	20+20	15	50	32,5cde	40	70	55fg
H. bacteriophora	100	20+20	25	35	30cde	45	65	55gh
H. bacteriophora	200	20+20	15	60	37,5ef	50	70	60ghi
S. carpocapsae	50	20+20	30	50	40ef	55	85	70hi
S. carpocapsae	100	20+20	40	60	50f	60	80	70hi
S. carpocapsae	200	20+20	35	70	52,5f	65	85	75i
S. bicornutum	50	20+20	5	0	2,5ab	5	5	5ab
S. bicornutum	100	20+20	10	15	12,5abc	15	25	20bcde
S. bicornutum	200	20+20	25	25	25cde	30	30	30de
H. indica	50	20+20	5	5	5ab	10	10	10abc
H. indica	100	20+20	10	10	10abc	25	25	25cde
H. indica	200	20+20	15	15	15ab	25	25	25cde
Control	0	20+20	0	0	0a	0	0	0a

Table 2. The efficacy of entomopathogenic nematods against *Zabrus* spp. larvae at 20 ºC in laboratory

Nematod	Dose (IJ/ cm^2)	Larva used 1st and 2nd year	7th day count			10th day count		
			Death (%) 1st year	Death (%) 2nd year	Death average (%)	Death (%) 1st year	Death (%) 2nd year	Death average (%)
S.feltiae Endemic	50	20+20	25	15	20abc	25	15	20b
S.feltiae Endemic	100	20+20	50	10	30bcde	50	45	47,5de
S.feltiae Endemic	200	20+20	50	20	35bcde	55	50	52,5def
S.feltiae Commercial	50	20+20	20	25	22,5abcd	25	30	27,5bc
S.feltiae Commercial	100	20+20	35	35	35bcde	40	35	37,5cd
S.feltiae Commercial	200	20+20	50	20	35bcde	55	50	52,5def
H. bacteriophora	50	20+20	30	50	40bcde	55	50	52,5cde
H. bacteriophora	100	20+20	40	35	37,5bcde	50	40	45cde
H. bacteriophora	200	20+20	60	70	65g	70	80	75gh
S. carpocapsae	50	20+20	35	55	45defg	50	60	55def
S. carpocapsae	100	20+20	55	50	52,5efg	55	55	55def
S. carpocapsae	200	20+20	40	80	60fg	80	90	85h
S. bicornutum	50	20+20	30	30	30bcde	45	45	45cde
S. bicornutum	100	20+20	30	30	30bcde	45	45	45cde
S. bicornutum	200	20+20	45	45	45defg	75	60	67,5fg
H. indica	50	20+20	15	15	15ab	20	20	20b
H. indica	100	20+20	40	40	40cdef	55	65	60efg
H. indica	200	20+20	25	35	30bcde	55	65	60efg
Control	0	20+20	5	0	2,5a	5	0	2,5a

Table 3. The efficacy of entomopathogenic nematods against *Zabrus* spp. larvae at 25 ºC in laboratory

Nematod	Dose (IJ/ cm^2)	Larva used 1st and 2nd year	7th day count			10th day count		
			Death (%) 1st year	Death (%) 2nd year	Death average (%)	Death (%) 1st year	Death (%) 2nd year	Death average (%)
S.feltiae Endemic	50	20+20	25	15	20abcd	25	25	25b
S.feltiae Endemic	100	20+20	15	25	20abcd	45	55	50def
S.feltiae Endemic	200	20+20	15	30	22,5bcde	50	70	60efg
S.feltiae Commer	50	20+20	20	10	15abc	30	30	30bc
S.feltiae Commer	100	20+20	15	10	12,5ab	35	25	30bc
S.feltiae Commer	200	20+20	30	40	35bcdef	45	45	45cde
H. bacteriophora	50	20+20	55	65	60gh	60	70	65fg
H. bacteriophora	100	20+20	15	60	37,5cdefg	50	80	65fg
H. bacteriophora	200	20+20	40	60	50fg	50	80	65fg
S. carpocapsae	50	20+20	45	30	37,5cdefg	50	50	50def
S. carpocapsae	100	20+20	60	60	60gh	60	60	60efg
S. carpocapsae	200	20+20	95	60	77,5h	100	90	95i
S. bicornutum	50	20+20	40	40	40defg	60	60	60efg
S. bicornutum	100	20+20	45	45	45efg	70	70	70gh
S. bicornutum	200	20+20	45	20	32,5bcdef	90	80	85hi
H. indica	50	20+20	45	45	45efg	35	40	37,5bcd
H. indica	100	20+20	45	45	45efg	50	55	52,5defg
H. indica	200	20+20	20	45	32,5bcdef	60	80	70gh
Control	0	20+20	0	0	0a	0	0	0a

S. carpocapsae at the rate of 200 IJs/cm^2, and *S. feltiae*-Endemic and *H. indica* at the rate of 50 IJs/cm^2 provided the highest (85%) and the lowest (20%) mortality, respectively at 20°C. *H. bacteriophora* at the rate of 200 IJs/cm^2 with 75% mortality and *S. bicornutum* at the rate of 200 IJs/cm^2 with 67.5% mortality followed *S. carpocapsae*. They did not differ from each other statistically (Table 2). At 25°C, *S. carpocapsae* at the rate of 200 IJs/cm^2 was the one producing the highest mortality with 95% while *S. feltiae*-Endemic at the rate of 50 IJs/cm^2 was causing

25% mortality which was the lowest (Table 3). *S. bicornutum* at the rate of 100 and 200 IJs/cm^2 with 70% and 85% mortality and *H. indica* at the rate of 200 IJs/cm with 70% mortality followed *S. carpocapsae* at the rate of 200 IJs/cm^2, which were at the same group statistically.

CONCLUSIONS

As a result, *S. carpocapsae* showed the best efficacy against *Zabrus* spp. and it was followed by *H. bacteriophora* and *S. bicornutum.* Field trials with these nematodes showed high efficacy against *Zabrus* spp. should be planned for future studies.

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EVALUATION OF PERFORMANCES OF SELECTED SOME WINTER CHICKPEA GENOTYPES UNDER MEDITERRANEAN CLIMATE CONDITIONS

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ABSTRACT

This study was carried out to investigate the yield and yield components of some promising chickpea genotypes at Cukurova conditions. The experiment was performed according to complete randomized block design with four replications. This research was conducted with 9 genotypes (7 promising lines and 2 registered cultivars as a control orginated in Turkey) at Çukurova University, Agricultural Faculty, Field Crop Department during winter season of 2015/2016 and 2016/2017. According to average of experiment years, plant height, first podding height, number of branches per plant, number of pods per plant, number of grains per plant, grain weight per plant, 100-grain weight, and grain yield varied from 61.1 to 72.0 cm, from 22.7 to 35.4 cm, from 9.5 to 12.4 number plant^{-1}, from 56.6 to 103.5 number plant^{-1}, from 70.8 to 133.6 number plant^{-1}, from 27.3 to 45.5 g, from 32.5 to 44.0 g, from 1951 to 2690 kg ha^{-1} respectively. There were positive and significant correlations between grain yield and plant height, number of branch, number of pod, grain weight per plant, 100-grain weight.

Keywords: *Chickpea, Yield, Agronomic Traits, Genotype, Correlation*

INTRODUCTION

Cultivation of chickpea is widely spreaded in the worldwide and it is pulses the second most important after dry bean for sowing area in the world. It has high protein, carbonhydrate, mineral and vitamins and it is used as canning, boliling or roasting in human diets. Chickpea is most important pulses in Turkey. In Turkey planted area and average grain yield for chickpea are 360.0000 ha and the 128 kg ha^{-1} respectively (Anonymous, 2016). Chickpea has a great grain production in Mediterranean of Turkey. Chickpea is traditionally planted at early spring in the mentioned region, but grain yield is very low 800-900 kg ha^{-1}). Chickpea can be grown as a winter crop in the rotation with cotton and maize at Mediterranean region and it has a great grain production in this region, but Ascochyta blight is a serious problem for winter chickpea production because of warm and rainy climate condition at autumn sowing. Therefore grain yield can considerably decrease. However, grain yield increases greatly if cultivars with the tolerant to Ascochyta blight are grown as a winter crop at the Mediterranean conditions. The average grain yield in chickpea was obtained in between 2000 kg ha^{-1} and 3000 kg ha^{-1} (Mart and Anlarsal 2001.,Yucel and Anlarsal, 2012) for winter crop at coastal region. Some researcher reported that yield and yield compenents are greatly influenced by rainfall and temperature in flowering and podding, Khanna-Chopra and Sınha,1987; Yücel et al., 2012; Lake et al., 2016). Grain yield per plant was positively and significantly correlated with plant height, branches number per plant, pods number per plant and 100-seed weight (Toker et al., 2004, Gul et al., 2013; Pandey et al., 2013). Therefore, new chickpea cultivars which are tolerant to Ascochyta blight, favourable to harvesting machine, high yielding capacity, large seeded must be improved to spread cultivation of winter chickpea in the region.

The objective of this study revealed the performance of some promising chickpea genotypes under Mediterranean climates. Therefore, some selected chickpea genotypes at this region were investigated for grain yield and yield components and relations to among some traits.

MATERIALS AND METHODS

The experiment was conducted at research area of Department of Field crop of Agricultural Faculty of Cukurova University (35^0 18' E, 37^0 o1 N;23 m above sea level) during 2015/2016 and 2016/2017 experimental years. This area has Mediterranean climate at Turkey. Average annual total precipitation and mean temperature of this area are 625 mm and 18.7ºC according to long-terms respectively. Meteorological values were given in Table 1. In Balcalı the soil of research area was sandy-loam in textures. Values of pH and salt content were 7.78 and 0.33 mmhos cm^{-1}, respectively.

Table 1. Values of monthly average temperature and total precipitation of Adana.

Meteorological Parameters	Years	November	December	January	February	March	April	May
Mean Temperature (℃)	2015-2016	24.6	18.8	13.4	20.2	21.7	27.5	27.1
	2016-2017	23.2	14.1	13.9	17.7	21.1	24.7	27.4
	Long term	14.8	10.5	9.3	10.0	12.9	17.3	21.6
Total Rainfall (mm)	2015-2016	10.5	0.6	138.4	83.1	54.3	1.8	87.9
	2016-2017	11.9	147.1	52.0	0.8	65.4	65.9	45.9
	Long term	86.4	115.6	95.0	82.5	61.1	50.3	43.4

The research material was seven promising line (FLIP 09-51 C, FLIP 09-24 C, FLIP 09-17 C and FLIP 09 -29 C, FLIP 06-118 C, FLIP 06-111 C, FLIP-05-88 C) and two check cultivars orginated Turkey (Seçkin and Hasanbey). The promising lines were selected from a lot of number lines orginated ICARDA (International Center for Agricultural Research in the Dry Areas) in Syria. The experiment was organized in randomized complete block design (RCBD) with four replication. Each plot was sown in rows of 5 m lenght and 4 rows with a spacing of 45 cm between rows. Plant to plant distance in row was 6 cm. Each genotype was planted at the end of November in both years. The fertilizer was applied at a rate of 30 kg N ha^{-1} and 70 kg P_2O_5 ha^{-1} before sowing. The plot was harvested at the beginnig of June and the middle of June in the first and second experimental years respectively.

The traits were measured on ten plants selected randomly from each plot. Plant height (cm), number of branches per plant, number of pods per plant, grains per plant, 100-grain yield (g), grain weight per plant (g) and grain yield (kg ha^{-1}) were investigated.

The data were variance analiysed combined over experiment years according to the randomized block using MSTATC programme. Differences among the average were found using Duncan multiple range test at 0.05 probability level.

RESULTS AND DISCUSSION

Values of plant height and first podding height of genotypes for 9 chickpea genotypes in different experimental years were presented in Table 2. According combined years, the study showed that plant height was significantly affected by genotypes and it varied from 61.1 (09-17 C) to 72.0 cm (09-24 C). The years were significantly affected the plant height. The mean value for this trait was lower in first experimental year (63.5 cm) than in second experimental year (70.2 cm). Field emergence date of genotypes in the first year was later (January 5) compared with second year because of late rainfall. Genotype x year interaction was significant

for plant height. The highest value was achived by genotype 09-24 C with 72.0 cm followed by 05-88 C and 06-111 C while the lowest value was obtained from 09-17 C with 53.5 cm.

Table 2. Mean values for plant height and first pod height of 9 chickpea genotypes*

Genotypes	Plant height (cm)			First pod height (cm)		
	2015/16	2016/17	Mean	2015/16	2016/17	Mean
09-51 C	60.8 de	63.9 cd	62.3 de	28.5	24.1	26.3 d
09-24 C	66.6 cd	77.4 a	72.0 a	35.2	35.1	35.1 a
09-17 C	53.5 f	68.8 bc	61.1 e	22.5	23.1	22.7 e
09-29 C	59.4 e	69.3 bc	64.3 cde	27.2	28.6	27.9 cd
06-118 C	66.2 cd	70.0 bc	68.1 abc	30.3	29.5	29.9 bc
06-111 C	66.4 cd	73.3 ab	69.8 ab	31.9	34.9	33.4 ab
05-88 C	66.7 cd	76.8 a	71.7 a	35.1	35.6	35.4 a
Seçkin	64.7 ce	66.5 cd	65.6 cd	33.9	28.5	31.2 bc
Hasan bey	67.0 cd	65.9 cd	66.5 bc	32.2	28.4	30.3 bc
Mean	63.5 b	70.2 a	66.8	30.7	29.8	30.3
CV(%)			5.6			22.9

*Means with a column and line for each trait followed by the some letters are not significantly different for Duncan's multiple range test ($p < 0.05$).

According to combined years differences among the genotypes were significant for the first podding height . The highest value was found for 05-88 C (35.4 cm) and 09-24 C (35.1 cm) whereas the lowest one was 09-17 C with 22.7 cm. The first podding height was significantly affected by years and genotype x years interaction.

The mean values of branches per plant and pods per plant of 9 chickpea genotypes in different experimental years were presented in Table 3.

Table 3. Mean values for number of pods per plant and number of grains per plant of 9 chickpea genotypes*

Genotypes	Number of branches per plant			Number of pods per plant		
	2015/16	2016/17	Mean	2015/16	2016/17	Mean
09-51 C	15.9	6.3	11.1	68.7	113.5	91.1 a
09-24 C	13.5	7.6	9.5	50.0	71.9	60.9 b
09-17 C	11.9	7.0	9.5	44.9	74.9	59.9 b
09-29 C	14.8	5.8	10.3	55.3	85.8	70.5 b
06-118 C	16.5	8.3	12.4	75.9	131.1	103.5 a
06-111 C	14.7	7.3	11.0	53.6	62.0	57.8 b
05-88 C	13.6	9.9	11.8	46.5	90.6	68.5 b
Seçkin	12.2	6.7	9.5	39.1	74.2	56.6 b
Hasan bey	15.1	7.9	11.5	57.8	73.9	65.8 b
Mean	14.2 a	7.4 b	10.8	54.6 b	86.4 a	70.5
CV(%)			22.9			23.8

*Means with a column and line for each trait followed by the some letters are not significantly different for Duncan's multiple range test ($p < 0.05$).

The branches per plant were not influenced by genotypes and genotype x year interaction in both years, but years affected the branches per plant. Branches per plant in the first experimental year (14.2) were higher than in the second experimental year (7.4). The number of branches per plant was varied between 9.5 and 12.4.

The mean of the pods per plant were significantly affected by genotypes. The highest number of pods was obtained in 06-118C (103.5) and 09-51 C (91.1) while the lowest value was found for Seçkin (56.6) Number of pods was higher in the second year than in first year. The high average temperature (27.5 ^{0}C) and low rainfall (1.8 mm) during the flowering stage in beginning April at the first year may cause lower the pods compared with the second year (Table 1). Similarly to our findings some researcher reported that high temperature and low rainfall affected negative the generative traits in various legumes (Bakry et al., 2011; Kanchan and Virender, 2014; Lake et al., 2016

Values of grains per plant and 100-grain weight of chickpea genotypes in different experimental years were given in Table 4. Differences among the genotypes for grains per plant were significant. The mean values of grains per plant varied 70.8 (06-111 C) to 133.6 (06-118 C). This trait was significantly affected by the years. Number of grains per plant was higher in the second year compared with first year as in pods per plant. High number of grains per plant in the second year may be due to the high pods per plant in the same year. The effect of genotypes was significant for 100-grain weight. The mean 100-grain weight varied from 32.5 g (06-118 C) to 44.0 g (09-51 C). The 100-grain weight was significantly influenced by years. The value of 100-grain weight in the second year was greater than in the first year. The high temperature and low rainfall during the stage of grain filling in April at the first experimental year may decrease the 100-grain weight.

Table 4. Mean values for number of grains per plant and 100-grain weight of chickpea genotypes.*

Genotypes	Nuımber of grains per plant			100-grain weight (g)		
	2015/16	2016/17	Mean	2015/16	2016/17	Mean
09-51 C	78.2	131.6	107.9 b	43.5	44.0	44.0 a
09-24 C	64.1	83.2	73.6 c	37.6	40.0	39.0 c
09-17 C	55.9	96.2	76.0 c	40.2	42.0	41.5 b
09-29 C	64.4	91.1	77.7 c	35.4	38.4	36.5 d
06-118 C	97.2	169.9	133.6 a	32.0	33.1	32.5 e
06-111 C	96.5	72.2	70.8 c	39.5	45.0	42.0 b
05-88 C	59.3	91.4	75.3 c	34.5	37.7	36.1 d
Seçkin	54.6	99.7	77.1 c	37.5	41.7	39.6 c
Hasan bey	73.3	105.0	88.6 bc	39.9	42.0	40.9 bc
Mean	68.4 b	104.5 a	86.4	37.8 b	40.5 a	39.2
CV(%)			26.6			4.2

*Mean s with a column and line for each trait followed by the some letters are not significantly different for Duncan's multiple range ($p < 0.05$)

Values of grain weight and grain yield of chickpea genotypes in experimental years were given in Table 5. There were significantly differences among the genotypes for grain weight per plant and it varied 29.6 (09-24 C and 09-17 C) to 45.5 g (09-51 C and 06-118 C). Grain weight per plant in the second experimental year due to high pods per plant, grains per plant and 100 grain weight was significantly higher than in the first experimental year. Genotype x year interaction was not significant for this trait.

As shown in Table 5, according to combined years' differences among the genotypes were significant for grain yield. Grain yield varied 1951(06-118 C)-2690 kg ha^{-1} (09-24 C). However, there were not differences between genotypes 09-24 C, 05-88 C and Seçkin. Grain yield was greater in the second year due to the low rainfall and high temperature during the flowering and

pod filling stage compared with the first year. Similar opinions were reported by some researcher (Kanchan and Virender, 2014; Meena et al., 2015; Lake et al., 2016.) Genotype x year interaction was significant for grain yield. The highest yield was achived by genotype 09-29 C with 2690 kg ha^{-1} followed by 05-88 C and 09-24 C in the second experiment year. The lowest yield was obtained from 06-188 C with 06-188 C with 1577 kg ha^{-1}. These results were in close agreement with the findings of some reports (Anlarsal et al., 1999, Yücel and Anlarsal, 2006; Mart et al., 2011; Ton and Anlarsal, 2016). The mentioned genotypes and check cultivars also showed tolerance to Ascochyta blight in both years.

Table 5. Mean values for seed weight per plant and seed yield of chickpea genotypes.*

Genotypes	Grain weight per plant (g)			Grain yield (kg ha^{-1})		
	2015/16	2016/17	Mean	2015/16	2016/17	Mean
09-51 C	36.4	54.2	45.5 a	1823 g-ı	2760 cd	2290 b
09-24 C	23.4	31.2	29.6 b	2078 f-h	3303 ab	2690 a
09-17 C	22.1	37.1	29.6 b	2039 f-h	2548 de	2294 b
09-29 C	22.1	32.5	27.3 b	1741 hı	3364 a	2552 ab
06-118 C	33.8	57.3	45.5 a	1577 ı	2324 ef	1951 c
06-111 C	27.2	32.6	29.9 b	1824 g-ı	3060 a-c	2444 ab
05-88 C	19.9	37.6	28.7 b	1984 f-ı	32.8 ab	2617 a
Seçkin	20.1	39.3	29.7 b	2018 e-h	3134 a-c	2657 a
Hasanbey	28.6	40.0	34.3 b	2196 e-g	2874 bd	2530 ab
Average	26.0 b	40.2 a	33.1	1939 b	2957 a	2448
CV(%)			25.9			11.2

*Means withi a column and line for each trait followed by the some letters are not significantly different for Duncan's multiple range test ($p < 0.05$)

Simple correlations coefficients between yield and yield components in chickpea were presented in Table 6.

Table 6. Simple correlations coefficients between yield and yield components in chickpea

Yield components		2	3	4	5	6	7	8
1.	PH	0.531**	0.299*	0.334**	0.244*	0.254*	0.065	0.577**
2.	FPH	-	0.099	-0.268*	0.293*	-0.324**	-0.106	0.123
3.	BPP	-	-	-0.169	-0.174	-0.184	0.359**	0.613**
4.	PPP	-	-	-	0.921**	0.933**	0.004	0.328**
5.	GPP	-	-	-	-	0.936**	-0.320**	0.209
6.	GWP	-	-	-	-	-	0.154	0.304**
7.	100-GW	-	-	-	-	-	-	0.362**
8.	GY	-	-	-	-	-	-	-

*, **, ns, significant at 5% and non significant level respectively .

1.PH: plant height(cm), 2.FPH: First pod height(cm), 3.BPP: Grains per plant , 4. PPP: Pods per plant, 5.GPP: Grains per plant^{-1} 6. GWP: Grain weight per plant 7.100- GW: 100- grain weight(g), 8.GY:Grain yield(kg ha^{-1})

As seen in Table 6, positive and significant correlations were observed between grain yield with plant height (0.577**), pods per plant (0.328**), grain weight per plant (0.304**), 100-grain weight (0.362**) and branches per plant (0.613**). These results were also similar to the results of Ton and Anlarsal, 2016, Kobraee et al., 2010, Biabani et al.,2011. The association of grain yield with first podding height had not signifficant. Grain weight per plant showed significant and positive correlation with plant height, pods per plant, grains per plant. Similar results were reported by Ali et al., 2009; Ali and Hassan, 2011; Samad et al., 2014, Hegde and Kumar, 2015, Samyuktha et al., 2017. However, in contrast to our findings, (Sing et al. 2017) recorded that seed yield per plant showed non-significant correlations with these characters except pods per plant. This present revealed that there was negative significant correlation between grain weight per plant and first podding height. The relationship between grain weight per plant and branches per plant was non significant. Similar findings were reported by Jha et al. Singh et al. 100-grain weight exhibed negative and significant correlations with grains per plant. Similar result was reported by Samyukta et al., 2017. On the other hand there were not significant correlations between 100-grain weight and plant height, first podding height, pods per plant, grain weight per plant. However, the correlation between 100-grain weight and branches number was positive and significant. Positive and significant relationships were found between plant height and first podding height, pods per plant , grains per plant, grain weight per plant.

CONCLUSIONS

The results of this research showed that the 09-24 C, 05-88C, 09-29 C, 06-111 C genotypes had high grain yield and their yields were similar to those of check cultivars (Seçkin and Hasanbey). They also showed tolerance to Ascochyta blight. On the other hand 100 grain weight of 09-51 C, 09-17 C, 06-111 C, 09-24 C was greater than those of check cultivar Seçkin. The mentioned promising genotypes should be tested in diverse environment of Mediterranean region in Turkey. The present study revealed that plant height, pods per plant, grain weight per plant and 100-grain weight and branches per plant may be considered for improving grain yield in chickpea.

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DETERMINATION OF PHYTOTOXIC RESPONSES IN TOBACCO (NICOTIANA TABACUM L.) EXPOSED TO HERBICIDE AND INSECTICIDE STRESSES

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Abstract

Plants are exposed to various stress factors in their natural environment that causes physiological and biochemical damage on the product quality. Pesticide stress is one of the most experienced abiotic stress factors. In this study the effect of herbicide and insecticide stress were tested simultaneously and in a separate manner on the tobacco plant. With this aim different doses of herbicide and insecticide were applied on the tobacco plants and collected leaves were tested for their total chlorophyll, carotenoid and malondialdehyde content as well as antioxidant enzyme activity. According to the findings stress applied groups has shown that chlorophyll content has decreased, malondialdehyde content has increased where antioxidant enzyme activity changes. These changes were more remarkable for the herbicide and herbicide+insecticide group.

Keywords: *Herbicide, Insecticide, Nicotiana tabacum, Antioxidant*

INTRODUCTION

Plants are exposed to several stress factors in their natural environment. The production of reactive oxygen species (ROS) increases in plants under stress. Increased ROS causes lipid, protein and chlorophyll damage in cells, leading to DNA and RNA damage which results in cell death, thereby causing harm for the plant. Enzymatic and non-enzymatic antioxidants in plants helps the plant to overcome stress conditions by regulating ROS levels (Mittler 2002; Çulha and Çakırlar 2011; Büyük et al. 2012).

Pesticides are one of the common stress factors that plants are often exposed to. Combination of pesticide can be used in various agricultural applications. These active substances are specifically designed to combat diseases and unwanted organisms (Yıldız et al). Plant pests may include insects, rodents, weeds or other undesirable hosting organisms (Ecobichon, 1998). Therefore, pesticides occupies an important space among other chemicals. An ideal pesticide should be be highly specific for the undesired target organism. However, most pesticides are toxic to several non-target organisms, including human beings (Klaassen, 2011).

Pendimethalin (N- (1-ethylpropyl) -3,4-dimethyl-2,6-dinitrobenzenamine) is a herbicide that is used for controlling the narrow- and broad-leaved weeds in areas where cotton, tobacco, sunflower and vegetables are grown (Appleby and Valverde, 1989; Sondhia 2012). This agent inhibits the steps involved in cell-wall formation and cell division, thereby leading to damage.

Methomyl (*S*-methyl *N*-(methylcarbamoyloxy) thioacetamidate) is a broad-spectrum insecticide on vegetables, tobacco, cotton, alfalfa, soy beans and corn. It is effective as both contact and systemic effects (Farre et al. 2002).

In the present study, a comparative evaluation has been performed to assess certain changes in tobacco resulting from separate and simultaneously application of two pesticides that are

commonly utilized in tobacco-growing areas. For this purpose, total chlorophyll, carotenoid, malondialdehyde (MDA) and glutathione (GSH) contents and glutathione reductase (GR) and glutathione S-transferase (GST) activities have been investigated.

MATERIAL AND METHODS

Plant growth and treatments

The plant materials for the study were "Oriental cv." tobacco seedlings supplied from farmers who grow tobacco in Adıyaman/Çelikhan. The seedlings (of approximately 40 days old) were planted in turf: perlite mix (3:1) pots and placed in a climate chamber at 30˚C with 65% humidity. Seedlings were divided into four different groups 10 days after the planted. The first group was the control group and treated with pure water only. The second group was treated with 3 different doses (7.5, 15 and 30 mM) of pendimethalin (Pend), the third group was treated with 3 different doses (4, 8, 16 mM) of methomyl (Met) and the fourth group was treated with 15 mM Pend+8 mM Met. Leaf samples were collected for analysis 10 days after these treatments.

Physiological and biochemical analyses

Determination of the total chlorophyll and carotenoid contens were made according to De Kok and Graham (1980) and Lichtenthaler and Wellburn (1983). The GST activity was determined according to Habig et al. (1974). The GR activity was made according to Carlberg and Mannervik (1985). The MDA content was analysed according to Heath and Packer (1968). The GSH content was determined according to Akerboom and Sies (1981).

Statistical analysis was performed using the SPSS 17.0 software. The differences between the averages at different stress groups were determined using Duncan's (1955) method.

RESULTS

Total chlorophyll and carotenoids content of leaves in tobacco plant exposed to pesticide stress

Total chlorophyll content has decreased compared to control group at all stress groups. Although the highest total chlorophyll content was found as 7.28 μg^{-1} g at control group, the lowest total chlorophyll content was found as 5.33 μg^{-1} g at groups of treated with 15 mM Pend+8 mM Met ($p<0.05$) (Fig. 1).

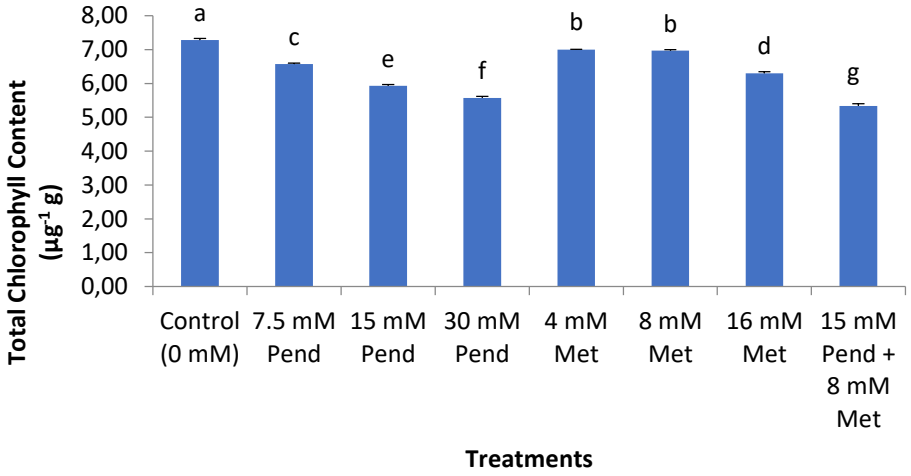

Figure 1. Changes in total chlorophyll content in tobacco leaves exposed to Pend and Met stresses. The different lower-case letters are significantly different from each other ($p<0.05$) among different concentration of Pend and Met according to Duncan's test.

Carotenoid content has decreased comparing to the control group at all stress groups. The highest carotenoid content was found as 3.21 μg^{-1} g at control group, the lowest total chlorophyll content was found as 1.98 μg^{-1} g at groups of treated with 15 mM Pend+8 mM Met ($p<0.05$) (Fig. 2).

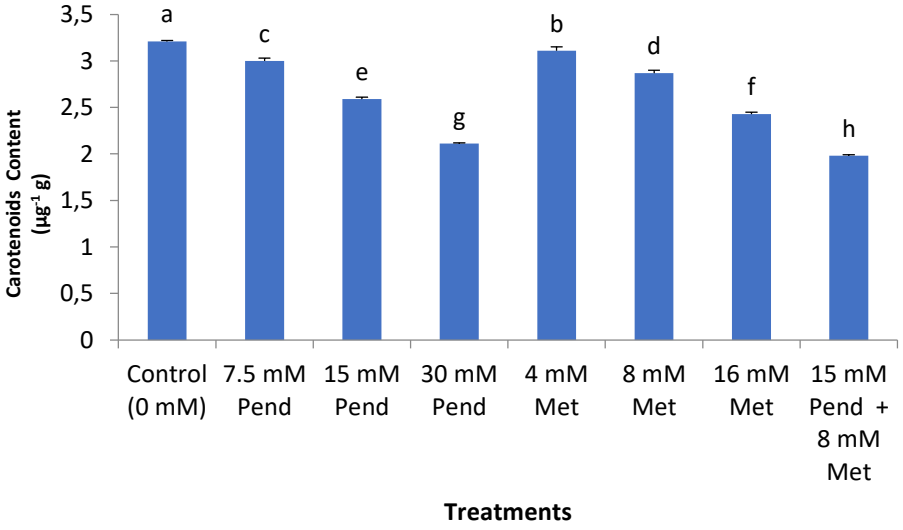

Figure 2. Changes in carotenoid content in tobacco leaves exposed to Pend and Met stresses. The different lower-case letters are significantly different from each other ($p<0.05$) among different concentration of Pend and Met according to Duncan's test.

MDA content of leaves in tobacco plant exposed to pesticide stress

MDA content of plants exposed to pesticide simultaneously was found to be higher compared to those exposed separately. The highest MDA content was found as 7.31 µmol MDA g^{-1} FW at groups of treated with 15 mM Pend+8 mM Met ($p<0.05$) (Fig. 3).

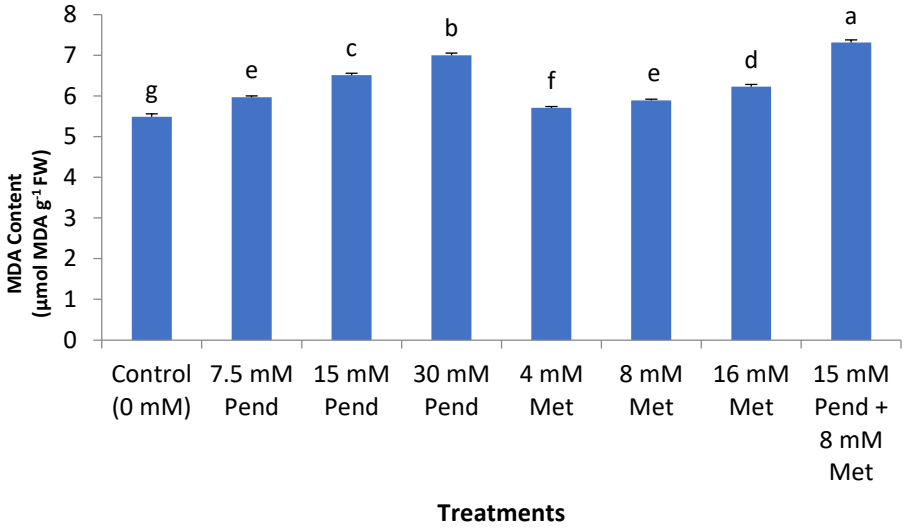

Figure 3. Changes in MDA content in tobacco leaves exposed to Pend and Met stresses. The different lower-case letters are significantly different from each other ($p<0.05$) among different concentration of Pend and Met according to Duncan's test.

GSH content of leaves in tobacco plant exposed to pesticide stress

GSH content increased compared to control at all stress groups. The highest GSH content was found to be 0.83 µmol $min^{-1}mg^{-1}$protein at groups of treated with 15 mM Pend+8 mM Met (*p<0.05)* (Fig. 4).

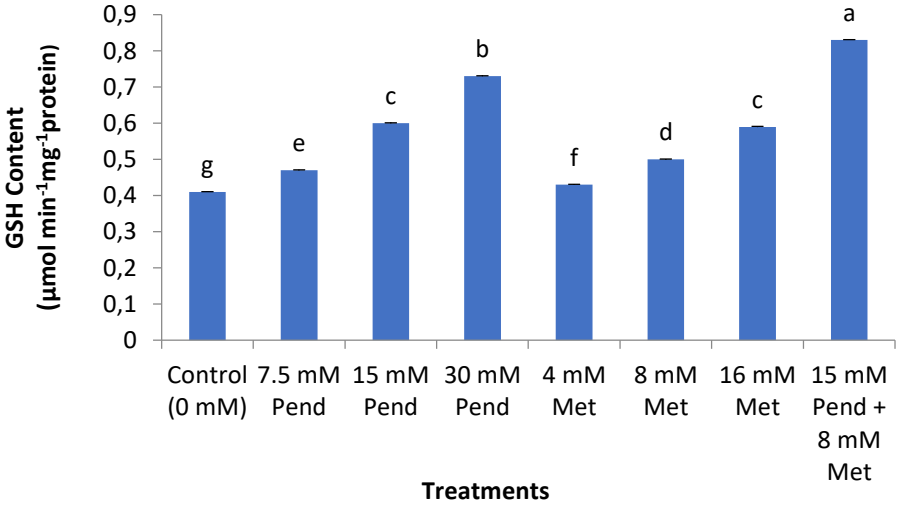

Fig. 4. Changes in total GSH content in tobacco leaves exposed to Pend and Met stresses. The different lower-case letters are significantly different from each other (*p<0.05*) among different concentration of Pend and Met according to Duncan's test.

GR and GST activities of leaves in tobacco plant exposed to pesticide stress

GR and GST activities of plants exposed to the pesticide simultaneously was found to be higher compared to those exposed separately. The highest GR activity was found to be 0.51 µmol $min^{-1}mg^{-1}$protein at groups of treated with 15 mM Pend+8 mM Met (*p<0.05)*. The highest GST activity was found to be 0.72 µmol $min^{-1}mg^{-1}$protein at groups of treated with 15 mM Pend+8 mM Met (*p<0.05)* (Fig. 5, 6).

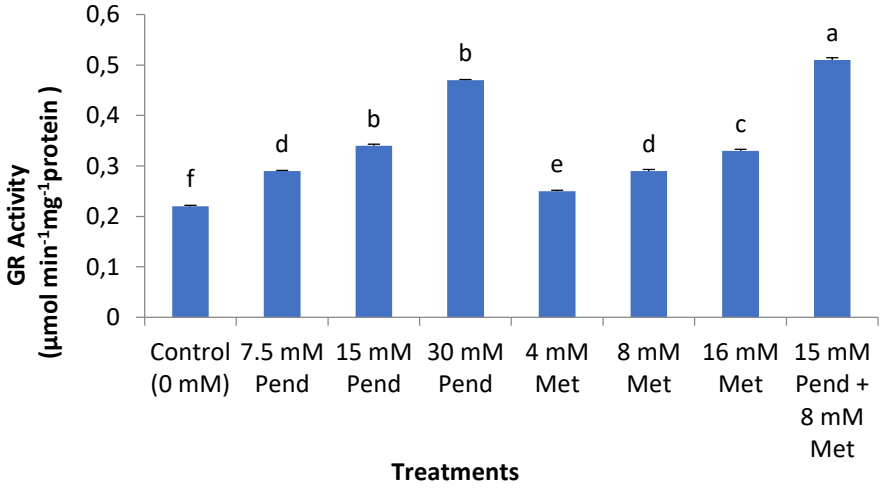

Figure 5. Changes GR activity in tobacco leaves exposed to Pend and Met stresses. The different lower-case letters are significantly different from each other (p<0.05) among different concentration of Pend and Met according to Duncan's test.

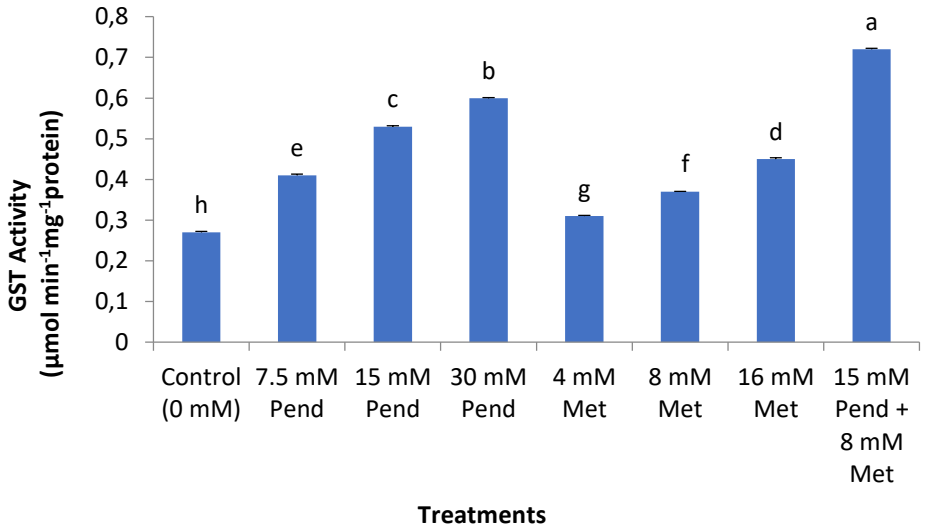

Figure 6. Changes GST activity in tobacco leaves exposed to Pend and Met stresses. The different lower-case letters are significantly different from each other ($p<0.05$) among different concentration of Pend and Met according to Duncan's test.

DISCUSSION

Environmental stresses might cause negative effects on plants. Pesticide stress is one of the most experienced abiotic stress factors. In this study the effect of herbicide and insecticide stress were tested simultaneously and in a separate manner on the tobacco plant. Chlorophyll content is accepted as one of the indicators of resistance of plant against environmental stresses. The results illustrated that total chlorophyll and carotenoid contents have decreased compared to control at all stress groups. This decrease in the pigment content can reflect pesticide-induced stress.

MDA is a final product of lipid peroxidation and an indicator of cellular damage in plants exposed to stress. There are studies in the literature reporting that MDA content increases under different stress conditions (Garkova et al.,2011; Qiu et al.,2014; Qing et al.,2015). The findings showed that MDA content increases compared to control group at all stress groups.

Antioxidant compounds play an important role on protection of cell against ROS and help plants to survive under stress (Mandal et al., 2009; Gill et al., 2013). This study shows that GST and GR activities and GSH content increases comparing to the control group at groups exposed to pesticides. Those changes were more remarkable for the groups that were treated with 15 mM Pend+8 mM Met.

CONCLUSIONS

As a conclusion, according to the results has showed that it is essential to raise awareness on pesticide use among farmers for the two stress factors that plants are exposed, either separately or often simultaneously during their growth which causes oxidative damage in the plant, and especially considering the unfavorable effects of pesticides on non-target organisms.

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COMPARATIVE ASSESSMENT OF LAJTHIZA AND BOVELLA'S WATER AT THE SOURCE, UP TO PRODUCTION-READY DURING 2017-2018

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Abstract

With population increasing and knowledge development, the requirements for drinking water quality and safety have been increased. Nowadays, the request for high quality and safety drinking water has been increased, parallel to industrial, agricultural, energy and aquaculture usage. Water is one of the main ingredients for consumption and for use in the food industry, it has a high strength solvent to salts therefore, depending on the chemical composition of the layers from which water passes, enriched with various substances and thus has a content of different chemical. Water used for consumption and industry should be drinking. The drinking water is characterized by parameters such as organoleptic, chemical and bacteriological. Water than from all other juices falling shrink to a certain temperature (+4 $^{\circ}$C) and then immediately abruptly begins to swell. When it freezes, it expands even more. For this reason the solid water is easier than its liquid state. So, instead of ice water swim by the laws of physics should sink to the bottom of it. Water should be easier and less mineralized. The amount of minerals dissolved in water is a basic indicator for the natural mineral water, the label in the form of parameter "dry residue at 180 $^{\circ}$C in mg/l". The lower this value is, the better is the water. This study takes into account the scientific evidence of the above, where the resources at any time of the year there are no variable composition and chemico-physical and to maintain equal treatment of the quality regulated by the technological process from storage, filtering varied and up on the packaging. Albania is considered a country with many natural water resources where there are many opportunities for the development of the water industry for human consumption

Keywords: *Water, Water features, Resources, Water quality*

INTRODUCTION

With the development and the population increasement, the demand for a better quality water is increased. Besides industrial usage, agricultural, energetic and thermo-energetic, fish industry and aquaculture, today also the demand for a drinkable and qualitative water is increased, especially in terms of personal hygena and in tourism. Water, not only is the main component of the human body, but he is also one of the three elements (earth, water, air) of life on our planet. The lack of drinking water to informal settlements and the more the risk of pollution from land existing as well as from the air, always adds more "drink water". Today more and more insurance to the consumer water is made in the form of packaged to ensure its quality. The variety of pollutants in the environment often causes the change of the physical, chemical and microbiological natural source. Increasing Food Standard of living increases the demand for bottled drinking water. The world market for bottled water has a consumption exceeding 89 billion liters per year. Western Europeans are the biggest consumers in the world of bottled water (85 liters/person/year). Classification of drinking water by the International

Organization for Water (IOW) is as follows: natural mineral water, spring water and purified water (surface water or groundwater). Also, IOW for bottled water bottle takes into account four categories as artesian water, drinking water (tap) water, gas and water wells. Water characteristics significantly affect the development of the various stages of the process of technological processes and the quality of nutrition, so it is necessary to use an adequate water. If you can not possess a suitable water for consumption, we should perform appropriate treatments to modify the characteristics or special operations, in order to avoid negative effects.

Among the key indicators necessary and drinking mineral water are:

The contentof dissolved salts in water (mg/ l)	< 50	< 500	500 ÷ 1500	> 1500
Natural mineral water containing:	very low	low	medium	high

Mineral salts in water in the form of cations and anions. Usually cations and anions in the most important are: Ca^{+2}, Mg^{+2}, Na^{+}, Fe^{+2}, $HC0_3^{-}$, $S0_4^{-2}$, Cl^{-}, F^{-}. Their content is necessary in certain limits, while the surplus is harmful to human health. In general, their content must be according to the following indicators, in mg/l: canions: $Ca^{+2} > 150$, $Mg^{+2} > 50$, $Na^{+} > 200$, $Fe^{+2} > 1$ and anions: $HC0_3^{-} > 600$, $S0_4^{-2} > 200$, $Cl^{-} > 200$, $F^{-} > 1$, content of free $C0_2$ according standardev should be > 250 mg/l.

In this article we are performed analysis and assessment Lajthiza and Bovilla water during 2017, some of these characteristics may come as a result of pollution of natural resources, the packaging that they are traded or technology of drinking water treatment.

MATERIALS AND METHODS

There are determined the physical-chemical parameters like pH, temperature, turbulence, conductivity and free clor.

Two are the methods used for the determination of physical-chemical parameters,the spekto-fotometrical method, UV-VIS and the volumetrical method (APHA1998). Also the microbiological lab determines total coliforms, fechal coliforms, fechal streptococci and general mycroflora in 22 degrees Celsius, 36 degrees Celsius and Clostridium. For microbiological analysis is used the technique of membrane filtering, where 100 ml of water is filtered with the help of a filtering device in vacuum, using membranic filters with 47-50 mm of diameter and pore size 0.45μm. The filters with the content of filtering water are put in a "petri dish" plates with willing dehydrated surfaces and according to case are put in thermostat or mari bath. We highlight that the methods used are in accordance with European standard 80/778 albanian standard STASH 3904:1997 (VKM 145,1998) for the water quality consumped by humans.

The sampling method

Samples for analysis were taken in accordance with the rules of sampling for analysis. They represent samples: water that is packaged in place, water packaged overseas (imports), as well as from the origin where packaged waters produced in place. Equipment used for the analysis of all physical-chemical indicators and bacteriological are:

Through these devices are defined as:

1. By conductometer determined electrolytic conductivity of water.
2. By pH-meter determined value of alkalinity.
3. By turbo-meter determined turbidity of the water.
4. By ammonium-meter determined NH^{4+} quantify.

5. By spectrophotometer determined the light absorption measures.
By analogy should make sure that the concentration of organic substances extracted by chloroform (ECC), as he polycyclic aromatic hydrocarbons, should not exceed 0.2 mg / l and not have the presence of pesticides (Schedule 1). Water to be evaluated as drinking must have a constant temperature, ranging from 6 to 12 ° C, there should be no abnormal taste or odor even at 60 °C. Also there should be no color and should be fine. The clarity of the water is an indication of lack of insoluble substances, however this feature is very relative because they are very few waters that have not suspended substances (Schedule 1). Evaluation of suspended substances can be performed with a measurement of turbidity by turbidimetrit Jackson. The analysis results are expressed in units Jackson (rotected) which can be converted into other units through equivalency factors. According to the norms of the World Health Organization maximum turbidity should be in the 5 rotected.
Chemical analysis should enable the removal of fecal contamination originated, those organic and inorganic type; such information is obtained by performing analysis for the determination of organic substances, ammonium chloride (organic pollution in action), nitrites and nitrates (organic pollution distinct) and inorganic elements that may be present due to infiltration of emissions coming from the surrounding industrial areas. It is important, both from the standpoint of the consumer, as well as suitability of use in any industry, defined as insoluble salts and their composition. For this purpose we need to know the power of a water classify in total, temporary and permanent.
The total power is determined by the concentration of calcium and magnesium salts. Temporary power is determined by the concentration of calcium and magnesium salts that are as bicarbonate, whereas the difference between these two types of power sets and found a permanent power of the calcium and magnesium salts that remain undigested in the water after it has been boiled.

RESULTS AND DISCUSSION

In this comparative study were obtained two types of groundwater in the country, Lajthiza and Bovilla water. Samples taken are taken from the source, the beginning of the technological process in the middle of the process and its conclusion. Chemical analysis carried enable the identification of fecal contamination originated, those organic and inorganic type; Such information is obtained by performing analysis for the determination of organic substances, ammonium chloride (organic pollution in action), nitrites and nitrates and inorganic elements that may be present due to infiltration of emissions coming from the surrounding industrial areas.

The quality of drinking water originating from Bovilla reservoir regularly followed by two laboratories, chemical and microbiological, set up near the treatment plant. These two labs perform regularly once or twice a day for about 19 tests physico-chemical parameters and six microbiological parameters (Schedule 1). In each laboratory carries untreated water and treated water; also measured several times a day some of the main parameters of water in the main stages of water treatment performance, the introduction, partitor, filters and exit the facility. At the same time, the electronic monitoring devices for untreated water and treated, automatic measured parameters, such as pH, temperature, turbulent, conductivity, chlorine free; data reflected in the central server, which supervises the entire process automatic water purification technology. We note that the methods used by us are in accordance with European standard 80/778 and STASH Albanian Standard 3904: 1997 (Decision 145, 1998) (Schedule 1, 2 and 3), the quality of water consumed by man.

Specific Bacteria

E.coli	250 ml	negative
Coliform bacteria	250 ml	negative
Fecal streptococci	250 ml	negative
Pseudomonas aeruginosa	250 ml	negative
Sulfite reducing sporeforming anaerobes	50 ml	negative

Schedule 1 – Physico-chemical evaluation of Bovilla water

Parameters	Measurement Unit	Normal	Untreated water of Bovilla	Treated water of Bovilla
Water temperature	^{0}C	8-15	11.7	10.93
Smell / taste	Normal	Acceptable	Normal	Normal
Concentration of H^+ ions	pH unit	6.5-8.5	8.15	7.89
Turbidity	FTU (NTU)	< 1.0	4.31	0.18
Electrical conductivity	µs/cm 20 ^{0}C	2500	280	340
The total alkalinity	mg/l $CaCO_3$		144.5	144.5
Total hardness	^{0} German	10-20	7.92	7.92
Free residual chlorine	mg/l Cl_2	0.5	-	0.76
Dissolved oxygen	mg/l O_2	≥ 8	8.70	10.50
Organic substances $KMNO_4$	mg/l O_2	5	1.07	0.55
Calcium	mg/l Ca	200	38.20	38.16
Chlorides	mg/l Cl^-	250	6.02	12.76
Sulphates	mg/l SO_4^{2-}	250	24.90	25.10
Ammonia	mg/l NH_4+	0.1	0.03	0.00
Nitrites	mg/l NO_2^-	0.5	0.01	0.00
Nitrates	mg/l NO_3^-	50	0.94	0.96
Phosphates	µg/l P_2O_5	-	48.96	20.60
Sulfides	µg/l H_2S	-	8.54	0.00
Aluminium	mg/l Al^{3+}	0.2	0.015	0.010
Iron	µg/l Fe	200	47.7	0.00

Drinking water plant Bovilla - Tirana, chemical water analysis (CMD .Nr 379

Data from monthly averages in Schedule 1 note that untreated water of Bovilla stands for the highest content of nitrite; all seasons, at 7-14 °C temperature ranges, features which is very acceptable especially in the hot summer season. The data of Schedule 2 and 3 shows that Lajthiza water has contents higher carbonates, of ammonium and organic matter during the hottest months, which when crossing the technological process of infiltration, all these indicators move on rates allowed standard.

Details of the above statements (Schedule 1, 2 and 3) indicate that:

T - The water temperature in the two measurements performed on two sources: Bovilla-Lajthiza ranging in T = 7.73 - 16.5 ^{0}C, this indicator is within the norm (norm 8-15 to 20 0C). pH of groundwater monitoring sources ranging: Lajthiza water from 7.1-7.4 to 7.89 - 8.15 of Bovilla water. The rate allowed for drinking water this indicator is within the norm: (pH = 6.5-8.5). Changes in two sources varies: 0.03 to 0.07 . Normal pH has Lajthiza water based on the final results.

Fp - Total Hardness Fp in water Lajthiza ranging 3.36 - 6.27 º German, while Bovilla water ranging in 7.92-8 º German. The water is within drinking water standard (optimal rate is 10-20 º Germany, where the maximum allowed content is 25 º German).

Ca - The content of calcium in Lajthiza water ranging 17.04 – 34.13 mg/l, while in Bovilla water content of Ca ranging in 38.16 - 38.20 mg/l. Boville water remains after processing of higher amounts of Ca, compared with Lajthiza water.

Schedule 2 – Lajthiza water in May, June and July

Parameters	Measurement Unit	Norma	DEPOT	Filter 1 mm	Filter 0.2mm	Final product
Water temperature	^{0}C	8-15	14.6	13.5	13.5	13.5
Smell / taste	Normal	Acceptable	Normal	Normal	Normal	Normal
Concentration of H^+ ions	pH Unit	6.5-8.5	7.3	7.2	7.2	7.4
T**ΔS**	-	-	60	70	60	60
Total Hardness	0 German	10-20	5.36 ^{0} Water	4.42 ^{0} Water	4.39 ^{0} Water	6.27 ^{0} Water
Calcium	mg/l Ca	200	42.1	38.2	29.7	16.7
Chlorides	mg/l Cl^-	250	14.07	13.6	12.9	1.1
Sulphates	mg/l SO_4^{2-}	250	12.6	11.38	9.5	9.5
Ammonia	mg/l NH_4+	0.1	0.0089	0.00	0.00	0.00
Nitrite	mg/l NO_2^-	0.5	0.0016	0.001	0.00	0.000
Nitrate	mg/l NO_3^-	50	0.98	0.86	0.67	0.60
Magnesium	mg/l mg	125	2.43	2.43	2.43	2.9
Hydrogen carbonate	mg/l HCO_3^-					57
carbonate	mg/l CO_3^{2-}	-	85.4	73.2	71.3	3
Iron	µg/l Fe	200	38,5	23.6	0.00	0.00
Organic substances	$KMnO_4$	5	066	036	0.12	0.01
Phosphates	µg/l P_2O_5	-	48.97	13.60	0.045	0.04
Sulfides	µg/l H_2S	-	8.54	0.006	0.0055	0.005
Aluminum	mg/l Al^{3+}	0.2	0.016	0.040	0.0055	0.005

Fe - The content of iron in two sources is: 38.5 - 0.00 µg/l Fe in Lajthiza water, and 47.7-0 µg/l Fe in Bovilla water. The final content is slightly above the recommended rate in the second stage of monitoring.

NH_4 - In the first stage of obtaining water from the water source of Lajthiza the ammonia content is 0.01 mg/l; content is below the max allowed: 0.05 mg / l. In the second post-processing phase the content of NH_4 is 0.00 mg / l. In the first phase the content of ammonia NH_4 in Bovilla water is 0.015 mg/l, while the ammonia content after production is 0.011 mg/l, above the recommended content.

Schedule 3 - Lajthiza water in December, January and February

Parameters	Measurement Unit	Norma	DEPOT	Filter 1 mm	Filter 0.2 mm	Final product
Water temperature	^{0}C	8-15	5-12	12.5	12.8	13.6
Smell / taste	Normal	Acceptable	Normal	Normal	Normal	Normal
Concentration of H^+ ions	pH Unit	6.5-9.5	7.1	7.06	7.09	7.1
T**ΔS**	-	-	60	70	60	60
Total Hardness	0 German	10-20	3.36 ^{0} Water	3.36 ^{0} Water	3.36 ^{0} Water	5.32 ^{0} Water
Calcium	mg/l Ca	200	36.2	33,04	29.1	16.8
Chloride	mg/l Cl^-	250	12.05	12.05	12.05	1.0
Sulphate	mg/l SO_4^{2-}	250	11.62	10.37	0.00	7.9
Ammonia	mg/l NH_4+	0.1	0.0074	0.00	0.00	0.0
Nitrite	mg/l NO_2^-	0.5	0.0015	0.001	0.00	0.0
Nitrates	mg/l NO_3^-	50	0.98	0.86	0.67	2.7
Magnesium	µg/l H_2S	125	2.83	2.76	2.43	3.0
Bicarbonate	mg/l Al^{3+}	-	75.4	68.2	88.3	3
Hydrogen carbonate						59
Iron	µg/l Fe	200	37,5	21.7	0.00	0.00
Organic substances	$KMnO_4$	5	066	036	0.12	0.12
Phosphates	µg/l P_2O_5	-	48.97	13.60	0.05	0.04
Sulfides	µg/l H_2S	-	8.54	0.006	0.006	0.005
Aluminum	mg/l Al^{3+}	0.2	0.016	0.040	0.016	0.005

Cl - The content of chlorine in Lajthiza water is 0.00 mg/l. In Bovilla water the content of Cl^- ranging from 6.02 - 12.76 mg/l. Cl^- content is much higher than in Lajthiza water.

SO_4 - The content of sulfates in Lajthiza water after processing reaches in 9.5 mg/l. In Bovilla water the content of sulphate is 24.9 mg/l. Sulphate content is within the recommended standard.

NO_3 - Nitrate content in Lajthiza water is small and varies between 0.98-2.7 mg/l. In Boville water the nitrates content varies between 0.94-0.96 mg/l. NO_3 content is within the recommended standard.

Analysis for microelements: Ni, Mn, Zn, Pb, Cu, Co, Cr.Cd. In this analysis has microelements content in two stages of monitoring: Ni = 0.005 - 0.002 mg/l, Mn = 0 - 0.001mg/l, Zn = 0.002 - 0.007 mg/l, Pb = 0 - 0.03 mg/l, Cu = 0.005 - 0.001 mg/l, Co = 0.007 - 0.04 mg/l, Cr = 0.045 – 0.000 mg/l, Cd = 0 - 0.001 mg/l.

CONCLUSIONS

Based on physical-chemical indicators in general result that those waters are suitable to be used for consumption. On the basis of studies conducted (Schedule 1, 2 and 3), referring to

the physical and chemical indicators, it turns out that the source country waters Lajthiza are suitable to be used for children.

During the study two sources of origin of the natural mineral waters of the country, Bovilla and Lajthiza, was met comparative evaluation between indicators of Marketing (TSM), bottled water (UAA) and the origin (UBA). Referring indicators environment (pH) of Lajthiza water, we can say that they have a pH value within EU norms which are 6.5 - 8.5, while refer to the content of salts, residue that dry, they have a content ranging from 120-220 mg/l (standard referred to 0-1500 mg/l). The exception is Bovilla water here,, where the salt content reaches up to 960 mg / l.

Another indicator of good quality of water Lajthiza is the indicator of strength. In general, natural mineral waters of our country have a low hardness, ranging from 6-11 German hardness grade, where the standard rate is 8-15 German hardness degrees. As such, they can be considered as water with low hardness. As such, they can be considered as water with low hardness. In the geological context, our waters are generally natural mineral water in the limestone cliffs descent, as it is the source of Lajthiza. So, in waters originating from limestone rocks and limestone-dolomite are rich in ion Ca^{+2}, $S0_4^{2-}$ while those with volcanic origins are rich in ion Mg^{+2} and $HC0_3^{-2}$.

The study conducted shows that in general there is an alignment between marketing indicators (label) and the packaged and fountain. Physico-chemical characteristics, microbiological and generally they are within standards.

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COLISTIN RESISTANT *ESCHERICHIA COLI* AND MCR GENE BY PLASMID-MEDIATED RESISTANCE

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ABSTRACT

The problem of antimicrobial resistance, which maintains its importance throughout the world, has once again attracted attention with its resistance to Escherichia coli isolates isolated from humans and animals. Colistin is the most effective antibiotic against carbapenem resistant Gram-negative bacteria. While the use of colistin was limited for veterinary treatment, colistin resistant Gram-negative bacteria were found at high rates in animal isolates. In humans, the use of colistin has been limited due to the impairment of renal function, which is now frequently used in the treatment of infections caused by multiple antibiotic resistant Gram-negative bacteria. The development of plasmid-mediated resistance provided by the mobilized colistin resistance (mcr-1) gene has further increased the importance of colistin, while the resistance of the colistin is thought to cause only long-term chromosomal mutations. Since the introduction of the mcr-1 gene in China in 2015, this gene has been identified in a variety of bacterial strains isolated from animals, animal food products, humans, and environmental samples. In this review, up-to-date information on the resistance of the colistin will be given.

Keywords: *E. coli, mcr, Colistin, Antimicrobial resistance*

INTRODUCTION

Recent years of metagenomic and genomic studies have revealed that antibiotic resistance genes are common. New and highly different genetic resistance genes can be identified in human, animal, agricultural land (animal faeces, soil, water and waste water systems etc.). The antibiotic resistance genes obtained from metagenomic libraries of human, chicken, pig, gull and cattle stool specimens were different from those described previously. The transferable colistin resistance gene is also defined in this way. The emergence of this resistance gene may be an important risk factor for the use of colistin in animals for treatment or prophylaxis. For this reason, there is a significant tendency to limit the use of colistin in animal production worldwide (Sun et al., 2017).

Colistin, also known as Polymyxin E, is an antibiotic of the polymyxin group produced by the *Paenibacillus polymyxa* bacterium. Polymyxin E is a polycationic peptide having both hydrophilic and lipophilic sites. The cationic regions interact with the bacterial lipopolysaccharide (LPS) layer in the outer cell membrane, which changes magnesium and calcium ions. Colistin is used topically in human medicine. However, it has been widely used in clinical medicine in previous years. Since

colistin causes significant nephrotoxicity, its use is limited (Caniaux et al., 2017). In recent years, it has been used as the last choice in the treatment of Gram-negative bacteria such as *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* with multiple antibiotic resistance (Sun et al., 2017).

Colistin has been used as a feed additive in the Asian and European countries, as well as for treatment and prophylactic purposes. Although approved by the FDA in the United States, it is not used in farm animals. It has been used as animal feed additive for body weight gain in livestock in Asian countries such as China, India, Japan and Vietnam (Kempf et al., 2016). It is frequently used in the treatment of infections in pigs and especially cattle in veterinary medicine. In Europe, it has been used to treat infections, which is caused by Enterobacteriaceae, of pigs, chickens, cattle, sheep and goats (Catry et al., 2015).

In recent years, the use of colistin is limited because of causing kidney toxicity in humans. It has begun to be resumed as a last opportunity in the treatment of carbapenem resistant bacteria recently. It is used effectively in the treatment of infections caused by bacteria such as *P. aeruginosa*, *Escherichia coli* and *Klebsiella* spp. It is also preferred in the prophylactic treatment of patients with respiratory tract infections and intensive care units (Caniaux et al., 2017).

Colistin resistance and recent emergence of mcr genes

Common use of colistin in food animals has been recognized as an important risk factor for the emergence and transmission of the plasmid-mediated colistin resistant gene *mcr-1*. Particularly in China, the use of colistin intensively in animal production may have brought to fruition of this resistance. Therefore, it has been proposed to reconsider and regulate the use of colistin in animal production (Rhouma et al., 2016). Brazil and China have forbidden the use of colistin as a feed additive seven months after the discovery of the *mcr-1* gene (Walsh and Wu, 2016, Sun et al., 2017).

Although classical resistance to colistin is rare, the SENTRY antimicrobial surveillance program identified the presence of a plasmid-mediated colistin resistance mechanism involving the *mcr-1* gene *E. coli* and *K. pneumoniae* between 2014 and 2015. *mcr-1* gene has been isolated from animal, human, and environmental samples (Liu et al., 2016), subsequently, its genetic variants (*mcr-2*, *mcr-3*, *mcr-4* and *mcr-5*) were identified. This plasmid-mediated gene has caused horizontal migration of the colistin resistance. The increase and spread of this resistance among bacteria in the Enterobacteriaceae family has reinforced worries (Xavier et al., 2016; Yang et al., 2017; Yin et al., 2017, Bardet et al., 2017).

Recent epidemiological studies of resistance to colistin have shown that *mcr-1* positive Enterobacteriaceae isolates are quite common worldwide in various animal hosts. Liu et al. (2016), first reported 21% and 15% *mcr-1* isolation in *E. coli* from pigs and raw meat in China. Also, the 1611 *E. coli* strains isolated from chickens between 1970 and 2014 were detected as only three *mcr-1* positive isolates (Shen et al., 2016)., 4438 *E. coli* isolates, which was isolated animal origin foods were investigated for colistin resistance gene *mcr-1* in another study conducted in China. Colistin resistance was detected in 16.8% of the *E. coli* isolates obtained from pigs and chickens in the years 2013-2014. It was found to be higher than those isolated in years 2007-2008 (5.5%) and 2010-2011 (12.4%) (Huang et al., 2017). In other studies, conducted in China, *mcr-1* positivity was found that 10.8% (10/93) of *E. coli* isolates producing Shiga toxin (Bai et al., 2016), 23.0% of carbapenem resistant *E. coli* isolates isolated from poultry (Wang et al., 2017), 5.11% of *E. coli* strains isolated from chickens (Yang et al., 2017).

High *mcr-1* positive *E. coli* was found in animal and food origin isolates, especially in turkeys (10.7%) and chickens (5.6%) in Germany (Irrgang et al., 2016). High colistin-resistance (45%) and *mcr-1* positive (13%) *E. coli* were reported in pig isolates in Japan (Kusumoto et al., 2016). In another study conducted in Japan, however, the ratio of colistin-resistant (1%) and *mcr-1* positive (0.02%) *E. coli* strains isolated from healthy animals was found to be low (Suzuki et al., 2016). In Vietnam, high *mcr-1* ratio (59.4%) was detected in stool specimens taken from chickens. This suggests that colistin is associated with the emergence and spread of *mcr-1* bearing bacteria because

of widespread use in poultry production (Trung et al., 2017). Although the *mcr-1* positivity in *E. coli* in Korea is low (0.1%), it has been determined that the prevalence of *mcr-1* has increased since 2013 (Lim et al., 2016).

mcr-1 positivity was reported in pigs and animal origin foods in the USA by 0.35% and 0.1%, respectively. The most interesting finding in this study is the detection of two *mcr-1* positive *E. coli* isolates from pigs. Because, colistin was not used as a treatment or feed additive in animals in the USA (Meinersmann et al., 2017). *mcr-1* positive *E. coli* isolates have been identified in South America since 2012. In Brazil, isolates of chickens (5%) and pigs (1.8%) were reported to carry the *mcr-1* gene, although some of them are colistin-sensitive (Fernandes et al., 2016). In Venezuela, two *E. coli* isolates isolated from pigs were found to be *mcr-1* positive (Delgado-Blas et al., 2016).

In South Africa, colistin resistant avian-pathogenic *E. coli* isolates increased from 4.5% in 2008-2014 to 13.6% in 2015 (Perreten et al., 2016). Only one *E. coli* isolate isolated from cattle in Egypt was found to be *mcr-1* positive (Khalifa et al., 2016).

It has been reported that *mcr-1* carrying *E. coli* and *K. pneumoniae* which is isolated from blood, urine samples of patients, and that all strains are expanded spectrum β-lactamase producing bacteria (Liu et al., 2016; Du et al., 2016). Colistin resistance, which can be transferred in NDM-1 producing carbapenem resistant Enterobacteriaceae strains, was found to be 10% in the United Kingdom. In Italy, the *K. pneumoniae* isolates had a colistin resistance of 57%, and the majority of these *mcr-1* strains had a low minimum inhibitory concentration (MIC) value (4 µg / mL) (Caniaux et al., 2017). Among the enterobacterial isolates isolated from urinary tract infections in Switzerland, the *mcr-1* gene was found to be low (Liassine et al., 2016), also Enterobacteriae which is isolated bacteraemia of the *mcr-1* gene was not detected (Nordmann et al., 2016a).

329 Enterobacteriaceae isolates for *mcr-1* and *mcr-2* genes has investigated by Sarı et al., (2017) in Turkey, and found that the isolates did not carry these gene regions. Similarly, Guducuoglu et al. (2018) investigated *mcr-1*, *mcr-2*, and *mcr-3* genes in carbapenem-resistant Gram-negative bacteria, indicating that isolates do not carry these gene regions.

In addition to the *mcr-1* gene, plasmid-mediated colistin resistance *mcr-2*, which is 76.7% similar to this gene, was detected in *E. coli* isolates from bovine and porcine origin in Belgium (Xavier et al., 2016). Its prevalence was found to be higher (20%) than the isolates carrying the *mcr-1* gene (13%). This gene was not detected in other studies of Germany and Switzerland where it was investigated (Roschanski et al., 2017; Buess et al., 2017). *mcr-3*, which is a 45% nucleotide analogue to the *mcr-1* gene and 47% nucleotide analogue to the *mcr-2* gene, is another colistin resistant gene was identified in porcine *E. coli* isolates in China (Yin et al., 2017). Fukuda et al. (2018) reported that *mcr-3* positivity of 8.3% in isolates of 120 isolates from pigs. One of the carbapenem-resistant *E. coli*, which is isolated from cattle, in Spain was found to carry both *mcr-1* and *mcr-3* genes (Hernández et al., 2017; Kluytmans, 2017). *mcr-4* gene, which is initially detected in *Salmonella enterica* serovar *Typhimurium* strain of a pig in Italy, and later also followed in three *E. coli* isolates is described by Carattoli et al. (2017) and Teo et al. (2018). Borowiak et al. (2017) identified *mcr-5* gene as a novel phosphoethanolamine transferase gene in *Salmonella Paratyphi* B dTa+ isolate 13-SA01718 with whole genome sequencing. This gene region was also identified in *E. coli* isolates from pigs in subsequent studies (Fukuda et al., 2018; Hammerl et al., 2018).

Epidemiological studies on the emergence of *mcr-1* mediated colistin and *mcr*-like genes in animals show that it is a selective agent of the use of colistin in food animals and contributes to the emergence and transmission of *mcr-1*. Colistin is not absorbed in the gastrointestinal tract of animals (Rhouma et al., 2016). Therefore, the accumulation of colistin or its metabolites in faeces may significantly increase the colistin-resistant, *mcr* carrying bacteria in agricultural ecosystems. Some studies also have shown that international trade in animal commerce or exotic animals, such as reptiles, is a significant influence on the spread of *mcr-1* mediated colistin resistance (Grami et al., 2016; Unger et al., 2017).

Detection Methods in Clinical Laboratory of mcr Resistance E. coli

Tests used to determine colistin resistance can be grouped as phenotypic and genotypic. Phenotypic tests reveal the presence of colistin resistance in Enterobacteriaceae and nonfermentative Gram-negative bacteria. All molecular tests have been designed to identify the *mcr-1* gene and other variants, regardless of phenotypic colistin resistance especially in the Enterobacteriaceae, (Caniaux et al., 2017, Osei Sekyere, 2018).

Chromogenic media (CHROMagar COL-APSE, SuperPolymyxin™ and LBJMR etc.) have been developed to identify polymyxin-resistant Enterobacteriaceae or Gram negative non-fermentative bacteria from environmental factors as well as clinical samples such as stools and blood. SuperPolymyxin™ has been shown to have 100% sensitivity and specificity. In particular, it can detect polymyxin resistance both direct isolates and clinical specimens such as feces or rectal swabs. Such mediums allow rapid and easy identification of the colistin resistance bacteriae. It has an important place in prevalence studies both human and veterinary medicine (Caniaux et al., 2017; Osei Sekyere, 2018).

Disk diffusion test and E-test are inadequate to detect colistin resistance after evaluation by European Committee on Antimicrobial Susceptibility Testing (EUCAST). Currently, only a broth microdilution method (BMD) is recommended as a reliable test for to detection of colistin resistance, although it is laborious and time-consuming (Caniaux et al., 2017, Osei Sekyere, 2018). EUCAST has determined the clinical cut-off limits of the colistin for Enterobacteriaceae, *Pseudomonas aeruginosa* and *Acinetobacter* spp. as sensitive ≤2mg/l, resistant >2mg/l (EUCAST, 2018). Clinical and Laboratory Standards Institute (CLSI) has determined the clinical cut-off limits for *Pseudomonas aeruginosa* and *Acinetobacter* spp. (susceptible ≤2mg/l, intermediate = 4mg/l, and resistant ≥8mg/l) (CLSI, 2017). In addition, CLSI has determined 2 mg/L epidemiological cut-off values for *Escherichia coli*, *Klebsiella pneumoniae*, *Raoultella ornithinolytica*, *Enterobacter aerogenes* and *Enterobacter cloacae* from the wild-type and other isolates (Chew et al., 2017; Vasoo, 2017).

Rapid diagnostic kits and automation systems have been developed by different companies to identify the colistin resistance. The sensitivity and specificity of these tests and automation systems are shown in Table 1 (Caniaux et al., 2017; Osei Sekyere, 2018).

Real-time PCR, multiplex PCR (mPCR), CT103XL microarray and loop-mediated amplification (LAMP) assays have been used to identify *mcr* genes in culture, clinical and stool samples (Caniaux et al., 2017; Osei Sekyere, 2018)

Table 1. Tests of identify *mcr* genes and colistin-resistant Gram-negative bacteria*

Diagnostics	**Species**	**Sensitivity (%)**	**Specificity (%)**	**References**
Screening and culture-based methods				
Broth microdilution (BMD)	Enterobacteriaceae	71.4 - 81.0	NS	Chew et al., 2017
CHROMagar COL-*APSE*	Enterobacteriaceae, Gram-negative non-fermenters	100	100	Abdul Momin et al., 2017
LBJMR media	Enterobacteriaceae, Gram-negative non-fermenters	100	100	Bardet et al., 2017
MIC Test strip® (MTS) (Liofilchem, Italy)	Enterobacteriaceae, *P. aeuginosa*, Acinetobacter spp.	NS	NS	Matuschek et al., 2018
MICRONAUT MIC-Strip® (MERLIN Diagnostika Gmbh, Germany)	Enterobacteriaceae, *P. aeuginosa*, *Acinetobacter spp*.	NS	NS	Matuschek et al., 2018

SensiTest™Colistin (Liofilchem, Italy)	Enterobacteriaceae; Gram-negative non-fermenters	NS	NS	Esposito et al., 2017
SuperPolymyxin™	Enterobacteriaceae, Gram-negative non-fermenters	86.0 - 100.0	100.0	Carretto et al., 2018; Matuschek et al., 2018
UMIC (Biocentric, France)	Enterobacteriaceae, *P. aeuginosa*, *Acinetobacter* spp., Gram-negative bacilli	NS	NS	Abdul Momin et al., 2017; Bardet et al., 2017; Nordmann et al., (2016a,b)
Automated commercial MIC testing platforms				
MICRONAUT-S (MERLIN Diagnostika Gmbh, Germany)	Enterobacteriaceae, *P. aeuginosa*, Acinetobacter spp.	NS	NS	Matuschek et al., 2018
MicroScan	Enterobacteriaceae, Gram-negative bacilli	100	NS	Chew et al., 2017; Jayol et al. 2018
BD Phoenix/ Phoenix 100™ (Becton Dickinson Diagnostics, USA)	Enterobacteriaceae; Gram-negative non-fermenters	91.87k	NS	Jayol et al., 2017a; Jayol et al., 2017b; Carretto et al., 2018
Sensititre™ (ThermoFisher Diagnostics)	Enterobacteriaceae, *P. aeuginosa*, *Acinetobacter spp*., Gram-negative bacilli	95.2 - 100	NS	Chew et al., 2017; Jayol et al., 2018; Matuschek et al., 2018
Vitek 2 (BioMerieux)	Enterobacteriaceae	95.2	NS	Chew et al., 2017
Molecular methods				
Conventional PCR	Enterobacteriaceae	100.0	100.0	Imirzalioglu et al., 2017; Jayol et al., 2017a; Jayol et al., 2017b; Bernasconi et al., 2017; Esposito et al., 2017; Abdul Momin et al., 2017
Multiplex PCR	Enterobacteriaceae	100.0	100.0	Osei Sekyere, 2018; Rebelo et al., 2018
Real-time PCR	Enterobacteriaceae and non-fermenters	100.0	100.0	Nijhuis et al., 2016
Whole-genome sequencing	Enterobacteriaceae	100.0	100.0	Bernasconi et al., 2017; Imirzalioglu et al., 2017; Rebelo et al., 2018

* This table has summarized from the studying of Osei Sekyere, (2018); NS: Not specified.

CONCLUSIONS

The identification of colistin-resistant genes is important to understand the development and molecular basis of the colistin resistance and to contend with this resistance. The discovery of new resistance genes by effective molecular assays will provide us with more accurate and precise information as it will improve the described mechanisms.

Nowadays, there is a worldwide trend to limit or even ban the use of colistin in animal production. Clearly, limiting the use of colistin is expected to greatly reduce selective pressures and thus control the plasmid-mediated colistin resistance. However, limiting or prohibiting colistin in animal production will not fully solve this important problem. Because the problem of antibiotic resistance is complex and can be affected by many different causes. Despite the fact that *mcr-1* positive *E. coli* isolates have been identified in the USA, it has been shown that colistin resistance may be affected by external factors. A comprehensive examination of these non-colistin factors will provide effective strategies and preventive measures to reduce the colistin resistance in the animal production system worldwide. Due to the diversity and complexity of the mechanisms associated

with polymyxin resistance, it is highly difficult to develop innovative, accurate and rapid tests that are clinically important. Simultaneous detection of carbapenem resistance should be considered to make the researches on colistin resistance clinically more useful.

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IMPACT OF VINIFICATION TECHNIQUES ON THE CONTENT OF POLYPHENOLIC COMPOUNDS OF WHITE WINE FROM CV. PULËZ DURING TWO DIFFERENT VINTAGES

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Abstract

Wine production techniques and agronomic practices influence the flavors and bouquet of white wines. Various studies have shown that these factors influence the content of phenolic compounds that are essentially responsible for the taste and aroma of wine. The purpose of this study is to investigate the influence of vinification tecniques in dynamic changes of phenolic compounds in white wines produced in two differente vintages. For this study, was taken a autochthonous white grape, Pulëz, cultivated in Berat area. For the production of wines were followed two different vinification schemes (fermentation with and without skins). Prior to fermentation, the must was macerated at 5°C for 24 hours. *Saccharomyces bayanus BC* commercial yeast was used for both fermentations. Every three days the phenolic compounds of wines were analyzed by spectrophotometric methods, such as the index of polyphenols, flavonoids and color parameters. The obtaned data were subject to ANOVA statistical analysis. The obtained results shows that vinification techniques gives a higher values in the index of polyphenols, flavonoids and color parameters with a significant difference with $P \leq 0.05$ Test Tukey. While different vintages have not significant differences in polyphenolic components. Based on the results of this study, we conclude that the application of two vinification techniques influences the increase of these constituents especially when vinification is carried out in the presence of the skin.

Keywords: *White wine, Fermentation, Polyphenols, Flavonoids.*

INTRODUCTION

Phenolic compounds are important constituents of white wine that can affect its stability and color. Many phenolic compounds in white wine derive from grapes; and specific phenolic compounds are found in different concentrations in different grape tissues (pulp, membrane and seeds) (Nordestgaard, 2011; Silva et al., 2005; Ivanova et al., 2012). It is generally accepted that these compounds are of great importance for the varietal character of a wine (Ugliano et al., 2006, Zafrilla et al., 2003; Gil-Muñoz et al., 1999). For these reasons, the application of

various grape processing techniques, especially the method by which the juice extracted, may affect the phenolic profile of white wines.
The process of fermentation with skins for a variable time, with the purpose of extracting poliphenols, stimulates the increasing of their quantity that improves the body and the flavor of white wine (Olejar et al., 2015; Olejar et al., 2016; Ramos et al., 1999).
Nowadays, the interest in phenolic compounds has been increased mainly due to their different chemical structure and biological activity in the prevention of same chronic or severe diseases (Soto Hernandes et al., 2017; Mitić et al., 2010). Many studies have shown that phenolic compounds in wine may have the potential to naturally prevent same major diseases such as cancer, cardiovascular and neurodegenerative diseases such as Parkinson and Alzheimer's, this is also one of the reasons for applying new vinification techniques to in order to increase the quantity of these ingredients in white wine (Aguilera et al., 2016; Österbauer et al., 2005).

In recent years, different versions of fermentation with skins in white wine have been studied to obtain wines of interesting and distinct types (Olejar et al., 2016, Cadota et al., 2012). Such technologies have been proved successful in obtaining complex flavors and aromas, potential increased antioxidant phenolic activity, and improved maturation and stability (Aleixandre-Tudo et al., 2015; Lomolino et al., 2010).

The main purpose of this study is to evaluate the dynamics of changes at the content of polyphenols, flavonoids and colors during fermentation (with and without skins) of Pulëz variety (*Vitis vinifera* L.) in two different vintages. The presence of skins during fermentation will increase the extraction of polyphenols in white wine, so the study of these phenolic parameters is of great importance as it will contribute to the evaluation and application of new production techniques of white wine.

MATERIALS AND METHODS

Vinification

A grape variety (Vitis vinifera L.) were obtained to carry out this study, Pulëz (from Berat area) in two different vintages 2015 and 2016, harvested at optimal ripeness and conditions within the quality parameters according to Table 1. These samples were transported to the Food Research Center of Biotechnology and Food Faculty.

The amount of grape taken in this study was 100 kg in each vintage, which was split into two 50 kg lots. The experiments carried out in this study were in two schemes of fermentations, with (Pulëz 2) and without (Pulëz 1) skins. The experimental tests were treated with a dose of 5g/hl SO_2 and placed in cold maceration for 24h at 5°C. After cold maceration, Pulëz 1 (fermentation without skins) of both vintage was pressed. The obtained juice was added 2 g/hl of pectolytic enzyme for better rendering and left for static decantation (48h at 5°C). Subsequent withdrawal of the juice from the lees and inoculation of 20 g/hl of S. bayanus BC yeast and fermented at controlled temperature of 14-18ºC for 12 days.

The other experimental test of Pulëz 2 (fermentation with skins) of both vintages after the maceration was left at rest until it reached room temperature. The juice was inoculated with 20 g/hl dry yeast S. bayanus BC and fermentation was done with skins until its completion (12 days). Fermentation performance was checked every day (% of sugar and temperature) and every three days samples were taken for analysis of total polyphenols index, total flavonoids content and color intensity. Samples prior to analysis were centrifuged to remove solid suspensions.

Determination of total poliphenols index (TPI).

The total polyphenols index was determined by Cetó, it is a spectrophotometric method. Samples were diluted and the absorbance was measured at 280 nm, characteristic of benzoic acid present in all polyphenols (Cetó et al., 2012).

Determination of total flavonoid contents (TF).

The total flavonoid content was determined by spectrophotometric method (Zhishen et al., 1999). This method based on the formation of complex flavonoid-aluminum. At 510 nm was measured the absorbance. The concentration of the total flavonoid compounds in the wines was expressed as catechin equivalent (mg/L). The samples were analyzed in triplicate**.**

Determination of color intensity.

Chromatic characteristics of the wines were determined by spectrophotometric method. The color intensity was calculated as the absorbance measured at 420 nm. The color intensity assessment refers to the numerical value determination of the white wine chromatic characteristics (Beccheti, 1999).

Statistical analysis

Samples were analyzed in triplicate. The analysis of the ANOVA has been carried out to study the effect of each of the treatments on the content of the total polyphenol index, total flavonoids and color intensity of the white wine studied. To establish differences between means, the Tukey Test ($P \leq 0.05$) was applied. The Statistix program, Version 9.0 (Analytical Software) has been used.

RESULTS

During this study, an autochthonous variety Pulëz (from the Berat area) was taken in consideration in two different vintages 2015 and 2016. We have work with two different fermentation schemes with and without skins, for the extraction of polyphenols in white wine. The quality parameters of the grape used (Pulëz variety) are shown in the following table:

Table 1. Quality parameters of grape var. Pulëz in two different vintages (2015-2016)

Variety	Sugar °Brix	Total Acidity (g/L tartaric acid)	pH
Pulëz (Berat 2015)	22.09 ± 0.01[a]	3.62 ± 0.01	3.3 ± 0.02
Pulëz (Berat 2016)	21.19 ± 0.00	4.89 ± 0.03	3.46 ± 0.05

a – Mean + SD

As can be seen, from the table 1, in vintage 2016, the maturity indicator is slightly lower than in vintage 2015, this is seen in the lowest sugar content and the highest content of total acidity and pH. This change is due to climatic conditions in the two years.

Total Polyphenols Index

Evolution of the total polyphenol index (TPI) values is shown in Table 2. As can be seen from the table the value of the TPI during both vintages is doubled in fermentation with skins.

Table 2. Extraction of polyphenols during the fermentation performance with and without skins, in two different vintages (2015-2016) for Pulëz grape variety.

Variety	Vintage	Days of Fermentation	Total Polyphenol Index
Pulëz 1*	2015	0	10.27 ± 0.53[a]
		3	19.25 ± 0.67
		6	13.37 ± 0.45
		9	13.05 ± 0.78
		12	14.45 ± 1.59
Pulëz 2*	2015	0	10.27 ± 0.53
		3	14.65 ± 2.29
		6	27.35 ± 0.5
		9	32.63 ± 0.5
		12	28.80 ± 0.5
Pulëz 1	2016	0	4.95 ± 1.22
		3	5.91 ± 0.11
		6	6.95 ± 0.05
		9	3.35 ± 0.13
		12	4.81 ± 0.11
Pulëz 2	2016	0	4.95 ± 1.22
		3	8.15 ± 0.04
		6	9.33 ± 0.05
		9	9.93 ± 0.06
		12	11.86 ± 0.02

1- without skins, 2 – with skins, a – Mean + SD

Thus, the maximum value of TPI during 2015 in fermentation without skins is 14.45 while in fermentation with skins this value increases to 28.8. During 2016 the maximum values of TPI in fermentation without skins is 4.81, whereas in fermentation with skins this value increases to 11.86.

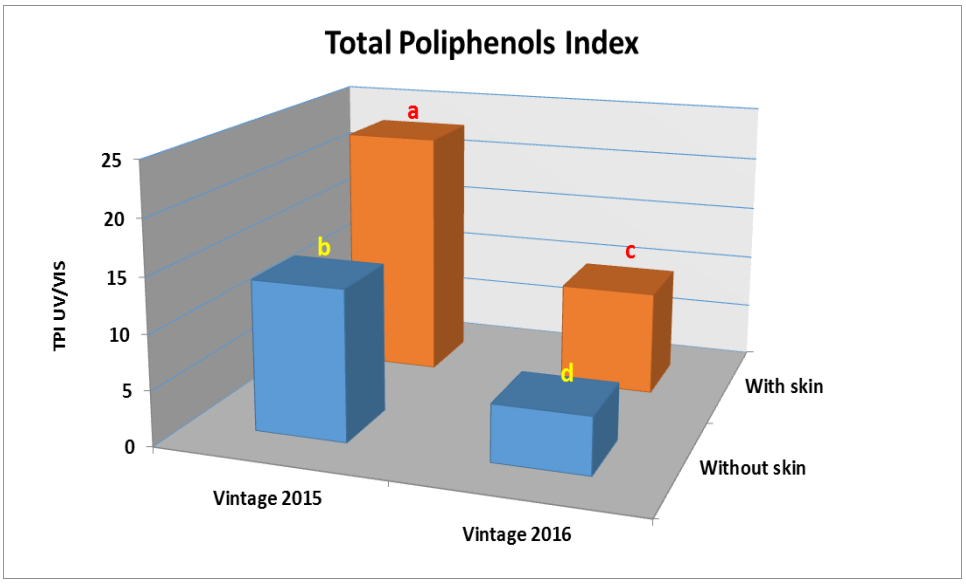

Figure 1. Performance of the TPI of white wine from Pulëz grape variety in fermentation with and without skins during two different vintages 2015 – 2016 (Tukey Test, $P \leq 0.05$).

From the statistical analysis Anova factor design was found that the fermentation scheme with skins generally represented a significant difference of $P < 0.05$ for both vintages. This difference for the total polyphenol index is shown in Figure 1.

Total Flavonoids

The table below shows the values of the total flavonoid (TF) content during the white wine fermentation process for two vintages. From the table it is noted that the flavonoids content is doubled in fermentations with skins, for both vintages.

Table 3. Extraction of total flavonoids during fermentation performance with and without skins in two differences vintage (2015-2016) for Pulëz grape variety.

Variety	Vintage	Days of Fermentation	Total Flavonoids (mg/L)
Pulëz 1*	2015	0	261.87 ± 2.52[a]
		3	171.02 ± 0.55
		6	179.68 ± 4.46
		9	245.48 ± 2.76
		12	243.17 ± 3.82
Pulëz 2*	2015	0	261.87 ± 2.25
		3	229.56 ± 4.52
		6	453.90 ± 8.18
		9	602.83 ± 0.29
		12	591.80 ± 9.12
Pulëz 1	2016	0	32.83 ± 3.02
		3	26.80 ± 1.06
		6	18.06 ± 2.48
		9	30.76 ± 0.82
		12	38.21 ± 1.81
Pulëz 2	2016	0	32.88 ± 3.02
		3	65.09 ± 2.41
		6	97.95 ± 1.04
		9	89.76 ± 2.76
		12	109.73 ± 2.35

1- without skins, 2 – with skins, a – Mean + SD

Thus, in vintage 2015 the value of TF in fermentation without skins is 243.17 and in fermentation with skins this value increases to 591.80. In vintage 2016 the value of TF in fermentation without skins is 38.21 and in fermentation with skins this value increases to 109.73.

Fermentation with the skins for both vintages showed that there was a higher significant impact with a $P \leq 0:05$ as also shown in Figure 2.

Meanwhile, during fermentation with and without skin, the intensity of the color does not differ much from one another, which means that fermentation in the presence of the skins does not stimulate the oxidation of wine.

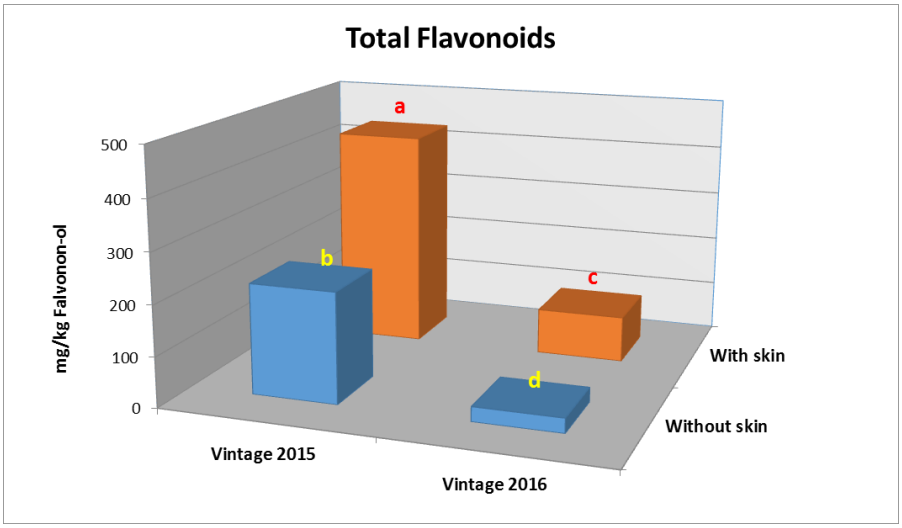

Figure 2. Performance of the TF of white wine from Pulëz grape variety in fermentation with and without skins during two different vintages 2015 – 2016 (Tukey Test, $P \leq 0.5$)

Color Intensity

The data in Table 4 show that the intensity of color at the end of the fermentation decreases in all the experiments carried out in two vintages.

Table 4. Extraction of color index during fermentation performance with and without skins in two differences vintage (2015-2016) for Pulëz grape variety.

Variety	Vintage	Days of Fermentation	Color Intensity
Pulëz 1*	2015	0	1.39 ± 0.01 [a]
		3	0.41 ± 0.01
		6	0.27 ± 0.01
		9	0.12 ± 0.01
		12	0.11 ± 0.01
Pulëz 2*	2014	0	1.39 ± 0.01
		3	0.41 ± 0.01
		6	0.27 ± 0.00
		9	0.27 ± 0.00
		12	0.21 ± 0.00
Pulëz 1	2016	0	0.20 ± 0.01
		3	0.25 ± 0.01
		6	0.38 ± 0.01
		9	0.20 ± 0.01
		12	0.29 ± 0.01
Pulëz 2	2016	0	0.20 ± 0.01
		3	0.29 ± 0.01
		6	0.43 ± 0.01
		9	0.16 ± 0.01
		12	0.27 ± 0.01

1- without skins, 2 – with skins, a – Mean + SD

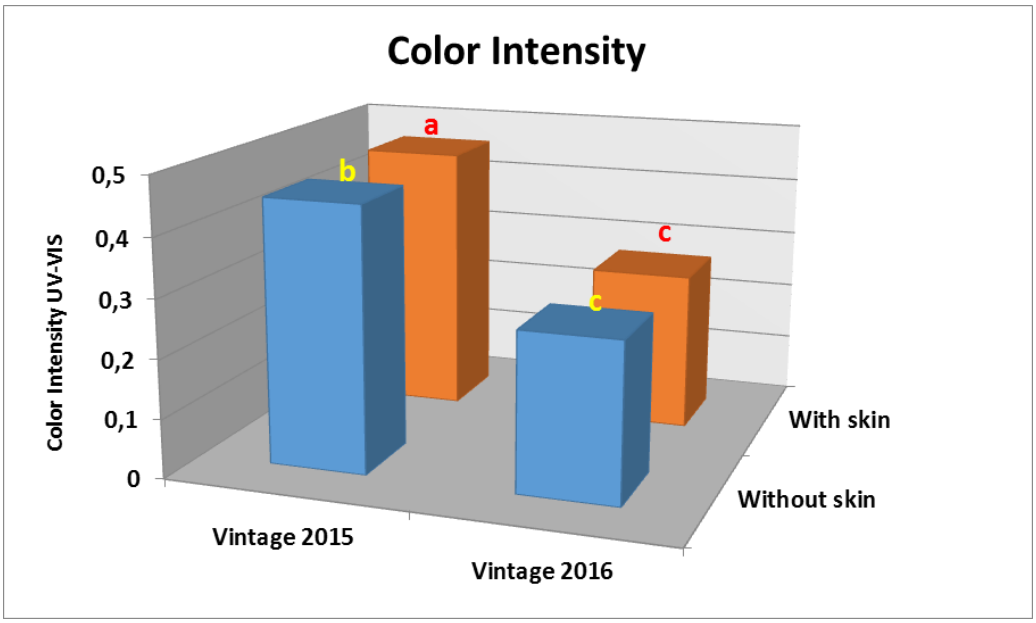

Figure 3. Performance of the Color Intensity of white wine from Pulëz grape variety in fermentation with and without skins during two different vintages 2015 – 2016 (Tukey Test, P ≤ 0.5)

Fermentation with the skins and without the skins according to statistical analysis of the design factor Anova and Tukey Test had no significative impact on this parameter but during tow vintages we have the significative difference (Figure 3). During 2015 the wine was more colored than 2016.

Table 5. Explanation of percentage variability of total polyphenols index, flavonoids and color intensity during fermentation performance with and without skins for grape varieties taken in two different vintages.

	Total Polyphenol Index	Total Flavonoids	Color Intensity
Fermentation	26.14*	18.51*	0.50^{NS}
Vintage	96.31***	70.14***	86.60^{NS}
Fermentation x Vantage	3.16^{NS}	6.32^{NS}	1.71^{NS}
Error	**1.39**	**5.04**	**11.19**

NS- not Significant, *** P ≤ 0.0001; * P ≤ 0.05

Table 5 shows the percentage of variability of the fermentation performance in the total polyphenol index content, total flavonoids and color intensity. As it is noticed, the fermentation scheme has a significant impact (P ≤ 0.05) on these components.

DISCUSSION

In white wine vinification, skin contact treatment is a process often applied to increase the wine's varietal character (Peinado et al., 2004; Selli et al., 2003). The organoleptic property of white and red wines comes as a result of phenolic compounds (Robichaud et al., 1990, Vidal et al., 2004). White wines in contrast to red wines are characterized by a low intensity aroma and

flavor. Chemical composition of grapes and respectively of the produced wines is complex and diverse. The concentration of this component depends on the variety, soil and climatic conditions, the applied growing practices, maturity and the winemaking technology (Fang et al., 2008, Ramos et al., 1999, Gil-Muñoz et al., 1999, García-Falcón et al., 2007).

Regarding the results obtained from this study, the application of two fermentation schemes indented from different vintages shows significant differences between them $P \leq 0.05$. The content of the total polyphenols index doubled the values when the fermentation occurred with the presence of the skin. The increase in this parameter at the end of the fermentation for white wine is in line with the ones realized by Lukic et al. (2017). This increase is due to the presence of skins during alcoholic fermentation by extracting these components from skins and seeds (Cadota et al., 2012). It was also noted that the impact of climate conditions from one year to the next presented significant difference ($P \leq 0.05$) in the content of total polyphenols, considering that in this study the origin and variety are the same. The vintage 2016 showed the lowest values in this parameter, however, during the use of fermentation with skins the amount of polyphenols total increased.

Flavonoids are the main compounds responsible for organoleptic properties (Alcalde-Eon et al., 2014). In this study, it was also noted that the white wines produced by vinification with presence of the skins represented content of flavonoids significantly higher for both the vintages (2015 – 2016). During fermentation with skins the increase in the content of total flavonoids in wine comes due to the extraction of these constituents from the presence of skins and seeds during fermentation. These results are similar to those achieved by Lukic et al. (2017).

Colour is generally the first organoleptic property of the wine that is perceived by consumers and it is, therefore, responsible for the consumers' first opinion on a given wine (Alcalde-Eon et al., 2014). Colour may condition the perception of the aroma, taste or mouthfeel properties of a wine (Morrot et al., 2001, Österbauer et al., 2005). Moreover, the color is considered one of the parameters that evaluate the oxidative stability of the wine (Beccheti, 1999). According to the results of this study, the wines produced in 2015 were more intense than those obtained in 2016; these values were within the established limits (Beccheti, 1999). The increase in color intensity is due to the extraction of the pigments found in the skin of the fruit during the fermentation with the skin. The conservation of these pigments by oxidation was not the result of the addition of SO_2, since the doses of SO_2 for both fermentations were the same, but it is assumed by the extraction of the phenols found in the grapes.

Use of the new schemes vinification in order to improve the quality of the wines, especially white wines is a necessary and important process for producing wines with high organoleptic quality. From the results of this study it is possible to evaluate and the importance of both oenological practices to improve the quality of white wine in relation to climate changes that may occur during one vintage to the other. Based on the vinification type, the fermentation with skin showed an increase in the values of components responsible for aromas and flavor in white wines. It has been observed that the increase of the levels of these compounds was more noticeable when the levels are lower. In addition, the use of these technological schemes can affect the increase in the amount of flavonoids in white wine, which can be useful from the phenolic point of view, and can improve the wine in terms of astringency and bitterness, especially when the wine is it produces from grapes that have not reached optimum maturity.

CONCLUSIONS

From this study, we conclude that the use of new fermentation schemes for white wines, fermenting with the presence of skins, promotes the growth of polyphenol and flavonoid contents. Increasing the content of phenolic components using fermentation with skin will improve the quality of the wine produced from unripe grapes. The information obtained in this

work about polyphenols component might be useful for nutritional studies to understand the bioactive effect of this wine in the human health. Fermentation with skin affect the organoleptic evaluation, because increase the components that is responsible for astringency, bitterness and color of white wine. Fermentation with skins results with significant impact on the content of phenolic components that are responsible and for the sensory characteristics of wines.

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FABRICATION AND CHARACTERIZATION OF CHITOSAN/GUM ARABIC/POLYVINYL ALCOHOL) NANOCOMPOSITE FILMS

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ABSTRACT

Recently, natural and synthetic polymeric biomaterials, as well as their composites, have found wide applications in regenerative medicine and tissue engineering due to their favorable chemical, physical, and biological properties such as biocompatibility, biodegradability, and non-toxicity. In this study, natural polymers; chitosan and gum Arabic were combined with polyvinyl alcohol (PVA), which is a biocompatible and biodegradable synthetic polymer, and nanosphere based nanocomposite films were fabricated through electrospraying technique. The morphological observations, determination of the average diameter and diameter distribution of nanospheres were conducted by scanning electron microscopy (SEM). The possible chemical interactions between the components and the existence of relevant functional groups were characterized with Fourier transform infrared spectroscopy (FTIR). Thermal degradation behavior of the nanocomposite film was investigated through thermogravimetric analysis (TGA).

Keywords: *PVA, Gum Arabic, Chitosan, Biomaterials, Electrospraying, Nanocomposite, Nanosphere*

INTRODUCTION

Natural polymers such as polysaccharides and proteins have a long history of biomedical applications, such as tissue engineering, wound dressings, and drug delivery systems, because of their unique properties like biocompatibility, biodegradability, and similarity to natural extracellular matrix. Composite materials, consisting of both synthetic and naturally occurring polymers, can also be developed for biomedical applications by cross-linking or blending the natural macromolecules with biodegradable and biocompatible synthetic polymers to enhance the chemical and mechanical stability (Hussain et al., 2016; Tan et al., 2015; Mogoşanu et al., 2014).

Nano-scaled materials such as nanospheres and nanofibers mimic the natural extracellular matrix and regarded as potential materials for applications in nanomedicine field which combines nanotechnology and medicine. High-voltage electrostatic technology, an environmentally friendly technique, can be used to manufacture nanospheres and nanofibers simultaneously. When the products are nanospheres, the technique is known as "electrospraying". On the other hand, when the products are nanofibers, the technique is known as "electrospinning" (Madhumathi et al., 2010; Tracy et al., 2016; Chen et al., 2018).

Chitosan, known for its absorption of exudes, anti-fungal, antimicrobial, anti-viral and wound healing properties, is a linear polycationic polysaccharide (Kim et al., 2011; Behera et al., 2017). The cationic structure of chitosan promotes electrostatic interaction with anionic groups (Ibekwe et al., 2017). Gum Arabic is an inexpensive, hydrophilic, nontoxic, biocompatible and

totally biodegradable polymer, and also a weak polyelectrolyte that carries anionic groups (Barik et al., 2015). PVA is a hydrophilic, nontoxic, biocompatible, and fully degradable synthetic polymer (Wang et al., 2011). The present study deals with the production and characterization of chitosan/gum Arabic/PVA nanocomposite films to combine the advantageous properties of these biopolymers by electrospraying technique.

MATERIAL AND METHODS

Materials

Glacial acetic acid and gum Arabic (*Acacia Senegal*) were purchased from Sigma-Aldrich Chemical Co. Chitosan (20 kDa, deacetylation degree 75%) was purchased from Santa Cruz. Ethanol (≥ 99.9 %) and PVA (MW = 30,000, fully hydrolized) were purchased from Merck.

Preparation of the nanocomposite film by electrospraying

0.25 g of chitosan was added in 5 mL of 1% (v/v) acetic acid solution and stirred continuously at 30ºC for 12 hours. Gum Arabic was dissolved in 2.5 mL of deionized water under constant stirring at room temperature for 12 hours. After complete dissolution, the prepared solutions were combined and stirred at ambient temperature for further 12 hours. 1 g of PVA was added gradually in deionized water and stirred at 80 ºC overnight. The combined chitosan and gum Arabic solution was added in 10 mL of aqueous PVA (10%, w/v) solution and stirred overnight at ambient temperature. The resulting clear gel was taken into a plastic syringe which has a 0.3 mm diameter metallic capillary tip.

The electrospraying system involves a syringe pump, a high voltage power supply, and a rotary drum collector. Rotational and axial speeds of the rotary drum collector can be controlled by a software. One electrode of the high voltage power supply was connected to the metallic capillary tip and the other connected to the rotary drum collector, used as the ground. The rotary drum collector was covered with an aluminum foil substrate. Rotational and axial speed of the rotary drum collector were 180 rpm and 1 mm/min, respectively. The composite solution, flowing at a flow rate of 1.5 mL/h, was subjected to a potential difference of 18 kV through the electrodes which are 10 cm apart. By the effect of the electrical field, the composite solution was moved to the collector as very small droplets, solvent was evaporated and a nanocomposite film consisting of nanospheres was obtained.

Structural and thermal analysis of the nanocomposite film

Analyses of the functional groups and possible chemical interactions between the constituents of the nanocomposite film were conducted by a Thermo Scientific/Nicolet IS10 spectrometer with a FTIR-attenuated total reflectance (FTIR-ATR) attachment. The film sample was dried at 40ºC in a vacuum oven for 24 hours prior to the analysis.

A Perkin Elmer/STA6000 thermogravimetric analyzer was used for the analysis of the thermal degradation behavior of the nanocomposite film. Percentage mass loss of the sample as a function of temperature were recorded. The sample was heated from 30ºC to 600ºC at a heating rate of 10ºC/min under nitrogen atmosphere.

Morphological analysis of the nanocomposite film

Morphological study of the nanocomposite film was conducted through a Carl Zeiss/Supra 40VP scanning electron microscope with an accelerating voltage of 5 kV. The film sample was sputter coated with gold/palladium alloy before SEM analysis. Diameter distribution of the

nanospheres were studied on an image analysis software by choosing 300 nanospheres at 10000X magnification and presented by a histogram.

RESULTS AND DISCUSSION

Structural analysis by FTIR

FTIR spectrum of the nanocomposite film, shown in Figure 1, was used to indicate the possible chemical interactions between the polymers and the existence of relevant functional groups. The broad band, seen at 3271 cm-1 is due to the characteristic absorption band of hydrogen bonded hydroxyl groups. N–H symmetrical vibration of chitosan which is expected to be seen between 3300-3500 cm^{-1} must have been masked by the broad O-H absorption band. This attributed to the overlapped NH_2 and -OH group stretching vibrations (Daoub et al., 2016; Hussain et al., 2016; AbdElhady et al., 2012). The absorption peak, seen at 2910 cm^{-1} is assigned to the vibrational modes of C-H groups (Fernandes et al., 2014). The peak, appeared at 1409 cm^{-1} is assigned to O-H in-plane bending of carboxylic acid groups. The peak, observed at 1561 cm^{-1} is attributed to symmetric deformation of $-NH3^{+}$ which might be formed by ionization of the primary amine groups ($-NH_2$) of chitosan by the anionic groups of the other polymers.

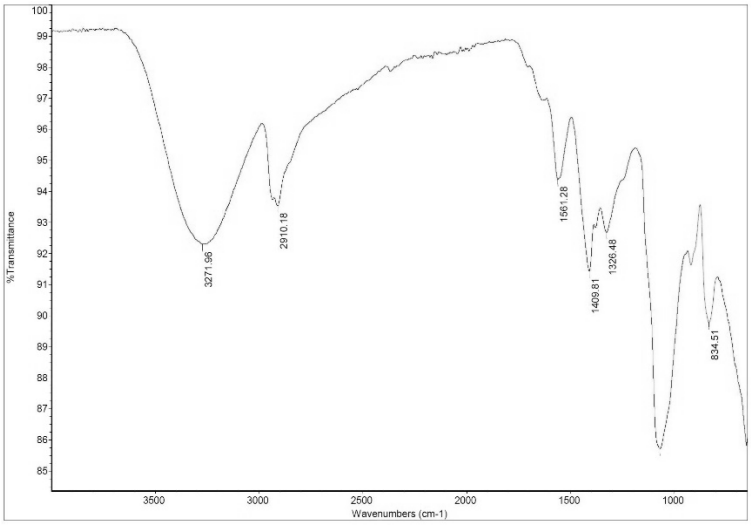

Figure 1. FTIR spectrum of the nanocomposite film

Analysis of thermal degradation behavior by TGA

The TGA curve of the nanocomposite film is presented in Figure 2. Based on Figure 2, the nanocomposite film is degraded thermally by three steps: water loss, major decomposition of polymer backbones, and degradation of the byproducts. The last step, take place between 350–600°C, is arising from the degradation of the byproducts of PVA, formed during its thermal degradation (Choo et al., 2016; Bozorgi et al., 2018). First degradation step, starting at about 100 ^{o}C is arising from the loss of adsorbed water to the polysaccharide structures. The second step between 200-350^{o}C, being the major decomposition step with a weight loss percentage of 69%, is due to the backbone decomposition of the polymers.

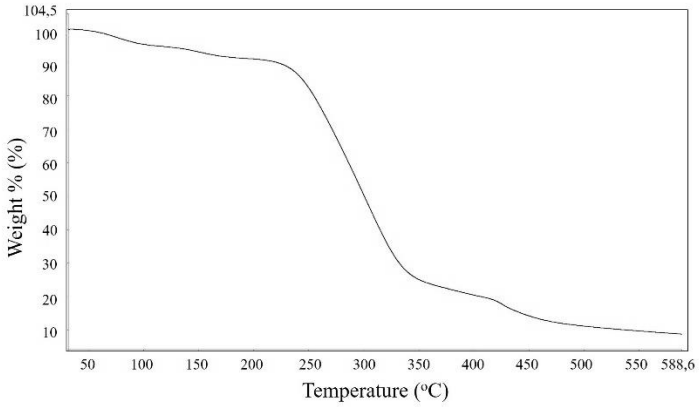

Figure 2. TGA curve of the nanocomposite film

Morphological study of the nanocomposite film by SEM

The SEM micrograph at 10000X magnification and the diameter distribution histogram of the nanocomposite film are presented in Figure 3(a, b). SEM observations demonstrated that the produced film consists of variable sized nanospheres. In addition, interconnected nanofibers which may be formed by the elongation of PVA through the effect of the applied electrical field, were present at a few regions. Diameter distribution histogram of the nanocomposite films revealed that 65% of the nanospheres are in the range of 200-550 nm and the average diameter was determined as 583 nm.

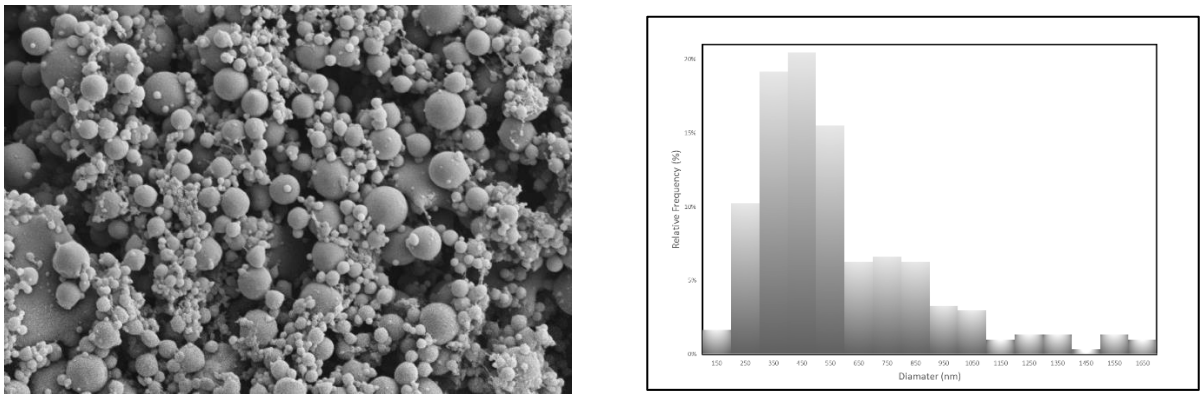

Figure 3. (a) SEM micrograph at 10000X magnification and (b) Diameter distribution histogram of the nanocomposite film

CONCLUSIONS

A ternary nanocomposite film, consisting of PVA, chitosan, and gum Arabic was developed in this study. FTIR spectrum of the nanocomposite film showed that there are ionic interactions among the native polymers. In addition, it was observed that the nanocomposite film exhibits only one major decomposition temperature (T_{onset}), as shown in the TGA curve of the nanocomposite film. These results indicate the compability of chitosan and gum Arabic, as the natural polysaccharides and PVA, being a synthetic polymer. The nanosphere based nanocomposite film, developed in this study, can be investigated for their potential use in drug release and wound healing applications in further studies.

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MODELING THE EFFECT OF TEMPERATURE ON PHYSICOCHEMICAL PROPERTIES OF OLIVE OIL

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ABSTRACT

Olive oil is used throughout the world, especially in regions around the Mediterranean Sea. Oils help to improve the taste, color and quality of the food, so it plays an important role in health and in life. The main goal of this work is to study the dependence of viscosity of olive oil on temperature, since is a fundamental characteristic property of all liquids. The dynamic viscosity of olive oil was experimentally determined as a function of temperature (20 to 60°C). Several physicochemical and rheological characteristics of olive oil were investigated. The studied parameters were: density, moisture content, pH, free acidity, electrical conductivity, dynamic viscosity and kinematic viscosity. The viscosity of olive oil was represented as a function of temperature by using two, three and multi-constant proposed mathematical models. The evaluations of the models were done by the correlation coefficient, percentage of average absolute deviation and standard deviation of the data.

Keywords: *Olive oil, Mathematical models, Density, Viscosity*.

INTRODUCTION

Olive oil, produced at a level of around 2.6 millions tones (Spain, Greece, Italy, Turkey, Syria, Tunisia and other), has a long history going back to pre-biblical times. It is produced and consumed mainly in Mediterranean countries, but demand is increasing in other countries in Northern Europe and in the US as a consequence of strong marketing of this oil. Olive oil is considered to be an essential ingredient of the healthy Mediterranean life style (Gunstone, 2002). Albania has been and continues to be a typically "agrarian" country, in which over 50 % of population lives and works in the rural areas, and which has as its major activity the agricultural sector, in which roughly 75-80 % of work' days are spent on farm (Leksinaj, 2008). The main olive growing regions include: i) the Ionic region, along the Southern Coastline, which accounts for 38.4% of the country's olive production, 28.5% of total olive groves and 28.8% of total olive trees; ii) the Adriatic region, along the Western Coastline, which accounts for roughly half of the country's olive production, total olive groves and total olive trees; iii) the Near-Adriatic region which accounts for 14.9% of the country's olive production, 16% of total olive groves and 20.3% of total olive trees; and iv) the Interior region, the production of which is minimal except for some areas where the microclimate allows for olive cultivation (FAO, 2008). In the year 2017 was produced 107.830 tons olives, increased with 8.9 % compared with the previous year. The highest level of olives production was achived in the prefectures of Berat with 32.410 tonnes, following by prefectures of Fier with 29.648 tonnes and Vlora with 14.881 tonnes (Instat, 2018). The olive oil sector is an important segment of Albanian primary production and agro industry. Main production areas of olives for olive oil are Fier, Vlora and the area between Elbasan and Tirana. In these areas, 90% to 95% of cultivars are for olive oil production. Olive oil industry can be divided in four main clusters, namely: small localized oil mills, small modern producers; medium-sized processors, and industrial producers and bottlers. Demand of olive oil and table olives is increasing, in parallel with revenues growth and urbanization (Leonetti et al., 2009). Viscosity is one of the most important

physical properties of a liquid system; the change of viscosity is linked to physicochemical oil properties (Fasina and Colley, 2008). This parameter can changes under temperature, pressure, and concentration influence, and all these changes can be modeled by some theorical equations (Ramakrishna et al., 1987). The dynamic viscosity of olive oil was experimentally determined as a function of temperature (20 to 60°C). The variation of the viscosity of olive oil with the temperature is analyzed applying the two, three and multi constant equations. The correlation coefficient for all the models obtained from the non-linear regression procedure was greater than 0.99.

MATERIAL AND METHOD

Olive oil is the oil obtained solely from the fruit of the olive tree, to the exclusion of oil obtained using solvents or re-esterification processes and of any mixture with oils of other kinds. Olive oil available in Albanian market was characterized for physicochemical and rheological properties. For this purpose we choose the olive oil from southern areas, because this is one of the main olive growing region. All oil sample were stored at room temperature (around 20°C) and in a dark place before analysis. The density and viscosity of olive oil were experimentally determined as a function of temperature (20-60°C). Physicochemical and rheological characteristics of olive oil were analyzed: density, moisture content, pH, free acidity, electrical conductivity, dynamic viscosity and kinematic viscosity. The moisture was calculated by sample weight loss at 105°C for a period of 24 h. The pH measurement of olive oil was obtained with a pH meter (PHS-3CW Microprocessor pH Meter) was calibrated with standard solutions buffered. Also we measured the electric conductivity with DDS-120W Microprocessor Conductivity Meter. The free oil acidity, a known weight of olive oil was dissolved in a mixture of diethyl ether/ethanol (1:1 v/v). The mixture was titrated with potassium hydroxide in methanol (0.05M), in the presence of phenolphthalein as indicator. Viscosity and temperature of olive oil samples were measured using the Digital Viscometer Model NDJ-5S with accuracy ±1%. The SP-1 spindle was operated at 60 rpm. In addition to the dynamic viscosity and density, kinematic viscosity by the formula was also determined. The Digital Viscometer gives indications for out of range operations when % (Torque) readings are ≤ 20% or ≥ 90%. The Electric model L-81 was used to increase the temperature of the oil samples to a specific temperature. All values were below the limits, were within the range established by European Guidelines. The dependence of the oil viscosities to temperature was modeled using two, three and multi constant equations. Equation 1 include the Arrhenius model, Law Power model and Natarajan model (two constant equations) that is commonly used to model temperature dependence of a property (Clements C. *et al*., 2006; Natarajan G. *et al*, 1989).

$$\mu = \mu_{\infty,T} \exp\left(\frac{E_a}{RT}\right) \qquad \mu = A(T - T_{ref})^B \qquad \mu = AT^B \qquad (1)$$

Where μ is the dynamic viscosity in mPa.s, $\mu_{\infty,T}$ is the viscosity at infinite-temperature in mPa.s, Ea is the exponential constant that is known as activation energy (J/mol); R is the gas constant (J/mol.K) and T is the absolute temperature Kelvin, T_{ref} is reference temperature of 273.15 C. Multi-constant formula known as Abramovic (three constant) and Clements (four constant) models that are represented in the following equations (Abramovic et al. 1998; Clements et al. 1992):

$$Ln\mu = A + \frac{B}{T} + \frac{C}{T^2} \qquad Ln\mu = A + \frac{B}{T} + \frac{C}{T^2} + \frac{C}{T^3} \qquad (2)$$

Where μ is the dynamic viscosity in mPa.s, T is the temperature in Kelvin. A, B and C are constants. The relationship between density and temperature can be expressed mathematically, while density decreases linearly with increasing temperature, is presented in equation 3 (Rodenbush et al., 1999):

$$\rho = a + b \cdot T \quad (3)$$

Where ρ is the density in g /cm^3, T is the temperature in C, a and b are constants.

According to Rodenbush et al., the dependence between density and viscosity can be expressed as illustrated (Rodenbush et al., 1999):

$$\rho = D + \frac{E}{\eta^{1/2}} \quad (4)$$

Office Excel 2016 software was used to carry out the effect of temperature on dynamic viscosity of olive oil by different mathematical models. The percentage of average absolute deviation of the olive oil at different temperatures together with standard deviations was obtained.

RESULTS AND DISCCUSION

The olive oil quality and behavior can be influenced by the cultivars, the degree of ripeness, the industrial processes extraction, and environmental conditions (Bento et al., 2002). Several physicochemical and rheological characteristics of olive oil were investigated. The physicochemical characteristics: density, moisture content, pH, free acidity, electrical conductivity, were determined following the analytical methods described in Regulation EEC/2568/91, amended (Commission Regulation, 1991). All parameters were determined in triplicate for each sample. By comparing our experimental data with standard value, we can see that they are roughly the same, with very little difference. This may come as a result of many factors that affect the quality of the oil and therefore the experimental results. Factors can be numerous, but we can mention the area of seed origin, storage conditions and stages of production of the final product. The density values of olive oil samples of southern region of crop 2017 was measured at 20ºC and found to be 0.9179 g/cm^3. Moisture of olive oil sample was 0.2%. pH and electric conductivity of olive oil sample were 4.1 and 0.045 mS, at 20ºC, respectively. The value of electrical conductivity obtained indicates that olive oil is truly an insulator as compared to other liquid insulators. Meaning is highly resistive to flow of electric charge. Also transformers or machines oil, coolants etc using olive oil as dielectric will last longer. Olive oil can be said to be very bad conductor of electricity (Ushie et al., 2014). A free acidity value and acid number of olive oil were 0.62% and 1.23. The acidity expresses the percentage content (in weight) of the free fatty acids in the oil under examination. The free oil acidity is a direct measure of the quality of the oil, thus reflects the condition from blossoming fruit and olive oil production. Setting a free oil acidity level lower than 0.8% can provide a useful standard to ensure that growers provide high quality undamaged fruit that is not harvested too late. The dynamic viscosity and kinematic viscosity values of olive oil sample of southern region were measured at 20ºC and found to be 68.4 mPa.s and 74.51 mm^2/s. Our value of dynamic viscosity of olive oil at 20ºC is in good agreement with other researcher. The viscosity influences by the wax content and composition, which is affected by cultivar, crop year and processing (Boskou, 2006).

Dynamic viscosity dependence of temperature and fitting of model to experimental data

The dependence of the oil viscosities to temperature was modeled using Equations 1-2. Equation 1 includes the Arrhenius model, Law Power model and Natarajan model (two constant equations). Equation 2 includes Abramovic and Clements models (three and four constant equations). The experimental data of olive oil, for dynamic viscosity (Pa.s) fitting by Arrhenius model is shown in Figure 1. The values of the estimated constants for Arrheniua Equation, correlation coefficients, percentage of average absolute deviation and standard deviation are given in Table 1, respectively.

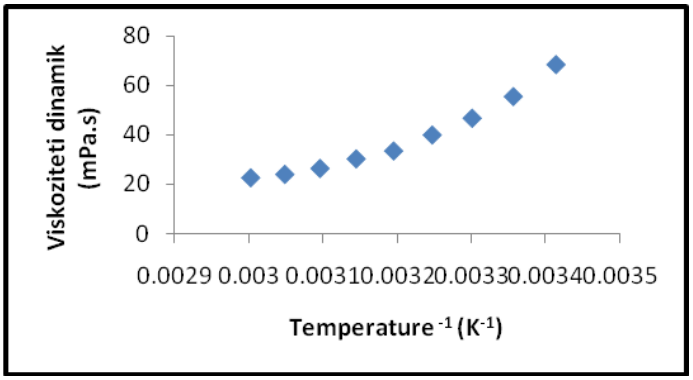


Figure 1. Effect of temperature on dynamic viscosity of olive oil by Arrhenius equation

The value of activation energy of the analyzed olive oil was 22.89 kJ/mol, which describe the sensitivity of viscosity to temperature changes. The correlation coefficient (R^2) for Arrhenius model was under value 0.99. The mean absolute percentage error was 5.87, below 10%, but standard deviation was higher than 10%. The experimental data of olive oil, for dynamic viscosity (Pa.s) fitting by Law Power model is shown in Figure 2. The values of the estimated constants for by Law Power Equation, correlation coefficients, percentage of average absolute deviation and standard deviation are given in Table 1, respectively.

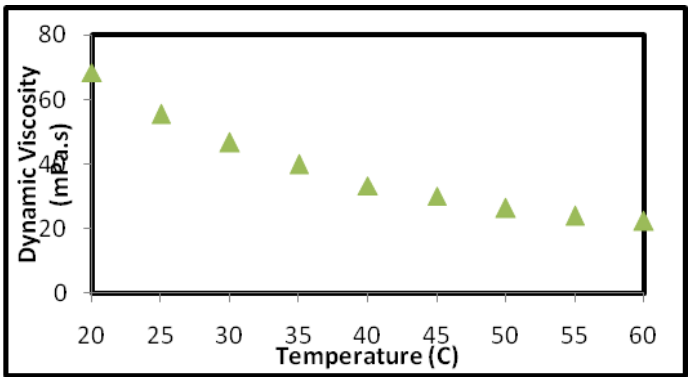


Figure 2. Effect of temperature on dynamic viscosity of olive oil by Law Power model

Figure 3 shows the olive oil μ (Pa.s) dependence of the temperature (K) and the fitting of the Natarajan model to experimental data (Equation 1). Table 1, resumes the results of Natarajan parameters, correlation coefficient, percentage of average absolute deviation and standard deviation

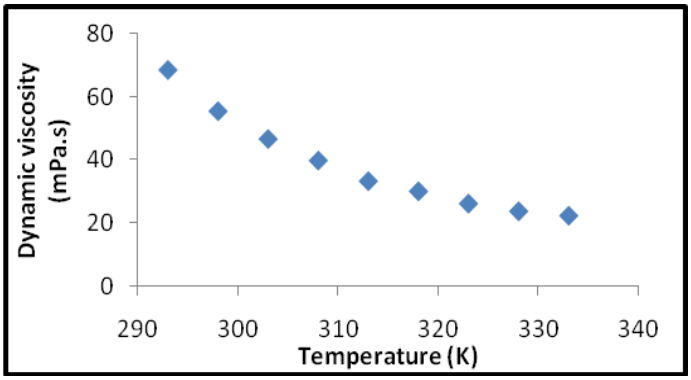
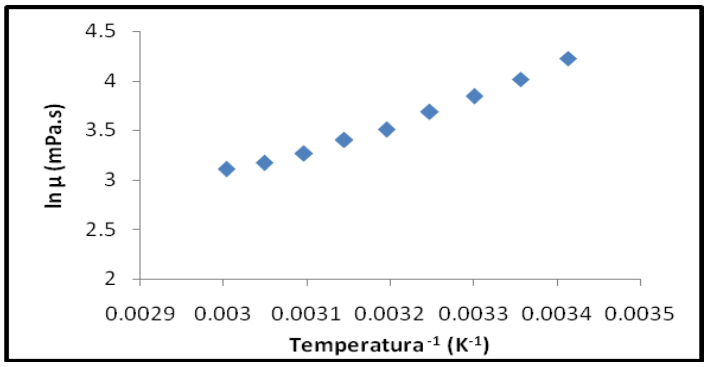
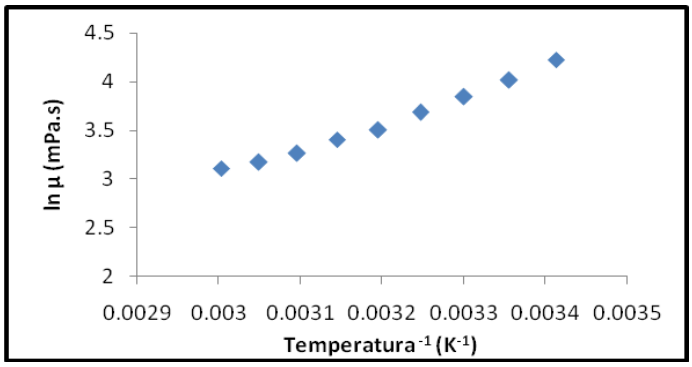


Figure 3. Effect of temperature on dynamic viscosity of olive oil by Natarajan model

Figure 4 shows the olive oil ln μ (Pa.s) versus (1/T) and the fitting of the Abramovic and Clements models to experimental data (Equation 2). Table 1 resumes the results of Abramovic and Clementes parameters, correlation coefficient, percentage of average absolute deviation and standard deviation.



(a)



(b)

Figure 4. Effect of temperature on dynamic viscosity of olive oil by (a) Abramovic and (b) Clements models

The percentage of average absolute deviation (AADP) and standard deviation were (SD) computed and used to compare the goodness of fit of the equations to experimental data. An equation with lower SD values gives a better fit to experimental data compared to an equation with higher SD values.

Table 1. Two, three, multi-constant values, Temperature range, R^2, AADP and SD of models studied

Mathematical model	A	B	C	D	Temperature Range	R^2	AADP	SD
Two constant	0.005	2755			293 - 333 K	0.988	5.88	13.97
	1606	-1.04			20-60 C	0.987	1.23	15.69
	3E+23	-8.8			293 - 333 K	0.984	0.53	14.46
Three constant	20.35	-13235	2E+06		0.0034 - 0.0030 (1/K)	0.999	0.11	0.39
Four constant	131.73	-11760	4E+0.7	-3E+09	0.0034 - 0.0030 (1/K)	0.999 - 1	0.05	0.38

Arrhenius, Law Power and Natarajan models (two constant equations) were not the best fit of dynamic viscosity of olive oil sample, because the percentage of average absolute deviation and standard deviation gives very high value. The correlation coefficients for Arrhenius and Natarajan models were under 0.99. According to the experimental data three and four constant equations are more suitable to describe the dependence of dynamic viscosity from temperature of olive oil sample. The R^2 values for olive oil were above 0.99, indicating experimental data fell on straight lines. All of the standard deviations were very low, which means that the viscosity values obtained were very stable.

The dependence of density and kinematic viscosity according temperature for olive oil

The kinematic viscosity can be calculated from the dynamic viscosity and the density. Because both, density and viscosity are highly temperature sensitive; it is possible to see the dependence between them. Furthermore, the density - viscosity dependence was analyzed, showing that a good estimation of the viscosity can be obtained from the measure of the density, which is a simple and time effective process (Esteban et al., 2012). Density of olive oil at different temperatures ranges of 0.9179 to 0.9307 g/cm^3. According to Rodenbush et al., when dealing with vegetable oils the dependence between density and viscosity can be expressed as illustrated in Equation (4). Kinematic viscosity of olive oil at different density ranges of 0.1158 to 0.2008 mm/s^2. The R^2 for both dependence obtained was greater than 0.99. The values of the estimated constants are shown in each equation, set in the graph.

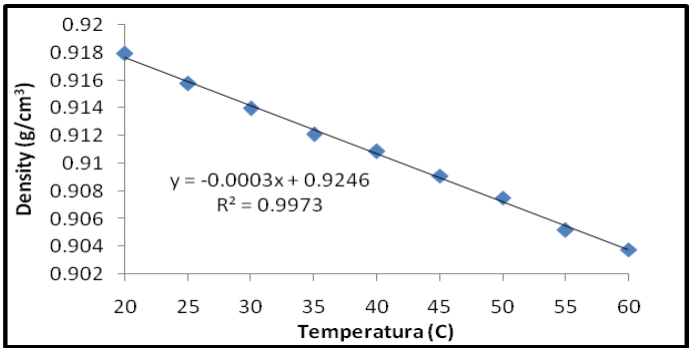


Figure 5. Effect of temperature on density of olive oil

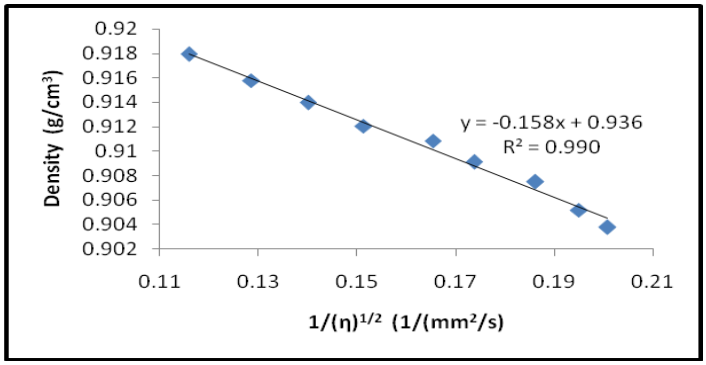


Figure 6. Effect of temperature on kinematic viscosity of olive oil

CONCLUSIONS

In this study the experimental results depend on oil nature and can be used as a way of characterizing the oil quality. The dynamic viscosity versus temperature of olive oil was measured and described by mathematical models. The correlation coefficient for all the models obtained from the non-linear regression procedure was greater than 0.95. However, comparisons of the correlation coefficient and percentage of average absolute deviation indicate that the temperature dependence of viscosity for the olive oil sample was best described by the Clements model. The results show that the experimental values of olive oil studied were comparable with the published values at the same temperatures.

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AREA OF DISTRIBUTION OF THE PATHOGEN PLASMOPARA HALSTEDII IN THE REPUBLIC OF BULGARIA

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ABSTRACT

Downy mildew/Plasmopara halstedii Farl. Berlese et de Toni is distributed in all the world where sunflower is produced. In Bulgaria, the pathogen is very important for sunflower production. Increasingly, scientists report the emergence of new, more aggressive races of the pathogen or return to "old". According to others, these races show resistance to the fungicides currently used. This requires annual research on the distribution range and diversity of the disease. Such surveys provide valuable information for both selection and practice - for zoning of varieties and hybrids. As a result of the study, three races of *Plasmopara halstedii* were isolated - 700, 731 and race 307. Their spread and attack rate are closely related to both the climatic conditions of the year and their resistance of varieties and hybrids cultivated in practice. It is particularly worrying that a "new" race was established in the DZI selection fields. After the test, it turned out to be a race 307 or a return to old races of the 300-group pathogen.

Key words: *Plasmopara halstedii, Races, Distribution*

INTRODUCTION

Downy mildew / Plasmopara halstedii Farl Berlese et de Toni is spread throughout the world where sunflower is grown. In Bulgaria, the pathogen is of great economic importance for the cultivation and zoning of sunflower as the main oil-bearing crop (Shindrova P 1998, 2000, 2005, Shindrova, 2006,). To a lesser extent, it is also important for the selection process aimed at creating sustainable hybrids. Gulyia T., 2007 reported that at least 35 races of the pathogen have been identified in the world that make the problem solving quite difficult. For a long time in our country there were races 700 and 731. Their distribution was largely due to the climatic conditions in the country and to the resistanc of cultivars and hybrids cultivated in practice. More and more researchers report the emergence of new aggressive races of pathogen (Tourvieill de Laborouhe D. et al., 2000; 2010) or the return of the "old". According to others, these races show resistance to the currently used fungicides (Viranyi, F. and T.Gulyia 1996). One of the most effective methods to solve this problem is the use of sustainable varieties and hybrids of sunflower. This is achieved with a purposeful selection work. For this purpose, annual research tours are carried out in all country to establish distribution and races diversity of the pathogen. These studies provide valuable information on both the selection process and the practice - for the proper zoning of varieties and sunflower hybrids.

MATERIALS AND METHODS

In order to determine the race variability of sunflower downy mildew in the major sunflower production regions in Bulgaria, field expeditions for collecting isolates of *Plasmopara halstedii* were organized in 2016 and 2017. Leaves from systemically infected plants were collected and used as inoculum. During the period of investigations, the inoculum used originated from: north-eastern, north-western, central-northern, central southern and central western (Table 1 and 2).

The inoculum collected from the above regions was used to infect the differential set lines applying the standard methodology Gulia et al. (1991).

For identification of the races on downy mildew was used the new nomenclature system with nine lines. These were grouped as follows:

- First group - HA-304 (D-1), Rha-265(D-2) , Rha-274 (D-3)
- Second group - DM-2 (D-4), PM-17 (D-5) и 803-1 (D-6)
- Third group - HAR-4 (D-7), HAR-5 (D-8) и HA-335 (D-9).

The race was identified on the basis of the response of the lines from each group (Tourvieill de Laborouhe et al. 2000).

The study was conducted in the phytopathological laboratory of the Dobrudzha Agricultural Institute under controlled conditions.

RESULTS AND DISCUSSION

During the first year of the investigation nine plant samples were collected and processed. The result from this investigation showed that only 2 races (pathotypes) were isolated – 700 and 731. Race 700 is established only in Northeastern Bulgaria (DZI, Staro Oryahovo and Brashlyan), while race 731 is established throughout the country (DZI / Dobrich / infection field, Kardam / Dobrich, Staro Oryahovo / Varna, Brashlyan / Rousse, Silanovtsi / Vratsa, Pleven, Radnevo, Ivailo / Pazardzhik and Sliven. *Plasmopara halstedii* is a pathogen which in our conditions does not exhibit a great variety of races. This could be due to the resistance of varieties and hybrids cultivated in practice and to the climatic conditions of the country.

Table 1. Distribution and race composition of (*Plasmopara halstedii* Farl. Berlese et de Toni) in sunflower during 2016

№	Location	Region	Race
1	DZI/Dobrich/infection field	North-eastern Bulgaria	700; 731
2	Kardam/Dobrich	North-eastern Bulgaria	731
3	Staro Oryahovo/Varna	North-eastern Bulgaria	700
4	Brashlyan/Russe	North-eastern Bulgaria	700; 731
5	Silanovtzy/Vratza	North-western Bulgaria	731
6	Pleven	Central- north Bulgaria	731
7	Radnevo	Central southern Bulgaria	731
8	Ivailo/Pazardzhik	Central southern Bulgaria	731
9	Sliven	Central western Bulgaria	731

When testing the samples collected in 2017 three races of the manna were registered - 700, 731 and race 307. The date in tabl.2 show that the following year a “new” race was found identified as a race 307. It was isolated from samples with origin from the region of DAI from breeding fields and materials from international exchange. One of the explanations for the occurrence of the new race in this very location can be the high breeding pressure on the pathogen, because in the breeding fields resistant genotypes are predominantly grown.

On the other hand, this may have been due to the intensive international exchange of breeding materials, which makes possible the transfer of the fungus, mainly through infected seeds, plant residues or soil particles.

Table 2. Distribution and race composition of *Plasmopara halstedii Farl. Berlese et de Toni* in sunflower during 2017

№	Location	Region	Race
1	DZI/Dobrich/infection field	North-eastern Bulgaria	700; 731
2	DZI/Dobrich/breeding field	North-eastern Bulgaria	307
3	Tervel/Dobrich	North-eastern Bulgaria	731
4	Brashlyan/Russe	North-eastern Bulgaria	700; 731
5	Silanovtzy/Vratza	North-western Bulgaria	731
6	Nova Zagora	Central southern Bulgaria	731
7	Radnevo	Central southern Bulgaria	731
8	Stoil voivoda/Sliven	Central western Bulgaria	731
9	Sliven	Central western Bulgaria	731

Race 700 is established in two places in Northeastern Bulgaria - (DZI - Gen.Toshevo and Brashlyan /Russe). In the rest of the region, race 731 was spread. It is particularly worrying that a "new" race was established in the DZI selection fields. After the test, it turned out to be a race 307 or a return to old races of the 300 group pathogen. This type of study is done to identify the most widespread and aggressive race of the pathogen. The next step is to identify sources and donors of sustainability. This is possible only on the basis of rich genetic diversity and purposeful selection work.

CONCLUSIONS

In the period 2016 - 2017 three races of the pathogen - 700, 731 and 307 were established in the territory of the Republic of Bulgaria. The most common race is 731, which is registered in all sunflower regions of northern and southern Bulgaria. Sustainable race diversity in *Plasmopara halstedii* during the period 2016-2017 is the result of a uniform crop structure and the use of resistance hybrids.

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THE NAME OF HAZARD INCREASINGLY IMPORTANT IN FOOD HYGIENE IS MODIFIED MYCOTOXINS

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ABSTRACT

Mycotoxins are toxic secondary compounds synthesized by certain fungal species that can grow in a variety of foods under specific conditions. In addition to animal products such as meat, milk and eggs, mycotoxins enter the food chain via cereal-based foods, which can accumulate in different organs or tissues. In particular, the main chemical structure of mycotoxins that undergo various metabolic changes in originated foods varies considerably. Mycotoxin derivatives, which cannot be detected by conventional analytical techniques as the structure changes in the plant, are described as "modified mycotoxin". Although the natural formation of modified mycotoxins has been shown in food and animal feed for a long time, there are not enough directives, regulations and recommendations for food and feed considering these modified species. In addition, little is known about the toxicity of these modified mycotoxins. In fact, the lack of analytical standards and reference materials has complicated their definition and partly limits the progress of research in this area. All of these effects can cause the modified mycotoxin components in food and feed to be taken into account. Given all these factors, the monitoring of the presence of these potentially dangerous metabolites is seen as an important activity in terms of food safety and provision of human/animal health. In this study, the definition of modified mycotoxins, their presence in food, possible damages and methods of protection and control have been examined in the light of current literature information.

Keywords: *Mycotoxin, Hazard, Modified, Food safety*

INTRODUCTION

Mycotoxins are toxic secondary compounds that can be synthesized under specific conditions by some fungal species which develop in a variety of food. The most common mycotoxins produced mainly by *Aspergillus*, *Fusarium*, *Penicillium* and *Alternaria* fungi are: aflatoxins; ochratoxin a (OTA); patulin; citrinin, trichothecenes: deoxynivalenol (DON), T2 toxin (T2) and HT2 toxin (HT2); fumonisins and zearalenone (ZEN). Mycotoxins can occur in the food chain due to fungal infections of plants consumed directly by humans or used as feed in livestock. Metabolism of imported mycotoxins can lead to mycotoxin accumulation in different organs or tissues entering the food chain via meat, milk or eggs (Accensi et al., 2006). Mycotoxins can undergo chemical reactions such as oxidation, reduction and hydrolysis as well as phase I and phase II metabolism, including amino acids, sulfate groups and second conjugation with glutathione. All these changes significantly change the chemical structure of the main component (Righetti et al., 2016). Mycotoxin derivatives, which cannot be detected

by conventional analytical techniques as the structure changes in the plant, are described as "modified mycotoxin". Even though the natural formation of modified mycotoxins has been shown in foods and animal feeds in the last ten years, there are no adequate directives, regulations and recommendations for food and feed, taking these modified derivatives into account. In addition, little is known about the toxicity, toxicokinetics and potential in vivo hydrolysis of these modified mycotoxins. When modified mycotoxins enter the intestinal tract, they are disrupted by gastric acids, small intestine enzymes or large intestinal microbial activity. This leads to the release of free and unbound mycotoxins in the human gut and thus mycotoxin accumulation (Marin et al., 2013; Dellafiora et al., 2016). For this reason, the release of the modified metabolites of mycotoxins in the human intestine has led to a more comprehensive assessment of the risk of exposure to these compounds. Modified mycotoxins such as myco-estrogens, trichothecenes, and fumonisins can be detected in cereal-based foods and feeds by non-conventional methods. More specifically, modified mycotoxin subspecies such as deoxynivalenol-3-glucoside (DON-3G), zearalenone-14-glucoside (ZEN-14G), HT-2-toxin-3-glucoside (HT-2-3G) diacetoxysirpenol (NAS-3G), mono-acetoxysirpenol-3-glucoside (MAS-3G), neosolaniol-3-glucoside (NEO-3G) and fumonisins-esters can be determined (Boevre et al., 2015). Toxic effects of ZEN, NIV, T2 HT2, DON and fumonosin were investigated in vivo and in vitro. The European Food Safety Authority (EFSA) reviewed the potential effects of modified mycotoxins on human health and called for analytical data left open until October 2014. In fact, the lack of analytical standards and reference materials has complicated their identities and partly limits research progress in this area. All of these effects can cause to underestimating the total content of mycotoxins in food and feeds. For this reason, the monitoring of the presence of these potentially dangerous metabolites remains one of the main tasks for food safety and human/animal health (Righetti et al., 2016). In this review, the definition of modified mycotoxins, their presence in food, possible damages and methods of protection and control will be discussed.

Modified Mycotoxins

Modified mycotoxins were first uncovered by Gareis and Bauer (1990) with the discovery of the breakdown of zearalenon-14-glucoside (ZEN-14G) during digestion in pigs. Initially the modified mycotoxins were expressed as non-detectable molecules by standard routine analysis. Metabolism of mycotoxins by plants can occur in part and leads to the production of components called modified mycotoxins. Three phases of chemical modification of these xenobiotic compounds can be observed during plant metabolism. Phase I processing involves reduction, oxidation or acetylation of the main mitotoxin and the emergence of a higher level of toxicity resulting in the activation of the derivatized molecule. Phase II is based on the formation of more hydrophilic compounds through the enzymatic conversion of more reactive groups such as conjugation, glucosidation and sulfation and facilitating the elimination of modified mycotoxins and thus the reduction of toxicity. Stage III involves the cleavage of mycotoxins to root or binding to cell wall of the plant. However, mammalian and fungal metabolites derived from mycotoxins, which can not be detected in routine analyzes, are also other modified mycotoxins that are thermally generated during the processing of food, but which are not produced principally by plants (Dellafiora et al., 2016). Following this complexity, all modified mycotoxins were systematically defined in four hierarchical steps by Rychlik and Humpf (2014). In the first step, 'free', 'matrix related' and 'modified' mycotoxins were distinguished. Then, the modified mycotoxins were separated into 'biologically' and 'chemically' modified derivatives. The biologically modified compounds are then distinguished as being 'functionalized', 'conjugated' or 'modified differently'. At last, a distinction is made

between the 'plant', 'animal' and 'fungal' conjugates for biologically conjugated mycotoxins (Table 1).

Table 1. Systematic Classification of Modified Mycotoxins by Rychlik and Humpf (2014)

Level 1	Level 2	Level 3	Level 4	Example
Free mycotoxins				Deoxynivalenol, zearalenone, 3/15-acetyl-deoxynivalenol
Matrix-related mycotoxins	Complexes, physically dissolved or entrapped			
	Covalently bound			
Modified mycotoxins	Biologically modified	Functionalized (phase I metabolites)		
		Conjugated (phase II metabolites)	Conjugated by plants	Deoxynivalenol-3-glucoside
			Conjugated by animals	Deoxynivalenol-3/8/15-glucuronide
			Conjugated by fungi	Zearalenone-14-sulphate Deepoxy-deoxynivalenol Nordeoxynivalenol A-C Deoxynivalenol-sulphonate, Nordeoxynivalenol A-C
	Chemically modified	Different modified Thermally formed Non-thermally formed		

Because of all the mentioned reasons, all of mycotoxins are ignored in food and feed. For this reason, the monitoring of the presence of these potentially dangerous metabolites has become very important nowadays. In this frame, liquid chromatography coupled with mass spectrometry (LC/MS) has been accepted as the gold standard for the determination of available quantities for at least ten years (Righetti et al., 2016). In general, there are three different approaches to the analysis of modified mycotoxins: direct and indirect determination, and also non-targeted analysis. Direct analysis provides advantages when conventional, standardized analytical methods are appropriate, but they should be optimized and adjusted according to their structurally related chemical properties as compared to free mycotoxins. The major disadvantage of the direct assay is that up to now only the reference substance is available for the analysis of a few modified mycotoxin (e.g., DON-3-Glc). During the malting process, a slight increase in DON3G was reported before germination despite a significant drop in DON and ADON. However, during the drying of malt, no significant changes were observed for DON or ADON, but variable results were reported for DON3G (Lancova et al., 2008).

Presence of Modified Mycotoxins in Foods

A limited number of studies have revealed the presence of modified mycotoxins in foods. The products which are mainly affected in the foods are cereal-based products which are at the initial stage of fermentation or a malt process. Studies conducted between 2010 and 2014 to detect mycotoxins have generally focused on grains. Lattanzio and Visconti (2012) reported decreased incidence in tricothecenes and decreased mean concentrations, also: DON3G (55.85% µg / kg), 15ADON (31.37% µg / kg), 3ADON (22.15% µg / kg) and FUS-X (5.5% ug

/ kg). They also reported that the estimated concentration ratios of HT2-G / HT-2 and T2-G / T-2 were 27% and 24%, respectively. Also in the analyzed food, especially beer, is a grain product which is most exposed during the processing and has been examined by many researchers.

A limited number of studies to determine the potential presence of available information has been summarized by the Food Standards Agency of London (2016) as shown in Table 2. However, to our knowledge it has not been any efforts to presence of modified mycotoxins in Turkey. The studies focused on the detection of unmodified species of mycotoxins in general. Thus it is seen that the lack of informative data regarding the potential dangers of modified mycotoxins in Turkey.

Table 2. Summary of modified mycotoxin forms found in different products

Food matrix		**n (sample)**	**Conjugates**	**n (positive)**
Beverage	Pain / Ale	9	DON3Glc	5 (56%)
	Beer	21	DON3Glc	9 (43%)
Herbs and spices		30	β-ZEL, β-ZEL14Glc, ZEN14Glc, ZEN14Sulf	5 (17%)
Cereal products	Baby products	30	DON3Glc	3 (10%)
	Grain products	25	DON3Glc	6 (24%)
	Breakfast cereals	60	DON3Glc	7 (12%)

Mechanisms of Action

In a small number of studies of metabolism on body, the exact mechanism of bioavailability has not been established. Recent studies have identified the hydrolytic fate of D3G during in vitro digestion. D3G was found to be resistant to hydrochloric acid, and it was thought that mammals could not be hydrolyzed in the digestive tract. Some lactic acid bacteria located in the intestinal flora, such as Enterococcus durans, E. mundtii or Lactobacillus plantarum, recover the glucose from the DON, although it is not the hydrolytic effect of human cytosolic β-glucosidase. Although the potential cleavage of D3G is shown during digestion, in vivo studies are necessary to quantitatively describe the DON release from D3G. Initial results suggest that D3G is partially cleaved from DON associated with glucuronide in rat urine. There is little information about the metabolism and toxicological mechanisms of trichothecenin in humans and animals. The bioavailability of DON has been reported to be low in sheep and cows and relatively high in pigs. Seeing whether D3G shows an increased uptake relative to DON is the most interesting result of these studies (Berthiller et al., 2013). B-type trichothecene deoxynivalenol (DON), which is mostly produced by *Fusarium graminearum* and *F. culmorum*, is a substance frequently polluting cereal products all over the world. DON binds to the 60S subunit of the ribosomes to exhibit their biological activity and thus inhibit protein biosynthesis. DON also affects the transcription of pro-inflammatory genes and mRNA stability. In animals, DON causes symptoms such as vomiting, anorexia, weight loss and susceptibility to infectious diseases. In addition, DON affects intestinal integrity and changes the local intestinal immune response. In humans, DON is associated with gastroenteritis attacks (Nagl et al., 2014). Due to the wide variety of adverse health effects, DON has a significant economic impact and is a major concern for public health. As a result, some countries have set a maximum limit or threshold for DON in food and feed. However, masked DON forms arising from conjugation reactions during phase II metabolism in plants have been ignored in the legal regimes of many countries due to the lack of toxicological data. DON is one of the most common mycotoxins in cereal products worldwide. DON is a risk for human and animal health due to its wide side effect effects. Recently, new information on DON metabolism has led to

the use of the biomarker approach to assess human exposure. In some subpopulations, a significant proportion of the individuals tested have been claimed to exceed the maximum acceptable amount for DON. Since the modified mycotoxin deoxynivalenol-3-b-D-glucoside (DON-3-GIc) is partially cleaved during mammalian digestion, further evaluation of DON has been reported to further increase the total mycotoxin burden associated with exposure (Nagl et al., 2015).

Fusarium trichothecenes can be divided into two main groups: Type A and Type B tricothecenes Type A trichothecenes are defined by the absence of a keto (carbonyl) function in carbon atom 8 (C-8), whereas type B trichothecenes are defined by the presence of a keto function at C-8 lt. Also types A and B can be distinguished by the presence (type B) and absence (type A) of a hydroxyl function at C-7. The most concerning tricothecenes in wheat and barley growing areas are DON, nivalenol (NIV) and their acetylated derivatives from B-type tricothecenes. Within the *Fusarium graminearum* species complex, there are three major trichothecene phenotypes (chemotypes), 3-3-ADON, 15-ADON and NIV. Although the strains predominantly tend to produce 3-ADON or 15-ADON, non-acetylated trichothecene DON frequently accumulates in wheat seed, because wheat or fungal esterases (deacetylases) remove acetyl units from 3-ADON or 15-ADON (Alexander et al., 2011).

Various effect mechanisms for FX have been reported. In general, FX is known to induce a ribotoxic stress response that inhibits protein and DNA synthesis in eukaryotic cells. In detail, it causes degradation of eukaryotic poliribosomes at high concentrations in vitro. FX binds to the ribosomes and inhibits the formation of the second peptide bond but does not inhibit polypeptide chain initiation. Furthermore, FX stimulates the DNA strand breakage of dose-dependent and segmented Caco-2 cells. This action is stronger than the action produced by the metabolite NIV. Previously, FX has been reported to exhibit a poor clastogenic effect on Chinese hamster V79-E cells, although these mechanisms suggest that FX is genotoxic in intestinal cells. The mechanisms of action of FX have not yet been fully understood. Further studies are needed to elucidate this problem with various studies that need to be done (Otsubo et al., 1972; Ueno et al., 1973; Mizuno, 1975; Aupanun et al., 2017).

FX alone showed greater toxicity in various cell types, including U-937 macrophages, HL-60 cells, RAW 264.7 mouse macrophages, SF-9 insect cells and Caco-2 cells compared to NIV and other type B trichothecenes (Aupanun et al., 2017). Dual combination of DON and NIV at low concentrations exhibited synergistic toxicity in Caco-2 cells but exhibited antagonistic effects (DON-NIV-FX) in the inverse combination (Alessane-Kpembi et al., 2013). FX is highly toxic to organs containing actively proliferating cells. Following administration of low dose FX (0.1, 0.3 and 0.5 mg / kg BW) in mice, it has been reported to induce lymphocyte apoptosis in lymphoid tissues, including Peyer's patches, thymus and spleen. FX has been reported to have a higher emetic potential than other tested mycotoxins (DON, NIV, 3-ADON and 15-ADON) in minks (Wu et al., 2013). Administration of FX (3.5 mg/kg BW) orally to pregnant mice in fetus brains, especially telencephalon, induced apoptosis (Sutjarit et al., 2014). Besides such toxic effects, administration of FX (5 μg / area) alone in shaved guinea pig skin has been reported to induce erythema and curing caused by deteriorating fibrolytes and cilium cells (Yang et al., 2017).

In an investigation in which individual or combined toxicological effects of DON, NIV and their mycotoxins in multiple deoxynivalenol family, such as acetyl derivatives of 3-ADON, 15-ADON, D3G and FX deoxynivalenol, in vivo, were examined in human gastric epithelium (GES-1) GES-1 cells, and that 3-ADON is less effective at reducing cell viability compared to DON, while the main compounds of 15-ADON and FX in GES-1 cells are slightly stronger than DON and NIV. In general, the ability of mycotoxins to be toxic when assessed individually has been determined in an increasing order to 3-ADONA<15-ADON <DON <FX <NIV. It has been observed that the synergistic cytotoxicity of DON + 15-ADON, DON + NIV and DON +

FX at low and / or moderate inhibitory concentrations (IC10-IC70, IC10-IC80 and IC10-IC40) for the interaction types of mycotoxin mixtures are observed, respectively. It has been determined that 15-ADON + NIV and 15-ADON + FX produce nearly complete antagonistic cytotoxicity on the GES-1 cell model, while FX + NIV results in almost completely synergistic cytotoxicity (Yang et al., 2017). In another study, the effects of feeding with six different diets supplemented with DON levels increased for 45 days on the liver and gene markers of the zebrafish were investigated. In addition to these parameters, long term effects on fertility, juvenile larval swimming activity and global DNA methylation in embryos have been investigated. Adult zebrafish performance was not affected by DON added diets. Liver CYP1A mRNA levels were found to be significantly higher in fish fed with 2.0 ppm DON compared to control, 0.1, 0.5 and 1.5 ppm groups. The gene transcripts of CuZn, SOD and Cyclin G1 increased as the DON content in the diet increased. It was determined that fish fed with 1.5 ppm DON had 22% higher fertility than the control group. It was observed that increased larval swimming activity was observed in the high DON group. The results have been shown that DON undergo detoxification in the liver via the phase 1 system and cause deterioration of the oxidative stability. It has not been determined in this study that the effects observed in egg production and larval swim activity arose whether due to direct interaction of DON with the reproductive organs or oxidative imbalance of liver of the parent emerged as a secondary (Pestka et al., 1987). In this study, the mechanism of action of modified mycotoxins in the embryonic period cannot be fully explained.

Although mycotoxins have different toxic effects, it is a known fact that they all target the immune system. Mycotoxins have immunostimulatory or immunosuppressive effects depending on concentration and parameter. Modified mycotoxins, which are the products of subsequent cleavage of these toxic components, have been reported to have a similarly immunosuppressive effect (Petska et al., 1987; Bouhet et al., 2006). The immune system is the defense mechanism that is primarily responsible for organisms invading the body. Because of contaminated with mycotoxin feed intake, increased sensitivity to infectious diseases leads to problems such as a chronic infection reoccurrence and reduced vaccine efficacy (Pierron et al., 2016). In another study (Accensi et al., 2006), however, it was determined that the modified mycotoxins did not cause immunosuppression.

Future Perspectives

To fully explain the exposure to modified mycotoxins, a more detailed understanding of their occurrence is essential. This is linked to the development of analytical techniques that can detect these compounds in various food matrices. DON3G is a well-researched compound that clearly demonstrates the problem that can occur with high incidence of modified mycotoxins and relatively high concentrations in cereal and cereal-based products. Because of altered physico-chemical properties, modified mycotoxins generally behave differently when compared to free mycotoxins. DON3G is an indicator of how the modified mycotoxin can enter a different reaction when subjected to food processing stages compared to the free mycotoxin DON. The effect of common household techniques such as food processing, industrial processes, as well as boiling of rice or macaroni, can have a profound effect on the presence and concentration of modified mycotoxins and must be extensively investigated. In industrial processes the influence of common domestic techniques such as the processing of food, as well as the boiling of rice or macaroni, can have a profound effect on the presence and concentration of modified mycotoxins and must be extensively investigated. In addition to the processing of food in industrial process, common household techniques such as boiling rice or macaroni can have a profound effect on the presence and concentration of modified mycotoxins and should be extensively investigated. Synthesis of chemical standards, monitoring of formation, toxicity

assessment and toxicokinetic studies of modified forms for this area can be made. Different control strategies have been described to prevent the growth of mycotoxigenic fungi in the pre-harvest period to prevent the growth of mycotoxins in food, as well as different control strategies to prevent mycotoxin contamination: use of resistant varieties before harvesting, good agricultural practices, field management and use of chemical or biological detoxifying agents, timely harvesting and precisely tuned equipment usage and post-harvest storage conditions, modified atmospheres, fumigation, irradiation, use of mycotoxin modifiers. To the best of our knowledge, however, no inhibition, detoxification or neutralization technique is specifically described for modified mycotoxins. It is clear that necessary laws and regulations should be made for this area.

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A STUDY ON THE PREVALENCE OF SALMONELLA BY IMS-PCR AND CONVENTIONAL CULTURE METHODS IN BEEF MEATS SOLD IN ERZURUM, TURKEY

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ABSTRACT

In this study, the presence of *Salmonella* spp. and specific microbiological parameters and were investigated in 30 beef meat samples collected from markets and butcher shops. The specific microbiological parameters were determined using a conventional cultural method and the presence of *Salmonella* spp. in beef meat samples was determined using conventional and immunomagnetic separation (IMS)-polymerase chain reaction (PCR) methods. In addition, antimicrobial susceptibility of the isolates was revealed using the Kirby–Bauer disc diffusion method. The results indicated that 1 of the 30 samples were positive for *Salmonella* spp. by the conventional method, and 8 of the 30 were positive by the IMS-PCR method. These results indicate a high prevalence of *Salmonella* spp. in beef meat samples from Erzurum city, Turkey, and the general microbiological properties should be considered for public health. The results also show that the IMS-PCR technique was superior to the conventional method for detecting *Salmonella* in beef meat.

Keywords: *Salmonella, IMS-PCR, Beef meat, Microbiological*

INTRODUCTION

Meat is a widely-consumed food in world due to its unique aroma and flavor as well as containing important nutrients such as protein, B group of vitamins and iron. It has a critical importance for the growth and development of humans due to nutritional composition contained (Williams, 2007). According to data of Turkish Statistical Institute, beef meat production in Turkey was 1,149,262 in 2015 (COMCEC, 2017).

Meat is an ideal medium for the development of bacteria due to its neutral pH, rich protein content and high-water activity. The meat, which has a highly potential for production and consumption, is contaminated with a variety of pathogen micro-organisms such as, *Salmonella*, Listeria, Escherichia, Shigella and Staphylococcus because of production, transport and selling under unhygienic conditions (Zhao et al., 2001). The World Health Organization reports that one out of every 10 people in the world has been infected with foodborne infections and about 420.000 of these cases have been fatal. On the other hand, the number of high bacteria lead to shortening of shelf life by deterioration of meat and therefore it may cause the economic loss (WHO, 2015).

Salmonella, an ubiquitous, short rod-shaped, facultative and Gram-negative bacteria which pose a public health concern worldwide. The warm-blooded animals such as livestock, wildlife, poultry, and companion animals and humans are host for *Salmonella* spp (D'Aoust and Maurer, 2007; Jalil and Islam, 2012). This enteric bacterium can lead to Salmonellosis by consumption foods that have been contaminated with feces of wild or livestock animals (D'Aoust and Maurer, 2007). According to a report published in 2015 by the Centers for Disease Control and

Prevention (Enterotoxigenic), it is estimated that about 1.2 million people in the U.S. have been exposed to *Salmonella* infections, and that an average of 19.000 hospitalizations and 380 deaths occur from this infection between 2000 and 2008. In addition to the lack of records on foodborne *Salmonella* infections in Turkey, there is a limited number of studies on food containing this microorganism.

Today, there are many methods for isolation and identification of *Salmonella* spp from foods. The main methods used in the detection of *Salmonella* spp. are conventional culture, Polymerase Chain Reaction (PCR) technology, immunology-based, and bacteriophage-based assays (Jeníková et al., 2010). The conventional culturing method, have used from past until today, is laborious and time consuming because of taking 5-7 days (Lee et al., 2015). Pre- and selective enrichment stages followed by streaking onto selective agars and finally verifying the identity of isolates by biochemical tests are the reasons of this situation. The detection of *Salmonella* spp. has a great importance in terms of public health, more rapid and sensitive methods are needed. Despite the providing rapid results in molecular based methods, they have some disadvantages such as requirement of higher level skill, more time and labor in the preparation and removing of the mixture, and PCR inhibition due to possible contamination from food matrix (Liébana et al., 2014). Removing inhibitor components is the most important step in the success of PCR applications and there are several methods to accomplish this (Schrader et al., 2012). IMS is one of them, an effective method for separating target microorganisms from food matrix materials and other microorganisms found in floras. In this method, metallic spheres coated with antibodies specific to the target microorganism are removed by a magnet after adding into the pre-enriched sample. The techniques such as conventional culturing, ELISA, microscopy, electrochemical impedance technology, and PCR are used to identify the separated lysate in the next step (Liébana et al., 2014).

There are not enough studies on the presence of *Salmonella* spp. in beef meats offered for sale in markets in Erzurum, located in the east of Turkey. Therefore, in the present study, it was aimed to determine the presence of *Salmonella* spp. by both classical culture and IMS-PCR technique, and some microbiological properties in meats offered for sale.

MATERIAL AND METHODS

Materials

In total, 30 beef meat samples were collected from 19 different local markets and retail stores, distributed in Erzurum city, Turkey during the May-December 2016. All samples were transferred to the laboratory under cold chain within one hour and microbiological analyzes were carried out on the same day.

Microbiological Analysis

Ten g of each meat samples were transferred to sterile stomacher bag which contained 90 ml of sterile Ringers (Merck, 115525) ¼ solution and mixture was homogenized by a stomacher blender (IUL Instruments, Barcelona, Spain)for 90 s. A series of 10-fold dilutions was prepared in tubes containing 9 ml of sterile Ringer's ¼ solution. Then, each diluted sample (0.1 mL) was plated on proper growth media except for total coliforms (pouring method). Total aerobic mesophilic bacteria were enumerated on Plate Count Agar (PCA) (Merck, 105463), after 48-72 h at 32±1 °C; total coliforms on Violet Red Bile (VRB) Agar (Oxoid, CM0107B) after 48 h at 37±1 °C; Enterococcaceae on Kanamycin Aesculin Azide (KAA) Agar (Oxoid, CM0591) after 24 h at 37±1 °C; Micrococcaceae on Mannitol Salt Agar (MSA) (Oxoid, CM0085) after 48 h at 37±1 °C; Pseudomonas on Pseudomonas Selective agar (Merck, 1.07620) supplemented with CFC (Merck, 1.07627) after 48 h at 25 °C; Yeast and molds on Rose Bengal Chloramphenicol (RBC) agar (Merck, 1.00467) after 5-7 d at 25 °C.

Bacterial strain

Positive control used in PCR assay was obtained from Turkey Public Health Institution Microbiology Reference Laboratories (*Salmonella* Typhimurium RSSK 95091).

Isolation and identification of Salmonella spp.

Conventional Method

Isolation and identification of *Salmonella* spp. was performed according to ISO 6579: 2002. Briefly, 25 g of meat sample was transferred to filtered stomacher bags containing 225 ml of sterile buffered peptone water (Merck, 107228) and homogenized with masticator (Neutec Masticator, Neutec Group, Inc., Farm- ingdale, NY) for 90 s. Homogenized samples were incubated for pre-enrichment for 24 h. Then, 1 mL was transferred to tube containing Muller-Kauffmann tetrathionate/novabiocin broth (MKTTn) (Oxoid, CM1048) supplemented with novobiocin (Oxoid, SR0181) in 10 ml volume and 0.1 mL of pre-enriched solution was transferred to tube containing Rappaport-Vassiliadis (RV) medium (Merck, 1.07700) in 10 mL volume. For selective enrichment, the tubes with RV were incubated at 41.5 °C for 24 h and inoculated tubes with MKTTn were incubated for 37 °C for 24 h. Following the incubation, enrichment samples were streaked onto Xylose Lysine Tergitol-4 (XLT-4) agar (Oxoid, CM1061) supplemented with tergitol (Oxoid, SR0237) and Xylose Lysine Deoxycholate (XLD) agar (Merck 1.05287) and incubated at 37 °C overnight. The colonies with a black centre with pinky-reddish periphery on XLD agar and black or black-centered with a yellow periphery on XLT4 agar were evaluated as suspicious for *Salmonella*. Suspicious colonies of being *Salmonella* spp. were selected and identified by inoculating to tubes containing 7 mL of Triple Sugar İron Agar (TSIA) (Oxoid, CM0277B), Lysine İron Agar (LIA) (Oxoid, CM0381) and urea broth (Merck, 1.08483) using a inoculating needle. Following the incubation of tubes at 37 ºC for 24 h, typical reaction on TSIA (alkaline slant, acid butt, positive H2S and positive/negative gas) and LIA (alkaline slant, alkaline butt, positive H2S) and urea negative cultures were evaluated as suspicious for *Salmonella*. The other biochemical tests were performed by using GN Cards (BioMérieux, Inc., Craponne, France) including 64 different test substrates on VITEK 2 Compact system (BioMérieux) for verifying the isolates. For this purpose, the isolates were incubated at 37 °C for 24 hour on blood agar. A sufficient number of colonies from pure culture were suspended in a polystyrene tube containing 3.0 mL of sterile saline solution (0.45%, pH 4.5). TheMcFarland turbidity of solution was adjusted to 0.5 using a turbidity meter. Then, the suspension was loaded on the GN cards. İdentification of presumptive *Salmonella* spp. isolates was performed on VİTEK2 Compact System (BioMérieux, Marcy l'Étoile, France) within 3 h using fluorescence reading of GN cards. VİTEK2 Compact System Software identified *Salmonella* spp. with a level of 97-99% probability.

IMS Method

Salmonella spp. were separated from pre-enriched samples using Dynabeads® anti-*Salmonella* (ThermoFischer Scientific, 71002) according to the manufacturer's instruction to perform IMS-PCR technique. Briefly, 20 µl of Dynabeads® anti-*Salmonella* was pipetted into 1.5 ml of sterile ependorfs placed in the MPC-S rack. Then, 1 mL pre-enriched samples were added to eppendorfs. Eppendorfs placed on MPC-S rack were incubated with gentle agitation for 10 min, followed by inverting it at least five times to mix the samples and beads. Hereafter, a magnetic plate was placed on the MPC-S rack and recovery of the beads was performed for 3 minutes. After recovery, the supernatant was carefully removed. Following the removal of the magnetic plate from the MPC-S rack, the IMS beads-bacteria complex on the tube wall were washed with 1 mL wash buffer (PBS with 0.05% Tween-20). Washing process was repeated

twice. Buffer-washed dynabead-bacteria complex were diluted with 100 µl of Tris-EDTA buffer (pH, 8). This complex was maintained at -80 ° C until used in the PCR. DNA extraction from the Dynebead-bacterial complex was performed by the boiling method. For this purpose, 100 µl of Tris-EDTA buffer solution (pH, 8.0) containing dynebead-bacteria was boiled for 10 min. At the end of the boiling, the samples were cooled on ice and centrifuged at 10.000 g for 15 sec. The supernatant obtained by centrifugation was used as a directly DNA template.

PCR Method

PCR primers (invAFW: 5'-ACA GTG CTC GTT TAC GAC CTG AAT-3'; invARV 5'-AGA CGA CTG GTA CTG ATC GAT AAT-3') were used to specifically amplify a 284-bp genomic fragment of the invA gen, which is highly specific for *Salmonella* spp. for PCR assays of the isolates. The PCR amplifications were performed in a total volume of 15 µL solution containing 2 µl of template DNA, 1× PCR buffer (Sigma), 0.25 mM MgCl2 (Sigma), 200 µM (each) dNTP (Sigma), 10 pmol of each primer, 1.25 U of Taq polymerase (Sigma). The PCR cycle condition was an initial denaturation at 95°C for 10 min; 30 cycles of 95°C 30 s, 55°C 30 s and 72°C 30 s; and a final extention at 72°C for 5 min. The amplified products were detected by electrophoresis in a 1% agarose gel in Tris/Borate/EDTA Buffer (TBE, pH 8.3) pre-stained with ethidium bromide. Then agarose gel visualized in UV light using Gel Doc™ XR+ Gel Documentation System (BioRad, USA).

RESULTS

Microbiological Characteristics of Meat samples

The microbial counts (log10 CFU/g) data for meat samples collected from 19 retail markets are presented in Table 1.

As shown in Table 1, the total aerobic mesophilic bacteria count (TAMB), Pseudomonas, yeast and moulds, coliform bacteria, Enterococcus and Staphylococcus/Micrococcus counts of the 30 beef meat samples showed differences of <10–9.69, < 10–6.17, < 10–5.69, < 10–7.06, < 10–4.76, and < 10–5.52, respectively.

Table 1. Some microbiogical characteristics of beef meats

Total Aerobic Mesophilic Bacteria	Pseudomonads	Yeast and Moulds	Coliform	Enterococci	Staph-Micrococci
<10	<10	<10	3.60	<10	2.70
<10	3.00	<10	<10	<10	<10
4.78	<10	3.52	4.70	2.30	4.37
5.00	<10	<10	4.48	2.85	<10
5.00	<10	4.00	<10	<10	<10
5.06	2.85	4.04	3.60	3.41	4.63
5.27	3.78	4.56	3.62	<10	5.07
5.48	4.00	4.28	5.78	<10	5.32
5.59	2.52	4.42	4.70	2.70	3.74
5.72	2.00	<10	2.48	2.60	2.00
5.95	5.81	4.91	3.46	<10	4.78
6.00	3.30	<10	3.60	3.04	<10
6.00	4.20	<10	4.13	2.00	3.26
6.00	6.17	4.00	3.30	<10	<10

6.03	3.31	3.30	4.06	<10	4.23
6.18	3.70	3.95	5.20	2.90	4.69
6.41	4.02	5.28	4.85	3.85	3.57
6.45	<10	<10	4.49	<10	5.52
6.55	2.00	3.00	4.87	3.28	4.06
6.64	3.44	4.41	4.13	4.47	4.89
6.71	2.48	3.00	3.30	3.28	4.23
6.93	4.93	<10	5.85	3.97	4.36
7.00	<10	4.97	5.23	<10	4.48
7.06	3.43	4.09	7.06	3.78	5.11
7.26	6.15	5.69	4.53	1.30	4.40
7.36	<10	<10	6.32	<10	4.00
7.75	5.34	4.60	7.06	4.76	5.20
7.81	4.79	3.78	<10	3.00	5.17
8.09	4.78	4.70	3.96	1.60	4.98
9.69	<10	5.35	<10	3.30	4.85

The presence of Salmonella spp. In beef meat samples

30 of the one (3.33%) beef meat samples were positive for *Salmonella* spp. by the conventional method, whereas eight of the 30 (26.67%) samples were positive by the IMS-PCR method (Table 2).

Table 2. *Salmonella* spp. results that were determined by conventional and IMS-PCR methods

	Conventional	IMS/PCR	Both methods
n/N	1/30	8/30	0/30
%	3.33	26.67	0

DISCUSSION

The general microbiological quality parameters of the samples were determined with respect to TAMB, Pseudomonas, yeast and mould, coliform bacteria, Enterococcus and Staphylococcus/Micrococcus counts. According to the Food Standard, the presence of *Salmonella* spp., coliform and other bacteria are accepted as the hygiene index (ISO 2001, 2002). However, the results obtained here show that the samples did not meet the standards with respect to the presence of microorganisms. A number of bacteria shortens shelf life by deteriorating meat quality, resulting in an economic loss. Our results seem to be due to processing and storage conditions as well as cross-contamination after processing in the markets and homes.

Salmonellosis is a widespread, foodborne zoonotic disease in the world and has been reported to affect over 90.000 people in European countries each year (EFSA, 2014b). The agent is taken orally by especially foods contaminated with feces. In food, meat plays an important role in the emergence of diseases. Increased food contamination, immune system diseases, gastrointestinal disturbances, and age are associated with increased risk of salmonellosis cases with non-specific symptoms (Cianflone, 2008). Not only in Turkey, but also in most developing countries, the absence of an epidemiological surveillance system of salmonellosis cases makes it difficult to assess the prevalence effectively (KÄFerstein, 2003). However, the number of cases of gastroenteritis due to *Salmonella* spp. was 1993 in 2008, but

this number increased to 2307 in 2011, according to the data unpublished by the THSK Department of Communicable Diseases (THSK, 2015). Comparing the data obtained in this study with other studies reveals that meat is an important food source in contamination with *Salmonella* spp. It is thought that the difference of *Salmonella* spp. prevelance data between previous studies and present study may be due to factors such as hygiene and sanitation conditions, methodological differences using detection, transportation and storage conditions in the markets and localization (Straver et al., 2007, Li et al., 2013). Beef meat is contaminated with *Salmonella* spp. due to processing of meat in non-hygienic conditions, low personnel hygiene, uncleaned tools and equipment used in production in a proper manner, and cross-contamination reasons in markets where sales are offered (İseri and Erol, 2010). In the present study, *Salmonella* spp. was identified in 38 (37.25%) meat samples by IMS-PCR technique and in 30 (30.39%) meat samples by conventional method. According to the Turkish Food Codex Communiqué on Microbiological Criteria, it has been reported that *Salmonella* should not be present for both meat types (TFC, 2011). Adiguzel et al. (2012) reported that *Salmonella* spp. could isolated from 4 (2.86%) of the 140 beef samples collected from markets in Erzurum. In our study, the number of *Salmonella* spp. positive samples isolated by the conventional method was one sample (3.33%), whereas 8 samples (26.67%) of *Salmonella* spp. positive samples were found by IMS-PCR technique. In this study, the prevalence of *Salmonella* detected in red meat by IMS-PCR technique is similar to that of meat of the same type: 23.3% (21/90) in Egypt (Sallam et al., 2014), 20% (38/189) in Iran (Soltan Dallal et al., 2014) and 17% (13/78) in China (Yang et al., 2010). However, the prevalence is lower than in some countries: 62% (31/50) in Vietnam (Van et al., 2007) and 57.08% (240/137) in Ghana (Adzitey et al., 2015). All results indicate that poultry meat is an important source of danger for *Salmonella* spp. infections.

The study results show that the contamination rate of poultry meat with *Salmonella* spp. is higher than the beef. When IMS-PCR and conventional method used for detection of *Salmonella* were compared, IMS-PCR technique was found to be superior. The superiority of IMS-PCR over the conventional method may be due to concentrated the target bacterium in the samples, the removal of inhibitor components and the elimination of other groups of microorganisms. Similar results were obtained by Siriken et al. (2015) who performed detection of *Salmonella* spp in beef and poultry meat by both conventional and IMS method (36.00%, IMS; 25.33%, conventional). The IMS-PCR technique is a method that allows rapid detection of *Salmonella* spp. after IMS. Zheng et al. (2016) reported that IMS-PCR showed an accuracy of 98.3%.

CONCLUSIONS

In this study, 30 beef meat samples were analysed for the presence of *Salmonella* spp. The results showed that one of the 30 samples were positive for *Salmonella* spp. by the conventional method, while eight of the 30 were positive by IMS-PCR. In conclusion, combining the IMS and PCR methods was used effectively to isolate *Salmonella* spp. from beef meats than either method alone. Taken together, it is evident that beef meat is a serious public health risk in this region in terms of the presence of *Salmonella* spp. in beef meats and microbiological properties of beef meats.

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EFFECT OF BROILER GENETIC STRAIN ON MEAT QUALITY CHARACTERISTICS

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ABSTRACT

The present work aims to evaluate the effect of broiler genetic type on meat quality and sensory acceptability. Two commercial broiler strains were studied:a fast-growing strain (Cobb and Arbor) and the slow-growing poultry strain (JV), that has been genetically selected to improve production traits of broilers and maximize the profitability of chicken meat production. A post-mortem inspection showed that the general trauma rate was higher in both Arbor and Cobb strains compared to the JV strain ($p<0.05$). Physicochemical parameters evaluation showed a significant difference between the studied poultry strains ($p<0.05$). Statistical analysis revealed that pH was positively correlated with cooking loss ant the color parameter a* but it was negatively correlated with water loss, exudate and color parameter b*. Moreover, the textural analysis showed that the broiler from Cobb strain was significantly difficult to chew. In addition, no differences ($p> 0.05$) existed among breast meat from the different strains with respect to consumer acceptability of appearance and overall acceptability. Breast meat from Arbor strain was slightly preferred ($p< 0.05$) with respect to color, aroma and juiciness.

Keywords: *Broiler genetic type, Fast-growing strain, Slow-growing strain, Meat quality, Economic profitability*

INTRODUCTION

In Tunisia, poultry sector plays an important role in the national economy and make a significant contribution to food security. This development is accompanied by consumer's interest in broiler meat because it is cheaper, more versatile, and is perceived to give more health benefits than red meat (Pandurevic et al., 2014).

The poultry industry is always trying to change, adapt and evaluate its strategies to meet the consumer expectations meat products quality while also preserving a respectable economic profitability. The most factors directly related to meat quality are pre- and post-slaughter practices, bird age, strain, sex, environment and nutrition (Le Bihan-Duval et al., 2002).

The objective of this study was to evaluate the effect of broiler genetic type on economic profitability, meat quality and sensory acceptability.

MATERIAL AND METHODS

Experimental birds and samples preparation

The trial and bird slaughter were carried out at a poultry company –Tunisia. Laboratory analyses were performed in High School of food Industry.

Total of 1000 chicken from two commercial genetic broilers strains (fast-growing strain (Cobb and Arbor); slow-growing poultry strain (JV)) were used to examine strain effect on meat quality and sensory evaluation.

Trauma evaluation

A post mortem examination was made on 1000 broiler carcasses per trip for trauma evaluation. Causes of trauma in broilers were evaluated by three methods : Five Ws, Brainstorming and Weighted voting methods.

pH

The pH of the samples was measured by inserting electrodes into the breast muscle using a pHmeter system (Consort C830 pHmeter) (Le Bihan-Duval et al.2007).

Exudate, cooking and thawing losses

Exudate losse (EL) was determined according to Offer (1991) method. Breast is weighed 24 h after slaughter (raw weight), then, stored at 4 °C for 6 days in filmed container with an absorbent paper. Exudate loss was calculated as follow: %EL= [(raw weigh – weigh after 6 days of storage)/ raw weigh]x100

Cooking losse (CL) was determined 24 hours after slaughter, according to a modified methodology proposed by Cason et al. (1997). Raw breast meat samples were weighed and packaged, then steam-cooked in a water-bath at 80ºC for 15 minutes. After this procedure, the samples were cooled at room temperature and re-weighed. CL was calculated as the difference between the initial and the final weight.

Thawin loss (ThL) was determined by immersing breast slices in liquid ethanol, cooled to -18 °C (for 12 hours) and stored at -20 °C. The frozen slices were thawed at + 4 °C for 12 hours, wiped and weighed. %ThL = [(weight before thaw - weight after thaw)/weight before thaw] × 100.

Total fat content

Total lipids from the breast meat were extracted according to the method described in NT 53.15 (1984).

Sensory panel

Samples of cooked breast were presented to trained panelists (n=42) in balanced, random, monadic order in individual booths in a sensory laboratory. Numerological scale (1= dislike extremely and 7= like extremely) was used to evaluate: appearance, color, aroma, juiciness, taste and overall acceptabilityn of samples.

Color measurements

A Minolta Chroma Meter CR-300 was used to evaluate color parameters L*,a* and b* where L* represents degree of lightness (0 = black to 100 = white), a* represents green (−a*) to red (+a*), and b* represents blue (−b*) to yellow (+b*). The color values were measured at three different sites on the same sample after 24 h Slaughtering (Olivo et al., 2001).

Instrumental texture measurements

Texture measurements were determined using Texture analyzer (model TVT 6700 Perten). Double compression, shear and penetration tests were conducted according to Honikel (1998).

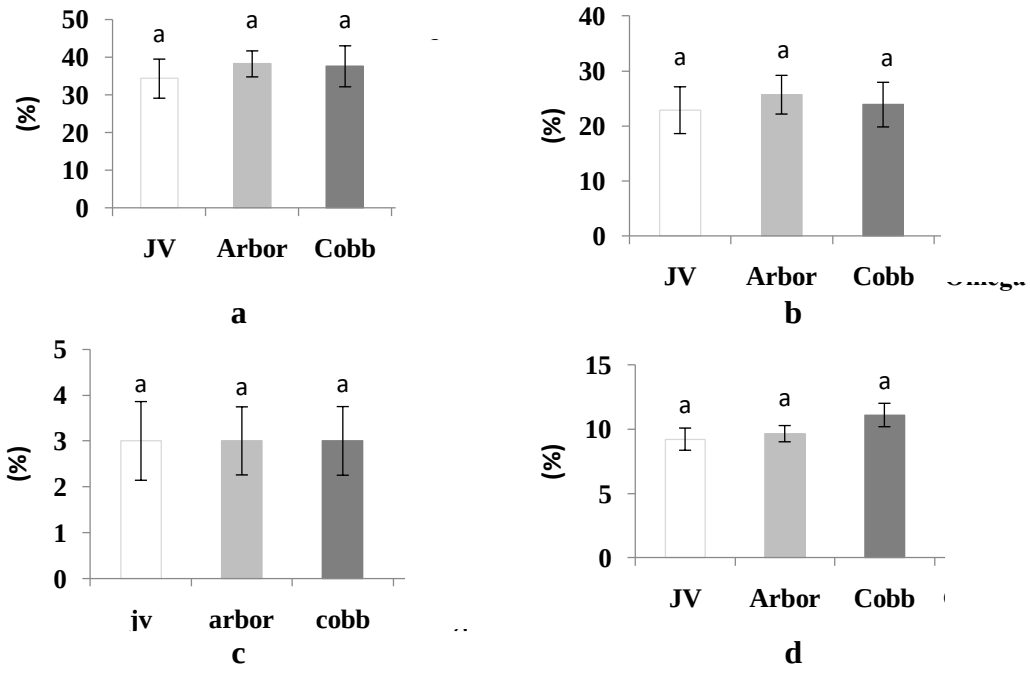
Statistical evaluation

Data were presented as the mean of triplicate± standard deviation (mean ± SD). The data were analyzed for statistical significance using Statgraphics Centurion XVI. Differences between treatments were assessed using one way ANOVA, followed by Tukey HSD post hoctest. P values below 0.05 were considered significant.

RESULTS

Trauma evaluation

Trauma represent the most frequent factors causing the deterioration of the organoleptic quality of broiler meats leading to significant economic losses. In this study, the trauma evaluation is carried out on wings, fillet and thighs (Figure 1).



For all values with the same letter, the difference between the means is not statistically significant

Figure 1. Trauma evaluation: (a) general rate; (b) trauma wings; (c) trauma fillet (c) and (d) trauma thighs

General trauma rate is higher in both Arbor and Cobb strains. An important traumatic injury is observed on chicken wings. Genetic strain does not have a significant effect on the trauma. The quality approach reveals many causes illustrated in the following figure.

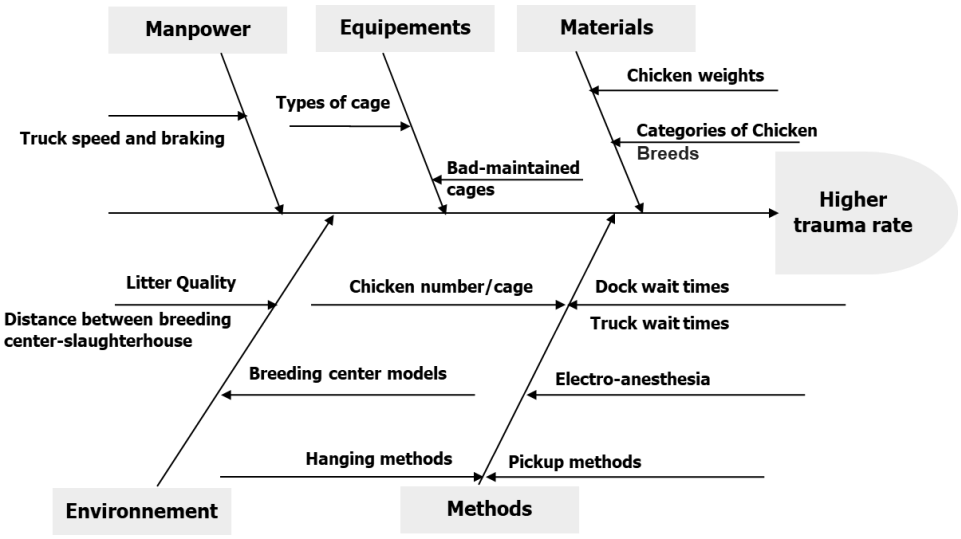


Figure 2. Ishikawa Diagram of meat trauma problem

Physicochemical parameters

Physical properties of studied broiler meat are presented in Table 1. According to results, Fast-growing strains present the highest exudate and cooking losses ($p<0,05$). Arbor strain present the highest total fat content about 0,79%.

the pH values were ranging from 5.5 to 5.8. Slow-growing strain (JV) has the lowest value of pH (pH= 5.80±0.06).

Table 1. Physicochemical parameters of broiler breast

	Strains		
	JV	Arbor	Cobb
Total fat content (%)	0.31^{a}	0.79^{b}	0.21^{c}
pHu	$5.80\pm0.06^{b.c}$	$5.63\pm0.08^{a.b}$	5.57 ± 0.02^{a}
%Exudate	42.41 ± 2.15^{a}	44.16 ± 0.49^{a}	68.57 ± 0.16^{b}
%cooking loss	26.15 ± 0.18^{a}	33.33 ± 0.20^{c}	30.26 ± 0.31^{b}
%thawing loss	21.92 ± 2.50^{a}	19.64 ± 5.97^{a}	17.29 ± 2.71^{a}

For all values with the same letter, the difference between the means is not statistically significant

Sensorial analysis

The results obtained in sensory analysis of the studied strains were presented in Figure 3. It was revealed a significant difference between samples in term of color, aroma, juiciness, taste and aftertaste. JV and Arbor strains have the maximum overall acceptability. The overall acceptability score for these samples were 5.5 and 5, respectively, on a 7-point hedonic scale. Also, it seems that the genetic strain did not affect the consumer acceptance.

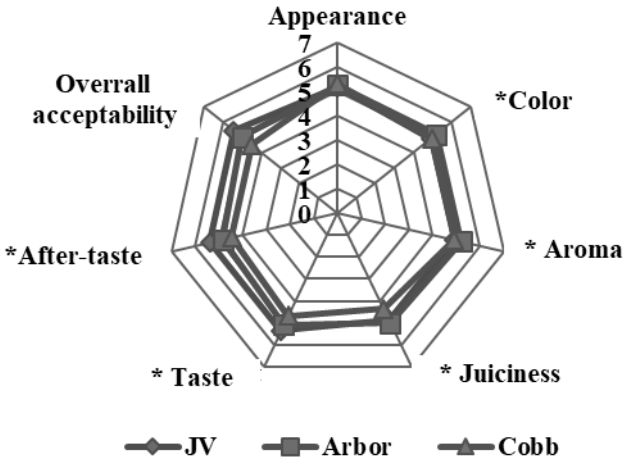
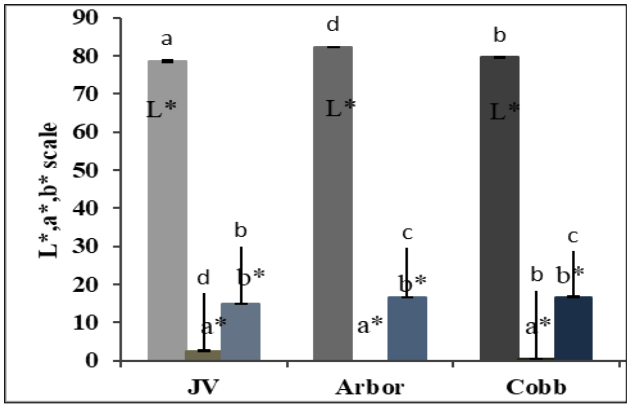


Figure 3. Sensory Evaluation of broiler filet of studied strains (* p<0.05)

Statistical analysis shows that there is a significant difference for the color components between the studied strains (figure 4).



For all values with the same letter, the difference between the means is not statistically significant

Figure 4. Cie L*a*b* parameters of broiler meat

Texture measurements were conducted to note that Cobb strain present a significant higher hardness, springiness, chewiness and resilence values. Shear and penetration tests show that Cobb has significantly the lowest values with an important adhesiveness (Table 2).

Table 2. Texture Parameters

	Strain		
	JV	Arbor	Cobb
Double compression test			
Hardness (g)	5255.00±76.00[a]	5505.00±4.00[a]	6906.00±7.00[b]
Fmax2(g)	4637.50±49.50[a]	4704.00±36.00[a]	6219.00±43.00[b]
Cohesiveness	0.66±0.03[a]	0.65±0.03[a]	0.76±0.01[a]
Springiness	0.50±0.00[a]	0.55±0.01[a]	0.61±0.01[b]
Chewiness	1728.50±47.50[a]	1981.00±121.00[a]	3251.50±30.50[b]
Resilience	0.32±0.00[a]	0.34±0.02[a]	0.44±0.01[b]
Shear test			
Fmax(g)	2142.00±52.00[a]	3183.50±7.50[b]	1704.00±51.00[c]
Wmax(g.min-1)	2552.89±50.59[a]	4611.99±287.87[b]	2142.77±431.73[a]
Penetration test			
Penetration Force (g)	94.67±1.78[a]	108.50±0.50[b]	84.50±0.50[c]
Adhesiveness	2.84±0.16[a]	3.08±0.27[a]	2.22±0.08[b]

DISCUSSION

The analysis of bird behavior on the slaughter lines indicate that heavy strains try to straighten themselves by flapping their wings (LeBihan-Duval et al., 2001). Hence the myoglobin content increases; which generates the appearance of hematomas and traumatic injuries.

After slaughter, postmortem glycolysis is activated and accumulation of lactic acid in the muscle is increased, which results in a decline in pH. This pH value is one of the important parameters for quality profiling of meat (El Rammouz et al., 2004). No significant strain effects were observed in muscle, suggesting that no protein denaturation issues existed within strain (Schilling et al., 2008). Previous studies have reported that lower pH (5.7) at 24 h postmortem indicates poor meat quality that is characterized by protein damage, lighter meat color, and reduced water-holding capacity, all of those being typical characteristics of pale, soft, and exudative meat (Van Laack et al., 2000).

Cobb strain show the lowest pHu value and the highest percentage of exudate loss. Indeed, heavy strains are characterized by a rapid decrease of pHu leading to increase exudate loss (Ngoka et al., 1982).

Boulianne and King (1995) report that pHu affect color. the pH value of the dark color pectorals is higher than the normal color pectorals. Thus, the luminance decreases and the red color index increases when pH increases. A low pH values lead to increase the width of

extracellular spaces and the reflection of the incident light (bloodless). Thus, fast-growing strains were lighter, less red and more yellow.

Although Arbor and Cobb strains belong to fast-growing strains, we note a significant difference between both of strains in term of texture measures. This suggests that the rearing mode does not affect the textural quality of broiler meat.

CONCLUSIONS

This study show that genetic strain does not have a significant effect on trauma meat apparition. However, fast-growing strains (Cobb and Arbor) present the highest general trauma rate. The quality approach reveals many causes related to : collection method, hanging method, type of cage and its maintenance, weight of chickens, distance between breeding center and slaughterhouse, type of breeding center, strains.

Physicochemical parameters (pHu, exudate, cooking losses).and sensorial analysis (CIE L*a*b* color scale, sensory panel, texture measurements) were significantly influenced by genetic strains.

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INFLUENCE OF THE NITROGEN FERTILIZATION ON THE YIELD OF TRITICALE GRAIN VARIETY VIHREN

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ABSTRACT

The aim of this article is to show the analysis of yields results and some of its components found in triticale, fertilized with different nitrogen rates. In this respect, during the period 2011-2013, at the Centre for Agricultural Research in Sredets the Polish experience of the block method has been used in 4 repetitions, with size of the harvest plot of 20 m^2 without irrigation. The subject of the study was triticale, Vihren variety, grown at three nitrogen levels: T_0 - (control) - without nitrogen fertilization; T_1- N_{60}; T_2- N_{100} and T_3- N_{140} on $P_{10}K_5$ background. Nitrogen rate has been introduced once before the active vegetation of the plants in the form of ammonium nitrate. Sowing has been carried out with seeding rate of 560 hp, after predecessor coriander. Except for the tested factor, the remaining agro-technological practices were in line with the triticale breeding technology adopted for the region. Grain yields (kg/ha) and some of its structural elements have been reported - number of stalks/m^2, plant height (cm), stalk length (cm), number of grains in a stalk, grain weight in a stalk (g) and the mass of 1000 grains (g). It has been found that grain yields increase from 10 to 38% when fertilizing with increasing nitrogen rates. The values of the indicators - number of stalks/m^2 and number of grains in a stalk increase and reach the maximum values for fertilization N_{14}. In the conditions of Strandzha, for Vihren variety growing the most efficient fertilization is with N_{10}.
Keywords: *Strandzha, Triticale, Fertilization, Yield*.

INTRODUCTION

Triticale */Triticosecale* Wittm./ has been an artificially created intergeneric hybrid that has been officially introduced into production for more than 50 years. It has a wide popularity as a forage, food and technical crops. It also has a number of agrotechnical advantages: the possibility of growing on poorer and acidic soils, high resistance to fungal diseases, very good drought- and winter resistance, less demanding to the dietary regimen. This makes triticale crops ecologically cleaner and cheaper to grow (Baychev, 2014). It is often considered as an alternative to wheat and other cereals, especially in areas with less favorable soil and climatic conditions (Kim et al., 2001; Ammar et al., 2004; Kolev et al., 2005; Kirchev et al., 2010; Cushla Mcgoverin et al 2011; Baychev, V. 2013.).

Method for optimal expression of biological opportunities and adapt to the soil and climatic conditions of different regions is to improve the technology of the crops /culture/. One of the main agronomic factors for this purpose is a mineral fertilizer, especially nitrogen nutrition. Kirchev et al., 2005; Gibson et al., 2007; Lestingi et al., 2010; Kuang, 20011; Hristov, 2014). Kirchev et al. (2014) establish that triticale cultivated in the Thrace area conditions showed greater responsiveness to increasing nitrogen fertilization, and in Dobrudzha area conditions there has been established a limit in the range $N_{120\text{-}160}$. Gushevilov (2001) determines the effect of lower and medium fertilization norms in the cultivation of triticale on gray forest soil and concludes that liming and fertilization are mandatory triticale cultivation measures. From provedeneni earlier studies (Tanchev, D. et al., 1989; Dimitrova-Doneva, 2007) on leached cinnamic forest soil in the Strandza area there has been established that on a background of

$P_{100}K_{60}$ an effective rate of fertilization of the Persenk variety with nitrogen has been $_{100\text{-}140}$ кg/ha, and when growing after preceding stubble, rape and sorghum: fertilization with 100 кg/ha nitrogen. Studies on the biological and economic qualities of triticale in different soil and climatic conditions and cultivation factors allows for a better use, especially in poor and acidic soils, for increasing the production of grain per unit area, respectively. increasing the attractiveness of these crops. The aim of our study was to determine the optimal fertilization rates in triticale for grain in the conditions of the Strandzha area.

MATERIAL AND METHODS

The study has been conducted in 2011-2013 at the experimental field of the MOA /the Municipal Office for Agriculture/ in the town of Sredets, (42034`27018`) at an altitude of 47 m a. s. l. and covers a terrain typical of the area. The soil type on which the experiment is based on, is leached cinnamic forest soil with a slightly clayey mechanical composition, a shallow humus horizon (20-25 cm), a humus content below 2.25%, a slightly acidic reaction, poorly loaded with phosphorus, and well-stocked with potassium.in the experimental field of the PES Sredetz (42034`27018`) at an altitude of 47 m a. s. l. and covers a terrain typical of the area. The soil type on which the experiment is based is leached cinnamon forest soil with a slightly clayey mechanical composition, a shallow humus horizon (20-25 cm), a humus content below 2.25%, a slightly acidic reaction, poorly loaded with phosphorus, and well-stocked with potassium.

The object of the study is triticale, Vihren variety grown at three nitrogen norms: T_0 – (control)- without nitrogen fertilization; T_1- N_{60}; T_2- N_{100} и T_3- N_{140} at a background of $P_{100}K_{50}$. The nitrogen norm is introduced once before the active spring vegetation of the plants, under the form of ammonium nitrate. The sowing has been carried out with a sowing rate of 560x / m2, after a predecessor of coriander. Except for the test factor, the other agro-technological practices are in line with the triticale breeding technology adopted for the region. The experimental design is a randomized block in four replications of $25m^2$. Grain yield (kg / ha) and some of its structural elements - number of triticale ears /heads//m^2, number of grains in ear, grain weight in ear (g) and mass per 1000 grains (g) have been reported.

Experimental data processing has been performed using the Microsoft Excel Xp programme package.

RESULTS AND DISCUSSION

The area of the experimental field belongs to the Strandzha climate zone of the Continental-Mediterranean climatic region. Its special features are determined by the joint influence of the Black Sea and the Strandzha Mountain. Characteristic of the region is the much softer and more precipitation winter, compared to the European-continental part. Spring is felt in the first half of March. In summer, although the temperature differences are not large, there are droughts in the period July-August. Spring is warmer and longer and autumn is cool. The microclimate of the experimental field does not differ significantly from that of the entire area, both in terms of temperatures and rainfall distribution.

The meteorological conditions during the three years of the survey (2011-2013) differ substantially in terms of both the average monthly air temperatures and the monthly precipitation values, compared to the climatic norm for the Sredets region, but are similar in terms of each other (Table 1).

The first harvest year (2010/11) has been characterized as moderately warm, with a temperature closest to the average temperature for the multiannual period, but with regard to moisture for the vegetation period and months, there are significant differences. The rainfall

measured during vegetation (488.1 mm) is significantly less than rainfall over the multiannual period (583.1 mm) and the other two years. Autumn - the winter period is characterized by rainfall close to the climatic norm and favors the initial development of culture. With regard to the great importance of sufficient rainfall in the spring for the production of triticale, especially in April-May, their distribution in 2010/11 is favorable, which is a prerequisite for high crop productivity.

In temperature terms, the second year (2011/12) of the study differs significantly. The winter months are colder and the average monthly temperatures in January and February are negative (-0.3^{0}C and -1.2^{0}C), but due to the snow cover, the frosting of the crops was not taken into account. Temperatures in April - June exceed the temperature range and set the period as warmer and worse temperature mode. Autumn is characterized by a lot of rainfall in October (when the highest rainfall is measured during this harvest year, 149 mm) and relatively dry November, the precipitation conditions in December-February lead to a good winter-spring stock of soil moisture. March-June are unevenly distributed, especially in May, when they are 117.8 mm, which is 57.7 mm above the May norm.

Table 1. Rainfall and average monthly temperatures during the vegetation of triticale in Sredets during the period 2011-2013

Precipitapion during the vegetation of triticale in Sredets for 2011-13, mm					Average daily temperatures during the vegetation of triticale in Sredets for 2011-13,^{0}C				
Years						Years			
Months	Perennial volues	2010/ 11	2011/ 12	2012/13		Perennial volues	2010/11	2011/12	2012/13
X	64	77.2	149	92.1		13.7	11.7	10.8	16
XI	70.1	60	0.6	12.9		9.1	10.5	4.6	9.2
XII	67.2	48.3	64.1	106		4.4	3.7	3.8	1.6
I	60.2	54.4	71.5	46.8		1.8	1.9	-0.3	2
II	47	34.6	46.2	49.8		3.6	1.6	-1.2	4.4
III	45.3	31	7.6	46.5		6.5	6.5	6.5	7
IV	48.7	89.1	47.6	26.8		11.6	10	13.9	13
V	60.1	42	117.8	19.5		16.8	16.3	16.9	18.8
VI	66.3	30.5	20.6	74.9		20.8	21.2	22.4	21
VII	54.2	21	10	27.6		23.3	24.7	25.9	22.5
∑	583.1	488.1	535	502.9	$\bar{X}$	11.16	10.81	10.33	11.55

The third harvest year can be defined as quite different in temperature relative to the multi-year period. The vegetation period is with higher average monthly temperatures than the other two vegetation periods, too. Except for December, temperatures in the other months are higher than the climatic norm. Particularly in April and May, when combined with less rainfall, they

create unfavorable conditions for forming more and better quality grain. The rainfall at the beginning of June somewhat compensates for stress and helps to obtain good yields.

The number of classical stems is a major component of yields in other cereals as well as in triticale (Skuodiene and Nekrosiene, 2009). In the present study, differences in its values were established in relation to the years and the level of the nitrogen norm (Figure 1).

The number of crop-ear stems is a major component of yields in other cereals as well as in triticale (Skuodiene and Nekrosiene, 2009). In the present study, differences in its values were established in relation to the years and the level of the nitrogen norm (Figure 1).

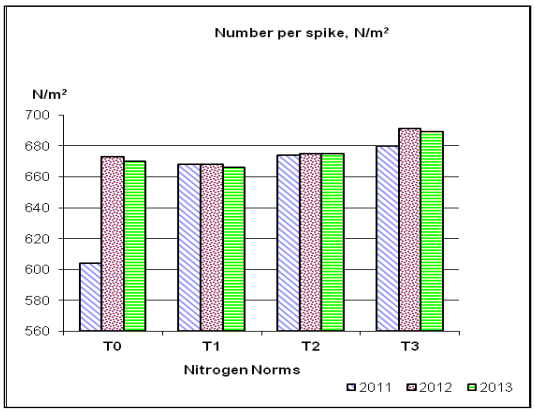

Figure 1. Number of ear stems

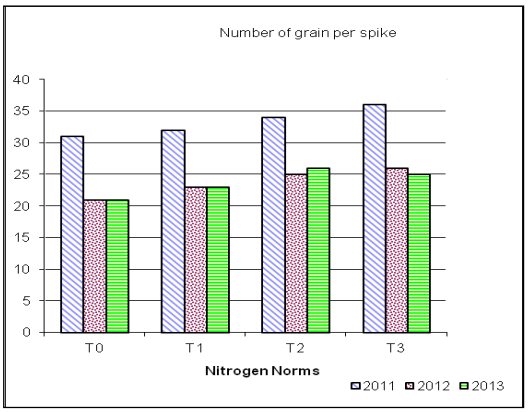

Figure 2. Number of grains in an ear

Table 2. Effect of conditions and nitrogen fertilization on some triticale yield components.

Sourceof variantion	Year			Fertilization			Interaction Year X Fertilization		
	MS	η (%)	Fexp	MS	η (%)	Fexp	MS	η (%)	Fexp
NS/m²	2392.33*	22.43	23.151	2903.556*	40.83	28.09892	1306.55*	12,64	36.74
NGS	938*	83.15	75.375	184*	16.31	9.8571	6*	0.53	0.160714
WGS	0.0037*	0.87	4.353	0.272222*	95.71	320.2614	0.004856*	3,42	5.712418
W100G	890.9858*	88.5	6838.124	62.4475*	9.35	479.3412	7. 145833*	2.15	54.85

In year 2011, 577 ears / m^2 have been formed, and in 2012-677 pcs./ m^2. When fertilizing with N_{140}, they have reached maximum values, with an average increase over the period of the net variation being 38pcs. (5.9%). By the data on the effect of year and nitrogen fertilization factors on the structural components (Table 2) there has been seen, that the influence of the factors has been statistically proven. The highest one is that of the fertilization (40.83%), followed by the interaction between fertilization and year - with 36.74%, and the year - with 22.43%.

The variation of the values of the number of grains in an ear (Figure 2) has been from 23.7 in 2012 to 33.3 in 2011. The impact of the year has been stronger than that of fertilization, since the change in the values is up to 10 grains in an ear, and under the influence of fertilization - up to 5 grains, the difference between the different levels of the nitrogen norm is 2-3 grains. The results are explained by the favorable combination of climatic conditions during the formation of the grain (February-March), and the possibility of absorbing nitrogen from the fertilizer norms. This is confirmed by the results in Table 2.

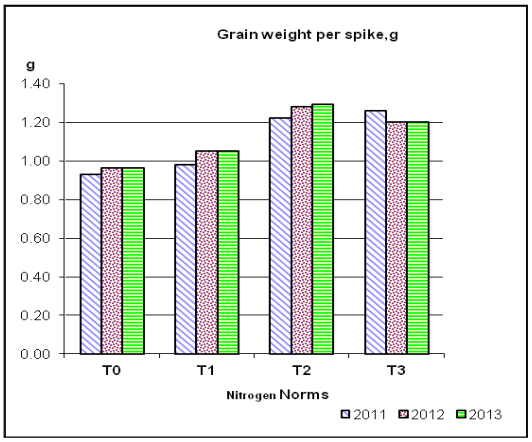

Figure 3. Weight of grain in an ear

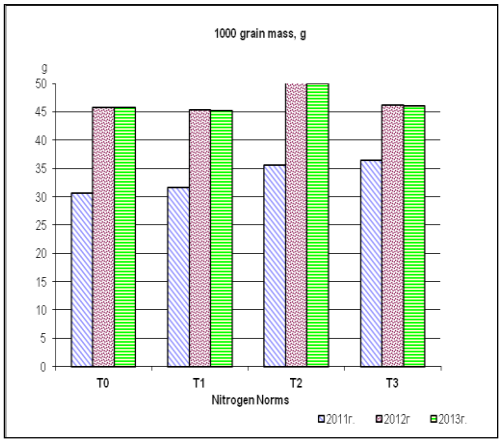

Figure 4. 1000 grains mass

When comparing the ear grain mass in variants (Figure 3), it has been found that the changes in the values by years are small and insignificant. The fertilizer variants form more grain in an ear. The greatest effect had had the average nitrogen rate (N_{100}), with the increase over the previous nitrogen norm (N_{60}), being the largest one: 22%. The most powerful impact has been the fertilization in 2011, when there is also the greatest increase in grain mass. Nitrogen treatment is the key factor for the grain weight variation in an ear (95.71%).

1000 grains' mass (Figure 4) is a qualitative feature, defining the value of seeds as a sowing material. According to some authors (Đekić 2014) nitrogen feed affects positively the mass of 1000 grains, while others (Dumbravă, 2016) consider that nitrogen fertilization increases the productivity of the triticale, but does not affect the mass of 1000 grains. The results of the experiment show that the absolute grain mass is positively influenced by the applied nitrogen fertilization, but with a different effect. In the case of T_2 fertilizers, it had grown by 5 - 6g compared to the unspent mass, and had slightly decreased with the fertilization with T_3 variants. It is striking the similar response (almost equalized values by variants) of the crop in years 2012 and 2013.

By the grain yield data (Table 3) is established, that all fertilized variants exceed in yield the non-fertilized ones, which indicates the impact of fertilization. It creates more favorable conditions for growth and development of plants and realization of their productive capacity in the agro-climatic conditions of the region. Without the use of nitrogen fertilization, the yield is the result of soil fertility and varies from 4720 in 2011 to 5500 kg / ha in 2013, which is almost in line with that obtained in 2012. Testing of increasing nitrogen norms (60, 100 and 140 kg / ha N) significantly increases grain yield with triticale in each year of the study, with the highest yield being obtained with fertilization with T_2 -7500 kg/ha in 2012 and 7300 kg/ha in 2013 with a relative increase relative to the net control of 36% and 32%, respectively, and T_3 - 7170 kg / ha in 2011 with an increase of 51%. On average, over the period, the application of T_2- N_{100}, has increased the yield with 200 kg / ha or 38% T_0.

The increase in yield relative to T^0 has been statistically proven. Low nitrogen fertilization leads to boosting yields in 2011 with 17%, in 2012- with 8%, in 2013 - with 7%, on average for the period - with 10%. Despite the fact that the application of nitrogen norms higher than N_{60} leads to a statistically significant increase in yield to N_0, the determination of the optimal nitrogen rate can only be made by calculating the reliable differences from the previous tested nitrogen norm. The relative yield surplus between T_2 and T_1 in years is as follows: 24.32%, 26.05%, 24.69%, and the average for the period (1430 kg) -24.7%. Average for the period, fertilization with T_3 leads to a decrease in the yield relative to T_2 with 160 kg.

Table 3. Grain yield from triticale for the period 2011-213, kg / ha.

Years	2011		2012		2013		Average for the period	
Variants	kg	%	kg	%	kg	%	kg	%
1.N_0 (cont.)	4720	100	5480	100	5500	100	5230	100
2.N_{60}	5550	117	5950	108	5900	107	5800	110
3.N_{100}	6900	146	7500	136	7300	132	7230	138
4.N_{140}	7170	151	7040	128	7000	127	7070	135
GD 5%	23.98		18.46		17.10			
0.1%	39.79		30.61		28.36			
0.01%	74.39		57.25		53.03			

In Table 4 there are presented data for the analysis of the yield variance. The power of fertilization factor is 92.5%, followed by the year 4.13%, and the interaction between them 3.37%. The smaller percentage of the year is probably due to the fact, that the study period includes three consecutive years with similar climatic conditions.

Table 4. Variety Vihren yield variance analysis.

Factor	SS	DF	MS	F exp	η	Fcrit	
						P=0.05	P=0.01
Total	383087.7	47					
Factor A Year	15264.67	2	7632.333	20.03237	4.13	3.26	5.25
Factor B Fertilization	341654.3	3	113884.8	298.9102	92.5	2.86	4.38
Interaction AXB	12452.67	6	2075.444	5.447361	3.37	2.36	3.35
Within	13716	36	381				

CONCLUSIONS

In the conditions of Strandzha, on leavened /leached/ cinnamic forest soil, single nitrogen fertilization on background $P_{100}K_{50}$. is an effective event, which increases the yield of Vihren grain variety on average for the period from 570 to 1840 kg/ha. The most effective is the one-time fertilization with 100 kg/ha of nitrogen, introduced early in spring. The values of the number of crop ears/m^2, and the number of grains in an ear, increase and reach the maximum values when fertilization N_{140}. The grain weight is most strongly influenced by the nitrogen fertilization; the mass of 1000 pcs. of grains - from the year.

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PREVENTIVE COMPONENTS THAT AFFECTIVE IN EFFECTIVENESS OF AGRICULTURAL ADVISORY SERVICES IN IRAN

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ABSTRACT

Technologies and farming methods are constantly changing. Farmers should be informed about how to use these innovations in their fields. Utilizing the competence of the graduate's agricultures in the form of Agricultural Advisory Services companies (AASC) is one of the best solutions to transfer knowledge and technology to farmers and accelerating in agricultural development. The main purpose of this study is study and identification of the problems that AAS is faced with. Statistical population of the study consisted of Agricultural Consultants (N=1731). Using the formula Cochrane, sample size was determined 306. Questionnaire was the data instrument. The appearance and content validity of questionnaire was obtained by comments of extension experts. Reliability coefficient of questionnaire was obtained 0.89 by Cronbach alpha. The results showed that AAS increased participation of stakeholders in decision making and planning. AASC also provided the specialized context fields in agricultural extension. Results showed that AASC increased accountability and responsibility in extension services. By using exploratory factor analysis barriers are classified in four factors, including Infrastructure, Policy-making, Socio - cultural and Financial. These factors could explain 62.86 % of variance in reduce effectiveness of AASC among farmers in West Azerbaijan province.

Keywords: *Preventive, Agricultural Advisory Services Companies, Factors, Effectiveness, Iran*

INTRODUCTION

Agricultural advisory (extension) services are a vital element of the array of market and non-market entities and agents that provide critical flows of information that can improve farmers' and other rural peoples' welfare. After a period of neglect, agricultural advisory services have returned strongly to the international development agenda. Apart from their conventional function of providing knowledge for improved agricultural productivity, agricultural advisory services are expected to fulfill a variety of new functions, such as linking smallholder farmers to high-value and export markets, promoting environmentally sustainable production techniques. (Anderson, 2008). In many developing countries, rural populations are heavily dependent on agriculture as well as different social services for their livelihoods. Yet access to adequate knowledge, improved technologies, financial services and other relevant social services remains a critical issue (Tchouawou and Colverson, 2014).

However, with regard to the implementation of demand-driven and participatory approaches, some success stories were registered with strategies used in agricultural extension. With the lack of a gender sensitive approach to service delivery, challenges still impede the implementation of market-led and participatory Extension Agricultural services systems. Many systems have put a greater emphasis on promoting various agricultural extension projects

without understanding the practical and cultural obstacles that prevent women from accessing the most needed services. This has largely resulted in women's unequal access to Extension Agricultural services in rural locations. Consequently, the need for Extension Agricultural services delivery systems focused on best-fit approaches has been underlined as they provide an opportunity for adapting Extension Agricultural services to different population groups with specific priorities and needs (Manfre et al., 2013).

The decentralization of extension services retains the public delivery and public funding characteristics of traditional centralized extension, but transfers the responsibility for delivery to local governments (district, county, etc.) in diverse ways. For instance, many Latin American governments undertook this approach in the 1980s and 1990s, and it is being initiated enthusiastically in several African and Asian countries. The main expected advantage of the approach is in improving accountability, as agents become employees of local government, which—if democratically elected—would be keen on receiving positive feedback on the service from the clientele-electorate (Anderson, 2008). Access and use of information plays a crucial role in any activity (Martiius and Stabingis, 2000). Today farmers couldn't have access to new technologies only through direct contact between researchers. But new organizations should be responsible for transferring information and technologies to the farmers (Lashgarara and Hosseini, 2008). Commercialization in agriculture requires demands for technical consulting services (Christoplos, 2008).

Agriculture is one of the most important economic sectors in Iran. Agricultural sector provides about a quarter in employment and 33% of exports in Iran (Manzoralibadi, 2009). Despite the important role of agriculture in food production, employment and exports, unfortunately rural community is being faced with numerous problems. Issues such as poverty, unequal income distribution, unemployment, low productivity, unskilled labor force, inadequacy in agricultural sector, lack of appropriate extension system abound in agricultural sector of Iran (Merzaiy et al., 2007). To increase agricultural production level, farmers are needed to have access to extension services. But despite the long term of starting agricultural extension programs, in Iran, millions of farmers have not been covered by public extension. FAO statistics in Africa show that two of every three farmers do not have access to public services. This ratio in Asia is three of every four people, Latin America six of the every seven people, and five of the six people in the Middle East (Shekara, 2001; Zamanipour, 2001; Lashgarara and Peshbien, 2004). A number of specific formats of extension operations emerged over recent decades in endeavors to overcome these widely acknowledged problems. These newer (and now, for some, not so new) approaches, which depart from the traditional public service models, entail institutional innovations and reforms, often pluralistic, where specific design features reflect attempts to overcome weaknesses inherent in earlier public extension efforts (Anderson, 2008).

Agricultural Extension Services have been widely criticized due to inability to perform assigned functions and the absence of expected effectiveness and efficiency. Therefore, major changes such as structural reform, decentralization and privatization are essential to agricultural extension (Birner et al., 2009). Rivera (2008) assumes that the agricultural extension in the public sector has been seriously criticized in many countries due to its inefficiency.

Today, the role of agricultural public extension in transferring of technology in agriculture has been questioned (Rasouliazar and Fealy, 2008). Ahmadi (2004) pointed out that negligence to capital and human factors in agriculture, lack of covering comprehensive stockholders in agricultural extension, limited resources, manpower and funds in public extension system, dearth of fitness levels of staffing and professionalism to the needs of farmers are the main problems existing in agricultural knowledge and information system of Iran (Ahmadi 2004). Other countries have different strategies to cover defects and weaknesses of public extension (Mandler, 2010). Policy-makers in these countries have reached an important consensus to find

other alternatives to public extension. One of this alternatives is the use of private companies to provide information and transfer technologies to farmers. Privatization of extension services refers to the services that extension staff in private organizations provides for those farmers who pay the cost of services. These services are being considered as supplement for public extension service (Hanchinal et al., 2001; Saravanan, 2001; Anderson, 2004).

Amirani (2001) states that the solution of these problems would be possible through consulting services. Privatization of extension services by contracts to farmers has been introduced as one of the strategies of restructuring the public extension system (Christoplos, 2008). Application of AASC integrated with other techniques to produce effective access to financial facilities and marketing for the product would increase production and improve performance of farmers production in fields (Smith and Munoz, 2002).

Benin et al. (2007) stated that the main purpose of an agricultural consulting services is to increase agricultural productivity by strengthening the technical skills of farmers, and also to monitor their activities through delivery information and consulting services to them (Benin et al., 2007). Anderson (2007) believes that consulting services are critical elements which provide key information and improve the welfare of farmers. He believes that the term "consulting services" refers to a complete set of agricultural organizations that facilitate and support participation of farmers and solve their problems in agricultural sector with transmission of information, skills and techniques.

Transferring to agricultural consulting services and conventional extension systems could enhance productivity in production organization like agricultural farms (Arbenz, 2004). Application of consultancy companies is meant to achieve goals such as: increased efficiency and faster economic growth, agricultural development and decrease of government intervention in executive decisions (Rasouliazar and Fealy, 2008). One of the important challenges that extension Planners are faced with it, is the issue of how to increase the level of effectiveness and efficiency of technical consulting services (Chipeta, 2006). Designing effective extension systems has always been indispensible to system designer and policy-makers. Sundberg asserted that effective counseling services have significant impact on performance and efficiency of farmers (Sundberg, 2005).

Ministry of agriculture in 2009 reported that West Azerbaijan province has a high capacity in agriculture production (Anonymous, 2008). But on account of its geographical situation (being mountainous) and scattered villages farmers have limited access to public extension, and a large number of farmers are deprived of obtaining extension services.

Accordingly, using AASC can solve many of this structural problems and bottlenecks of public extension system. According to statistics of Agricultural Engineering Organization over 1900 AASC have been formed and established in Iran. The largest number of Agricultural graduates in form of AASC was based in West Azerbaijan. The number of these graduates was 1731 that were based in 162 AASC companies (Anonymous, 2009). Considering the important role of AASC in providing extension services to farmers, it is necessary to identify obstacles and barriers that influence the effectiveness of this companies. Since these obstacles and problems will reduce the effectiveness of services and take false credits in extension services sector (Barret et al., 2005); therefore, the main goal of this study was identifying obstacles factors that reduce effectiveness of AASC in Iran. By identifying this problems, Policy-makers and Extension planners could have suitable strategies to solve their. And also AASC could increasing their effectiveness in process of delivery agricultural services to farmers.

Research Methodology

The methodology used in this study involved a combination of descriptive and quantitative research and included the use of correlation and descriptive analysis as data processing

methods. A questionnaire was developed based on interviews and relevant literature. The questionnaire included both open-ended and fixed-choice questions. A 5-point Liker scale ranging from 1 (strongly disagree) to 5 (strongly agree) was applied as a quantitative measure. Content and face validity were established by a panel of experts consisting of faculty members and experts in the Ministry of Agriculture. A pilot study was conducted with 30 rural people who had not been interviewed before the earlier exercise of determining the reliability of the questionnaire for the study. Cronbach's Alpha coefficient was 0.86 which demonstrated that the questionnaire was highly reliable. The research population included Agricultural Consultants that are offered advisory to farmers in the Provinces of West Azerbaijan (n = 1731). Using a Cochran formula, sample size was determined 306. Factor analysis statistical methods were used. Also for data processing Statistical Package of social Science (SPSS) version16 was used.

RESULTS AND DISCUSSION

The results of descriptive statistics show that the average age of consultants was 28.53 years, with 3.8 years work experience. The majority of them (72.2%) were male. Majority of respondents (81.4%) had a B.Sc in agriculture majors. Consultants have announced that a major method to earn income was monitoring the farmers' farm (49.3%). In addition 38.6 percent of the consultants preferred the method of "visit farms' farm". The results showed that (81.4%) of consultants have suitable place in AASC. The average distance of AAS center from city was 17 kilometers (Table1).

Table 1. Descriptive statistics of extension experts

Variables		n	%	Mean	SD.	Min.	Max.
Age				28.53	3.8	22	38
Age experience				3.22	1.55	1	7
Distance from city				17.5	89.9	2	34
Sex	Female	85	27.8				
	Male	221	72.2				
Education	Bachelor of science (B.Sc)	249	81.4				
	Master of Science	54	17.6				
	PhD	3	1				
Methods to obtain income	Inputs sale	55	18				
	Delivery advisory	151	49.3				
	Farm monitoring activities	35	11.4				
Educational methods	Office visit	9	2.9				
	Farm visit	118	28.6				
	Group methods	89	29.1				
	Use telephone	21	9.6				

Suitable office place in AASC	Yes	249	81.4				
	no	57	18.6				

Priorities attitudes of consultants about advantages of advisory services companies indicated that improving farm management skills of farmers was ranked as the first advantage (CV=0.184), also increasing access to demand-driven extension services (CV=0.185) was ranked as the 2th, and increasing participation of farmers in planning and decision-making process (CV=0.187) was in the next rank. Other findings are shown in Table 2.

Table 2. Advantages of AAS from farmers' perception

Advantages	mean	SD	Coefficient of variance (CV)	Rank
Increasing farm management skills of farmers	4.33	0.80	0.184	1
Improving access to Demand-Driven extension services	4.32	0.80	0.185	2
Increasing participation of farmers in planning and decision making process	4.26	0.80	0.187	3
increasing the specialty of extension services	4.36	0.82	0.188	4
Increasing responsibility of extension consultants	4.28	0.86	0.200	5
Increasing bargaining power of farmers for acquire information and services	4.15	0.90	0.216	6
Providing rural development fields	3.66	0.84	0.229	7
Increasing the extension services to farmers	3.78	0.87	0.230	8
Reducing cost in public sector	3.84	0.97	0.252	9
Increasing quality of extension services	3.79	1.02	0.269	10
increasing incomes of farmers	3.41	0.93	0.272	11
Improving in public extension situation	3.66	1.04	0.284	12

Strongly agree=5,agree=4, intermediate=3, Disagree=2, Strongly disagree=1

Factor analysis is a general term for some multivariate statistical methods whose main purpose was summary data. This method examines internal correlation in a large number of variables, and eventually is explained in the form of general operating and restricted categories. Performed calculations display that internal coherence is proportional (KMO=0.94) and the Bartlett statistics is significant (χ^2= 2467.047 and P=0.000). To determine the number of factors, special amount and percentage of variance was used.

Table 3 through using the ordinal factor analysis shows the classification of the factors into four latent variables. The variables were classified into infrastructure, policy-making, socio-cultural and financial. The basic idea of factor analysis is to find a set of latent variables that contain the same information. The classic factor analysis assumes that, both observed and the

latent variables are continuous variables. But, in practice, the observed variables are often ordinal. Results shows these four factors have been explain 62.86% of the total variance in reduce effectiveness of AASC (Table 3).

Table 3. Total variance explained

	Rotation sums of squared loading		
factor	total	% of Variance	Cumulative %
1	3.985	22.13	22.13
2	2.930	16.27	38.40
3	2.344	13.02	51.42
4	2.057	11.42	62.84

Table 4 explained variance by each of the factors reducing effectiveness of AASC. As it can be seen structure factors, policy-making factors, socio – cultural factors and financial factors were identified as main barriers in the effectiveness of AASC (Table 4).

Table 4. Classification of factors by using ordinal factor analysis

Factor name	**variables**	**Variance by factor (%)**	**% of Variance**
Structural	Lack of cooperation of other institutions and organizations(public) with AASC	0.642	
	Lack of expert and technical personnel in AASC	0.671	22.13
	Lack of coordination in the activities of public and private sector	0.666	
	Lack of necessary facilities (vehicle) by the consultants	0.601	
Policy-making	Lack of services to marginal farmers	0.623	
	Lack of subsidies and grants from the government for companies and farmers	0.731	16.27
	Lack recognition signed of AASC	0.713	
	Lack of executive power of AASC	0.590	
	Lack of monitoring and evaluation activities of AASC	0.664	
Socio-cultural	Unhealthy competition between advisory agencies	0.652	
	Lack of trust in advisory services companies	0.541	13.02
	Illiteracy of farmers	0.662	
	Little attention to the needs of women farmers	0.540	
Financial	High cost of consultancy services	0.715	11.42
	Lack of credit and financial power of farmers	0.719	

The first factor was called “structural factors”. This factor according to the special value (3.985), which is higher than other factors, could explain 22.13% of the total variance. The second factor was named policy-making. This factor according to the specific amount 2.93 could explain 16.27% of total variance. The third factor was named socio-cultural factors. This factors according to the specific amount 2.344 could explain 13.20 % of total variance. The fourth factor was named financial factor. This factor according to the specific amount 2.057 could explain 11.42% of total variance. Between these factors structural factors can cause the most to explain the variance in the reduce effectiveness of AASC by respondents. So should increase the effectiveness of AASC among farmers, necessary will be done some practices and pointed to items mentioned by policy-making and extension-planners (Table 2)

CONCLUSION

Advantages of using the services of AAS indicated that improving farm management skills and enhancing productivity of farmers are the main advantages of using AAS. Also offering consulting services based on demand of farmers and increasing participation of farmers in decision- making and program planning were identified as other advantages of counseling services. Therefore considering the cases and factors on the strengthening and developing of consulting services is very crucial, since AASC can solve many of the challenges of farmers and the agricultural sector. These findings also accord with studies of (Anderson, 2008; Saravanan, 2001; Shekara, 2001; Sadighi, 2004; Rezvanfar and Arabi, 2006).

Results from factor analysis shows that barriers were infrastructure components, policy-making, socio - cultural and financial factors. The most important barrier factor is the infrastructure factor. Factors such as lack of cooperation with AASC from other organizations (public organization), lack of specialists in the company structure, tasks interference with public extension sector, lack of communication infrastructure (roads and ICT), and also shortage of vehicles and equipment have been identified as barriers for infrastructure. Therefore to increase efficiency of AASC these issues should be resolved. Also it is necessary that the consultants should increase their technical competences. Also through the acquisition of funding sources it is necessary for AASC to provide equipment. Finally missions and tasks of each sectors (public, private) should be explained and determined. These finding also pointed by several authors, such as (Hung, 1992; Walker, 1993; Arbenz, 2004; Povellato and Scorzelli, 2006; Naderlof et al., 2008; Fealy et al., 2007).

The second barriers factor was named policy-making factor, that AASC face with. There are issues such as lack of livelihood and subsistence farmers to advisory services, lack of subsidies and financial assistance from the government to provide services to marginalized groups such as women and rural youth, lack of executive power of Advisory companies, and lack of sufficient credit for the signing of AASC from the other organizations. On the other hand the lack of assessment and monitoring sector has caused many problems for AASC. Undoubtedly providing appropriate plans and programs of government can enhance AASC. Use of specialized assessment and evaluation committees to review the performance of consultants and the increase of the executive power of AASC through obtaining funding, and the recognition of the sign' companies could reduce the problems that are classified as obstacles factors in policy. Research findings are in compliance with this studies (Walker, 1993; Beglarian, 2002; Rezaei 2005).

The third factor that barriers to the effectiveness of AASC among farmers was the socio-cultural factors. Unhealthy competition between AASC, lack of trust farmers towards them, the low educational levels of farmers and the problem of having access to women in order to delivery advisory services were determined as socio-cultural barriers. So to solve this problem AASC should increase their technical competences about farmers' issues in order to increase farmers' confidence and trust toward them. Also it is highly crucial that female consultants would be able to provide services to rural women. This finding also pointed by several authors, such as (Hang, 1992; Walker, 1993; Ahmadi, 2001; Pamela et al. 2003; Rasouliazar and Fealy, 2009; Waddington et al., 2010).

Financial factors such as high cost of consultancy services for farmers and lack of access to financial resources by farmers were identified as financial barriers to the effectiveness of AASC. So to reduce the financial barriers Governments must be considering strategies to provide funding sources to farmers to develop agricultural activities (such as loans). Moreover evaluation committee should be monitoring the services offered to farmers. Consultants also must use other methods to provide cost of services such contract among farmers at the end of the production process. Agricultural advisory services is a private sector to reduce problems of public extension sector and improve farm management skills of farmers in Iran. Providing

information and consulting services to farmers cause the increase of quality and quantity of agricultural products. According to these issues the following suggestions will be presented to increase acceptance and reduce problems of AASC. And increasing effectiveness of them. As it was stated some of preventing problems will be solved through reform and changes in the structure of AASC activities. Attention to these issues such as acquiring professional and technical skills by consultants and employing female consultants could solve a part of the problems which reducing the effectiveness of advisory services among farmers. Also the policy-makers should develop facilitate mechanisms such as (providing supportive policies and infrastructure development) to AASC.

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INVESTIGATING THE FACTORS AFFECTING THE SUSTAINABLE DEVELOPMENT OF WATER RESOURCES IN THE IRAN AGRICULTURAL REGION

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Abstract

The purpose of this study was to investigate the factors affecting the sustainable development of water resources in the agricultural sector of Urmia. The statistical population of this study is experts in agricultural service centers and Urmia Agriculture Jihad, whose number is 110 persons based on the information received. The method of sampling in this research was simple random sampling. Morgan's famous table was used for sampling; therefore, the number of statistical samples was 86. This research was a researcher-made questionnaire consisting of 5 units. The questionnaire was designed according to the hypotheses and objectives of the research. After validation and validity, it was provided to the statistical sample to answer the questions. For statistical analysis, inferential statistics methods have been used using 22Spss software.

Research findings shows that ecological and infstracture components affective in the sustainable development of water resources in the agricultural sector. Also research findings showed the most ecological factors that affecting the sustainable development of water resources in the agricultural sector is Fitness fitting with facilities and constraints and Covering irrigation canals to prevent waste and evaporate water and also the most the Infrastructure Influencing Factors on Sustainable Development of Agricultural Water Resources was mitigating river flows" and the "repair and reconstruction of water transfer channels" as well as the "prevention of increased water evaporation in Irrigation of farms (such as irrigation at appropriate times) was identified as the most important infrastructure components effective in increasing the sustainable development of agricultural water resources from respondents' point of view.

Keywords: *Effective Factors, Sustainable Development, Water Resources, Iran*

INTRODUCTION

Agricultural activities, while carried out at the present time, may lead to malnutrition and hunger for future generations. For this reason, farmers need to devote more time to thinking, planning and developing management in order to achieve a sustainable agri-agricultural unit. Water is the main factor behind agricultural development, which accounts for about 70% of the world's water consumption in agriculture. In many countries, including Iran, irrigation is one of the main components of food production (Heydari, 2011).

Investigating the status of water resources and its management in Iran indicates that the country is located in the world's arid regions and water shortage is the most important bottleneck of agricultural development (Keshavarz et al., 2005).

The only source of the main sources of water in the country is the annual atmospheric precipitation, which is accumulated in the form of surface waters in rivers and canals, and so on. (Karpisheh, 2011). Therefore, a comprehensive and comprehensive approach to the

improvement of sustainable water management and its effective factors is of crucial importance, and effective water management is crucial to sustainable development. (Dungumaro and Madulu, 2003). Due to its biological nature and its strong dependence on nature, agriculture is the largest consumer of water resources in most countries. In our country, 93.5% of the water resources are used in agriculture. In addition, water scarcity and pollution of water resources transfer agricultural water to other sectors and the low water use efficiency in agriculture as a crisis, which requires serious attention of authorities and public officials in the national arena. Also, increasing demand for water and increasing periods of drought and human impact on natural resources have posed a serious risk to the quantity and quality of water resources. In this regard, the importance of rational management and utilization of water resources in terms of sustainable development, especially sustainable agricultural development It can be said that the shortage of water is the greatest dilemma of the world in the present century (Heidari, 2011).

Water is one of the most important factors in Iran's agricultural production; the amount of rainfall in the country is such that in most areas without resorting to irrigation, agricultural activity is not possible. Also, agriculture not only does not have acceptable performance compared to other countries, not only in dryland areas, even in wetlands and modern networks, which receive enough water. Considering that Iranian agriculture is heavily dependent on irrigation; if the role of water is not considered in the development of the country, the food security of the country will be faced with serious problems (Yusufi et al., 2011).

Water resources management is very important in agricultural development. From three perspectives, water plays a key role in sustainable development. First, it is consumed as a final product.

Second, water is an important input element in many businesses. The third reason of this importance is the key role of water in living organisms on the planet. Sustainable socioeconomic development in low water countries is limited to the availability of water and the reduction of water quality. A resource in resource management can put development plans at various parts of the economy at high risk. Perhaps because of the key role of water in sustainable development, the fourth program of economic, social and cultural development of the country has the water of intrinsic value and capital for exploitation, conservation and recycling (SalimiFard and Mostafaei, 2012). The water workers in the city of Urmia, located in the center of the province of West Azerbaijan, have been suffering from water scarcity in the agricultural sector for some time now, due to the traditional and incorrect use of water resources by farmers.

The agricultural sector is of particular importance in this city. The main source of water supply for agriculture in this area is groundwater, but due to the excessive utilization of groundwater, the annual decline in these waters is significant, and the major problems are the unconventional harvest of the groundwater resources are more than the annual amount of nutrition. Unofficial statistics of the existence of more than 400,000 wells in the country it says that more than 130,000 unauthorized wells are harvested over 5 billion cubic meters of water. In 2005, China, India and Iran ranked first to third in excess of groundwater resources. According to the statistics reported by Iran Water Resources Management Company, the amount of harvested from unauthorized wells was 3 billion and 400 million cubic meters, which caused the depletion of groundwater table and negative balance in the country's large plains. On the one hand, unbalanced feeding and harvesting and subsequent droughts have put a lot of pressure on the reservoirs and lowered the level of underground aquifers and subsidence of the plains of the area (Khalili, 2016). Population growth and socio-economic development increase demand for services provided by the lagoon (Lake Urmia catchments) such as water and food. Surplus water depletion and undeveloped underground water management can lead to water shortages (Faramarzi, 2012). The decrease of atmospheric droughts and droughts in recent years has destroyed the Niligan Lake Urmia. With the approach of summer and heat season, 50-mm

reduction of rainfall during the current year of water in the West Azerbaijan, and especially Urmia, due to the dryness of the lake, experts and custodians of the water supply area warned of the occurrence of water crisis in the region. And the subject matter that has attracted much attention in this area, considers the development of outsiders and unauthorized splits outside of the city as one of the main threats to the area.

The reduction of water volume in Urmia reservoir to 155 million liters from 220 million liters and a sharp decrease in the water level of Urmia also poses a serious challenge as Urmia water now accounts for 40 wells and dams. The daily consumption of 192 liters per person in the year 90, although lowered to 176 liters in the year 92, but with a 20 percent drop in precipitation, it still exceeds global standards and water reserves in the province. Culture and management of water use are needed in all sectors, in particular Household and agriculture. Average water consumption in the country is 2000 liters in a second, while in winter this rate in Urmia will rise to 1,700 liters and in summer to 2,700 liters, which will be a serious challenge, along with a shortage of water reservoirs in Orumieh (Malikzadeh, 2014)

In this regard, the present study attempts to investigate the factors affecting sustainable management of water resources in the agricultural sector in Urumia so that the results of this research can finally be presented with effective solutions to increase the factors affecting the sustainable development of water resources in the agricultural sector. Urmia achieved.

This research indicates that the factors affecting the sustainable development of water resources affect Urumia agriculture.

RESEARCH METHOD

The purpose of this research is to apply applied research and also, from the point of view of the nature of the method, is a descriptive-survey research type. Finally, in terms of data collection, it is a type of field research (Azadanlou, 2009). The statistical population of this study is 110 experts of Urmia Agricultural and Urban Agriculture Jihad Service Centers, which includes all experts from experts in Agricultural Services and Jihad of Urmia. The sampling was simple random and based on Morgan's table, the sample size was determined. 86 people are determined. In this research, a researcher-made questionnaire was used to collect data and information. In addition, in order to formulate and set up a researcher-made questionnaire, firstly, an evaluation group (professors and experts) to prepare and set questions of the questionnaire, their relationship with the subject, the goals and hypotheses of the research, and the setting of questions in terms of the phrasing, clarity, induction. The researcher's opinion and expression were not given. After several times, the final questionnaire was prepared and the questionnaire questionnaire was prepared according to the independent and dependent variable used in the research. Considering the fact that the researcher made questionnaire in this research, such as Likert scale, Accordingly, the responses of respondents by giving scores are very low (score 1), low (score 2), average (score 3), high (score 4), Very much (score 5) was compared and analyzed.

RESULTS

According to data analysis, from 86 respondents to the questionnaire from Urmia service centers and Jihad, 45 people are 3.52% male employees and 41 persons, 7.47% female employees. Also, the frequency distribution of the respondents' age to the questionnaire in Urmia service centers and Jihad, the youngest age of 24 years and the oldest age is 58 years.

Regarding the status of education, it can be said that the highest frequency is for graduate degrees with 5.53% and the lowest frequency for higher education is 5.3%. Regarding the age

of activities in the organization, the highest frequency is related to age 32 with 15.1% and the lowest frequency is about 56 years old with 2.1%.

Regarding the frequency and frequency of education in Urmia service centers and Jihad, the highest percentage of agricultural education is with 2.30% and the lowest percentage is in the Watershed study program with 5.3%. The results also showed that the average work experience of respondents to the questionnaire was 83.14, 1, and 35 years, at the service centers of Urmia. The results showed that the mean of work in the water project respondents in the research is 32.1 and the lowest is 1 year and the highest responder is 2 years. Also, the average of respondents 'water experts in this study is 43.3 years and the lowest responder is 1 year and the highest responder is 5 years; and the mean of respondents' depression in the current study is 37.1 years and the lowest responder is 1 year and the highest responder Has 2 years.

Prioritizing respondents' views on the ecological factors affecting the sustainable development of water resources in the agricultural sector

Prioritizing respondents' views about the factors affecting the ecology of the sustainable development of agricultural water resources in Urmia showed that "the appropriateness of cultivation with facilities and constraints" and the "cover of irrigation canals to prevent waste and water evaporation" as well as exploitation From the wells that fit the facilities and constraints "as the most important components of effective ecology in the context of increasing the sustainable development of agricultural water resources from the viewpoint of respondents.

As the results show, the consistent fitting of cultivation with the facilities and limitations can increase the sustainable development of agricultural water resources. Undoubtedly, having proper cultivation facilities can provide the success of many projects in the agricultural sector. On the other hand, having measures and predictions of critical situations such as droughts and events that may occur under certain conditions, and will lead to certain measures in the conservation and maintenance of water resources in times of crisis such as droughts in increasing the proportion of crops with the constraints and facilities in the agricultural sector is effective.

In the field of knowledge of exploiters, water degradation and environmental impacts have been considered as an important factor in increasing the appropriateness of cultivation with the facilities and constraints in the agricultural sector. Undoubtedly, the type of behavior of each farmer against the items that is considered important for them can increase the water use efficiency of the agricultural sector to implement water conservation and preservation programs.

At the same time, the attention of experts as well as farmers to the environmental consequences of water resources for agriculture can double the importance of maintaining water resources with appropriate cultivation in agriculture for farmers. The results of the research are consistent with Amirkhani et al. (2010), because in Amirkhani et al. Researches between the variables of the meeting with the farmer in the village and the field, There is a positive and significant correlation between participation in classrooms and educational and educational lectures, scientific visits to networks and irrigation systems and tillage facilities, and watching television programs with farmers' technical knowledge in the field of optimal agricultural water managemen.

Table 1. Prioritization of ecological factors affecting the sustainable development of water resources

Items	Mean	Standard deviation	Coefficient of variance	Rank
Fitness fitting with facilities and constraints	4.16	0.76	0.182	1
Covering irrigation canals to prevent waste and evaporate water	4.22	0.80	0.189	2
Pipelining of wells in accordance with facilities and constraints	3.95	0.86	0.217	3
Prevent the destruction of springs and aqueducts	3.60	1.13	0.313	4
Fertile clouds of rain	3.16	1.10	0.348	5

Likert Spectrum: Very Low: 1 Low: 2 Medium: 3 High: 4 High: 5 Source: Research findings

Prioritizing Respondents' Views on the Infrastructure Influencing Factors on Sustainable Development of Agricultural Water Resources

The results of prioritizing the responses of respondents to the Infrastructure Influencing Influencing Sustainable Development of Urban Water Resources in Urmia showed that the term "mitigating river flows" and the "repair and reconstruction of water transfer channels" as well as the "prevention of increased water evaporation in Irrigation of farms (such as irrigation at appropriate times) was identified as the most important infrastructure components effective in increasing the sustainable development of agricultural water resources from respondents' point of view. Infrastructure factors as the basis for implementing many programs and projects can provide a platform for its success. Meanwhile, attention to controlling flowing waters in rivers is of great importance. When water transfer channels are rehabilitated, and in order to prevent evaporation of irrigation water properly and at the right time, experts will provide farmers with the necessary information on the sustainable management of water resources and ways to increase sustainable development of water resources in the agricultural sector, Water Importance Farmers and villagers are more concerned with improving their irrigation practices. The results of the research with AziziKhalkhali et al. (2009) According to research results, AziziKhalkhali et al., Household size, dependency, education level, aquaculture, annual agricultural income, extension contacts, social capital components, irrigation status of the region and participation status; farmers in the field of network management Irrigation is correlated with the issue of water resources management.

Table 2. Prioritization of the Infrastructure Operating Facility Effects on the Sustainable Development of Water Resources

Items	Mean	Standard deviation	Coefficient of variance	Rank
Containment of flowing waters in the rivers	4.02	0.75	0.186	1
Repair of water transfer channels	4.08	0.77	0.188	2
Avoid increasing water evaporation in irrigating the fields (such as irrigation at appropriate times)	3.36	0.70	0.208	3
Water rationing for agriculture	3.74	0.81	0.216	4
Rainwater harvesting	3.69	0.81	0.219	5
Improvement of farmland drains	3.81	0.93	0.244	6
Use of wastewater treatment	3.22	0.99	0.307	7

Likert Spectrum: Very low: 1 Low: 2 Medium: 3 High: 4 High: 5 Source: research findings

DISCUSSION AND CONCLUSION

As it was found out, attention to the terms of each of these factors is necessary in areas such as the appropriateness of cultivation with the facilities and constraints and the coverage of irrigation canals to prevent the loss of water, and the evacuation of water and prevent the destruction of springs and aqueducts, as well as fertilizing clouds of rain. These measures must be taken in relation to the social context that exists at the community level.

In this regard, attention to the economic consequences of

Reducing water resources in the region and the consequences that it can bring to farmers will be more effective. Because when farmers point out sources of funding and income for livelihoods in line, they focus on unproductive methods for exploiting water resources.

In order to implement irrigation development projects for sustainable development of agricultural water resources, attention should be paid to sub-structural issues, through the formation of research teams and development companies specializing in the management of flood waters in rivers and the restoration and rehabilitation of water transmission channels and Also, preventing the increase of water evaporation in irrigating farms (such as irrigation at appropriate times) can be effective in agriculture alongside farmers' communities.

Finally, it can be concluded that in order to achieve the long-term goals for sustainable preservation and sustainable development of agricultural water resources in Urmia, the factors affecting sustainable development ecology and infrastructure should be at the top of the issues of high-level managers and experts. According to the findings of the research on the ecological factor affecting the sustainable development of agricultural water resources in Urmia, attention should be paid to measures to protect water resources during low rainfall and adequate water storage for the agricultural sector, as well as covering irrigation canals and exploitation of well-connected facilities. Factors affecting the sustainable development of water resources. The use of consulting engineers and agricultural engineering companies to raise awareness among farmers about the sustainable development of agricultural water resources as well as the rehabilitation of water transmission channels and the restoration of drainage of farms.

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HYPOTHETICAL RADIOLOGICAL FINDINGS IN DOGS AND CATS SUFFERING FROM INSOMNIA

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Abstract

Sleep is a vital body function, regulating several biological phenomena. Deprivation studies are one of the ways used to examine the physiological functions and the regulation of the sleep. Sleep deprivation is a stressor, and its' effects depend on an individual's prior sleep deficit and distribution during the day. Sleep deprivation can be partial, total, acute, or chronic or specifically focused on one of the sleep phases. Sleep deprivation affects a large spectrum of vital systems such as thermoregulation, energy and mineral balance, and immunofunction. Based on the fact that sleep is a very important process for the normal development of many metabolic pathways, it is logical to think that insomnia has serious organism consequences. Functional irregularities of different organs are always reflected in structural changes that can be identified with imaging techniques. Imaging methods can also help identify problems of animals insomnia.

Keywords: *Radiology, Diagnosis, Insomnia, Sleep, Biology*.

INTRODUCTION

Definition of sleep, sleep architecture and sleep profiles

We all understand what it means to be asleep, but it is not always obvious whether observed animals are experiencing the same state. Sleep must be distinguished from circadian changes in alertness controlled by the suprachiasmatic nucleus and other body clocks. Most animals need to adjust their activity to optimal conditions of prey availability, predator threat, sexual opportunities, temperature and other variables affecting survival that vary with time of day. Adequate rest and sleep is essential for the welfare of young growing animals (Everson 1995; Rechtschaffen, 1998; Siegel; 2005). Sleep regulates the secretion of several hormones, such as GH and glucocorticoids (Steiger, 2002), and is essential for brain development (Mirmiran 1986; Morrissey et al., 2004; Siegel 2005). Defines mammalian sleep as an individual that sustains quiescence in a species-specific posture accompanied by reduced responsiveness to external stimuli, has a quick reversibility to the wakeful condition and characteristic changes in the electroencephalogram (Zepelin et al., 2005). The behavioural criteria consist of a lack of mobility or slight mobility, slow eye movements, characteristic specifies-specific sleeping posture, reduced response to external stimulation, increased reaction time, elevated arousal threshold, an impaired cognitive function and a reversible unconscious state. Electrophysiologically sleep is split into two main phases: rapid eye movement sleep (REM), also called a paradoxical sleep, or active sleep and non-rapid eye movement sleep (NREM), also called quiet sleep, orthodoxical sleep, or slow wave sleep (SWS). In principal, the smaller the animal and the northern it lives, the shorter the daily sleeping duration and the shorter the REM-phases. The stimulus threshold to wake up from REM-sleep is higher than

that of NREM, and thus a long REM-sleep period can be a threat to survival for prey species other than those rodents species that sleep in nests (Allison and Cicchetti, 1976; Elgar et al., 1988; Tobler, 1995). Primates often exhibit a mono- or biphasic sleeping rhythm while several other species have a polyphasic rhythm, sleeping all over the day. Typical examples of polyphasic short sleepers are our domestic ruminant species, such as cattle, sheep, and goats. The sleep cycle consists of one or several REM and NREM phases. The cycle length is species-specific. Sleep cycle lengths are short in farm animals, as in several prey animal species. Total daily sleeping duration also varies between species. These differences between species depend on several factors, such as time spent eating, and available food sources, digestion and rumination, and ecological niche (Allison and Cicchetti, 1976; Elgar et al., 1988;). Grazing animals, for example are assumed to sleep less, as they need more time to consume large amounts of low- calorie foods (Siegel, 2005). In mature mammals and birds, the sleep phase usually starts with NREM and deepens during the REM phase (Zepelin et al., 2005). The young of many terrestrial mammalian species sleep more and have more REM sleep than do older animals (Siegel, 2005). Sleep is essential for brain development, and REM sleep is connected to the early developmental phase (Mirmiran, 1986; Morrissey et al., 2004). Young animals have also higher need for energy conservation acquired through sleep. Precocial young mammals, such as bovine calves, are suggested to spend proportionally less in REM sleep from their total sleep time than do young altricial mammals (Siegel, 2005).

Functions of the different sleep phases and effects of deprivation

Sleep is a vital body function, regulating several biological phenomena. Many theories have been presented to explain the function of sleep and sleep phases. Most theories assume that sleep serves the same functions for all animal species (Siegel, 2005). Deprivation studies are one of the ways used to examine the physiological functions and the regulation of the sleep. Sleep deprivation is a stressor, and its' effects depend on an individual's prior sleep deficit and distribution during the day. Sleep deprivation can be partial, total, acute, or chronic or specifically focused on one of the sleep phases. Sleep deprivation affects a large spectrum of vital systems such as thermoregulation, energy and mineral balance, and immunofunction (Bonnet, 2005). If sleep loss is chronic, depriving individual animal totally from sleep or selectively from REM or NREM sleep, experimental animals will die within a month due to infections or metabolic disorders. Hormones, which are dependent on sleep, loose their secretion rhythm. Several sleep-related hormonal secretions, such as GH and prolactin, diminish, when sleep is deprived. The body temperature decreases before an individual falls asleep, but during sleep deprivation, the body temperature remains around the normal level (BONNET 2005). During NREM sleep, body metabolism slows, body temperature decreases, and oxygen consumption diminishes, so that energy is conserved (Shapiro and Flanigan, 1993). Brain glycogen storage is restored during NREM sleep (Benington and Heller, 1995) and anabolic processes accelerate (Shapiro and Flanigan, 1993). NREM sleep may also play a central role in neurogenesis; REM sleep occurs proportionally and absolutely more during early development, and secures neuronal development (Siegel, 2005). Biological rhythms, such as hormonal fluctuation and rest-activity or sleep rhythms, are generated by an internal system. Biological rhythms are classified as circadian, with a cycle of approximately 24-hours, and ultradian, with a cycle less than 24 hours. The main regulator, pacemaker, is in the suprachiasmatic nuclei (SCN). The SCN is situated in the hypothalamus, just directly above the optic chiasm (Buijs et al., 2003). The suprachiasmatic nucleuos functions already in fetuses such as in lambs (Yellon and Longo, 1987). The main regulators for the SCN are external light and feeding. Other factors that synchronize the circadian system are, for example, nutrition, hormone feed-back mechanisms, activity, and social cues (Buijs et al., 2003). Sleep onset stimulates GH secretion, but the hormones of the somatotrophic axis are also involved in sleep

regulation in a complex way. GHRH, for example, stimulates slow wave sleep and slow wave activity, and GH increases REM sleep. The secretion of GH increases during sleep independent of the circadian sleeping cycle, and sleep deprivation diminishes the GH release. In humans, however, a day time GH secretion increases after one night of sleep deprivation, thus partly compensating the loss.

Cytokines are proteins produced by leukocytes and other cells functioning as intracerebral mediators that may play an important role in immune and sleep regulation1. Several cytokines (*e.g.*, interleukin or IL, interferon alpha or IF-α and tumour necrosis factor or TNF) have been shown to promote sleep (Kapsimalis et al., 2008). There are however, other sleep-promoting substances called sleep factors which increase in concentration during prolonged wakefulness or during infection and enhancing sleep. These factors include delta sleep-inducing peptides, muramyl peptides, cholecystokinin, arginine vasotocin, vasoactive intestinal peptide, growth hormone-releasing hormone (GHRH), somatostatin, prostaglandin D2, and adenosine. There is evidence that cytokines play an important role in the pathogenesis of excessive daytime sleepiness (EDS) in a variety of sleep disorders and in sleep deprivation. Increased production of pro-inflammatory cytokines (IL-6 and TNF-α) have been noted during sleep deprivation causing excessive sleepiness. Viral or bacterial infections causing EDS and increased NREM sleep are associated with increased production of TNF-α and IL-B). Increased sleepiness and disturbed sleep in other inflammatory disorders such as HIV infection and rheumatoid arthritis are associated with increased amounts of circulating TNF-α. Several authors suggested that excessive sleepiness in obstructive sleep apnoea syndrome, narcolepsy, insomnia or idiopathic hypersomnia may be mediated by cytokines such as IL-6, TNF-α . The neuroanatomical substrates of REM and NREM sleep and wakefulness are located in separate parts of the central nervous system5. There are no discrete sleep-wake promoting centers but these states are produced by changes in the interconnecting neuronal systems modulated by neurotransmitters and neuromodulators. Insomnia is the most common sleep disorder affecting the population and is the most common disease encountered in the practice of sleep medicine. Insomniacs complain of difficulty initiating and maintaining sleep, including early morning awakening and non-restorative sleep occurring 3-4 times per week persisting for more than a month and associated with an impairment of daytime function. Acute insomnia may be associated with an identifiable stressful situation. Most cases of insomnia are chronic and co-morbid with other conditions which include psychiatric, medical and neurological disorders (Rechtschaffen, 1998).

The true function of sleep is still being discussed, but sleep undoubtedly affects the endocrine and metabolic systems and the immune function (Rechtschaffen et al., 1983; Bergmann et al., 1989).

Total sleep deprivation in animals

The first report on the total chronic sleep deprivation in rats dates back to 1962 (BERGMANN et al., 1989). The animals were kept awake for 27 days, which led to aggressive behaviour, decreased body mass gain and impairment of the startle response. The most detailed analysis of sleep deprivation was based on data deriving from well designed, several-year experiments conducted by Bergmann and Rechtschaffen (Webb 1962; Everson et al., 1989). The experiments were performed using the diskover- water method, with a rat being placed on a disk over a layer of water, and a polysomnograph signal setting the disk into motion whenever an initiation of sleep was recorded (Cirelli et al., 1999). The sleep deprivation obtained using this procedure made up 70–90% of the experiment time and led to the death of the animals within 2–3 weeks. In the course of the experiment, weight loss was observed despite an increased food intake, as well as pathological skin reactions on the tail and paws and a bad condition of the fur. Initially, body temperature was elevated, but it decreased during the period

preceding death. Plasma levels of the thyroid hormones decreased significantly and heart rate increased. At the same time, no stress symptoms, such as stomach ulcers, elevated ACTH or corticosterone levels, or decreased metabolic rate, could be observed during the experiment (Everson et al., 1989a; Cirelli et al., 1999). Rats died within 11–32 days (16–21 days on average) from the onset of deprivation, a period comparable to that of food deprivation with lethal effects (17–19 days). However, histopathological findings did not reveal any cause of death (28–30). The animals which survived acute deprivation (that were eventually allowed to sleep) showed a dramatic compensatory increase in the REM sleep (Lyamin et al., 2005). The other symptoms subsided within 24 hours, which indicates that the sleep deprivation did not exert destructive effects either on the cells, the neurons or the vital organs. Nonetheless, a complete recovery of the pre-deprivation levels of the particular sleep stages, or of the heart rate and body temperature, lasted several days (Marinesco et al., 1999; Lyamin et al., 2005). An interesting exception to the rule can be observed among marine mammals: despite the periodic, significant sleep restriction, they do not experience the recovery sleep that would be a typical reaction to prolonged wakefulness, as well as to 4 NREM or REM sleep deficiency, in terrestrial mammals. The seals, for example, when staying in the ocean, can function well for several weeks despite the fact that they exhibit a considerably low duration of the REM sleep. Their sleep architecture changes immediately after they come back to the land. Unihemispheric slow-wave sleep (characteristic of dolphins and whales) is replaced by alternate NREM and REM phases. The sleep time typical for terrestrial conditions is immediately restored, and no symptoms of developing the recovery sleep can be seen (Rampin et al., 1991). Similarly, no rebound sleep occurs in infant dolphins and their mothers who refrain from sleeping throughout the period from the delivery till the youngsters achieve some self-sufficiency, which can last several weeks (Newman et al., 2008). The ability to withstand sleep deprivation is dependent on the species-related natural sleep characteristics regarding the duration and quality of sleep. For instance, large ungulate herbivores have a short, shallow and intermittent sleep, while predators usually sleep long and deeply. The relationship between sleep deprivation and the level of stress has not been fully explained, although the latter may have a varying influence on the compensation for sleep deficits. In a study reporting on wakefulness maintained through immobilization for 0.5 to 4 hours, the recovery sleep became significantly shorter when the immobilization period reached its maximal duration (Siegel, 2008). Two-hour immobilization repeated on the consecutive days of the experiment produced similar effects. However a single 2-hour immobilization resulted in an 92% increase in paradoxical sleep within the following 10 hours, whereas a 2-hour wakefulness, maintained using standard methods (disk or gentle handling), did not significantly affect the sleep that followed (Rechtschaffen and Bergmann 2002). Rats appear to be particularly vulnerable to sleep deprivation enforced using the moving disk method, since in other animals (pigeons), the changes observed after 24–29 days of this procedure were not as severe as in rats (Newman et al., 2008). Other deprivation procedures were not lethal either to rats or other laboratory animals although this may have been due to the significantly shorter periods of deprivation under other experimental conditions or to the difficulties in achieving total sleep deprivation. In animal experiments, sleep deprivation induced an increased rate of systemic metabolism, which led to reduced body mass despite an increased food intake, even if the animals were provided with food that was rich in proteins and calories. Both in the total and selective deprivation of REM sleep in rats, the plasma concentrations of the thyroid hormones, mainly thyroxine and triiodothyronine, decreased considerably.

CONCLUSION

Hypothetical radiological findings

Based on the fact that sleep is a very important process for the normal development of many metabolic pathways, it is logical to think that insomnia has serious organism consequences. Functional irregularities of different organs are always reflected in structural changes that can be identified with imaging techniques. We think that there are many pathological and nonpathological conditions that provoke sleeplessness in dogs and cats. Imaging methods can also help identify problems of animal fatigue. To concretize these hypotheses, extensive studies are needed.

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COMPARISON OF DIFFERENT DNA MARKERS FOR SELECTION OF HIGH OLEIC TYPE SUNFLOWER GENOTYPES

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ABSTRACT

Sunflower is one of the most important oilseed crops due to high oxidative stability of its oil with high oleic acid content. Determination of high oleic sunflower by standard methods such as gas chromatography is time consuming and expensive. On the other hand, marker-assisted selection analysis with molecular markers associated with high oleic acid trait is a useful and powerful tool in order to facilitate sunflower breeding programs. For the purpose of genotyping the sunflower genotypes for high oleic content four molecular markers were used; SSR marker, HO PCR specific fragment, INDEL markers (FAD2-F4/FAD2-R1 and FAD2-NF/FAD2-NR and FAD2-IS-F/FAD2-IS-R primer set). The results showed that high oleic containing hybrids expressed a specific SSR band at 246 bp length and also HO PCR specific fragment at 870 bp length. INDEL marker FAD2-F4/FAD2-R1 has an insert specific band at 653 bp length. The results were also confirmed by estimating the fatty acid composition. The results of this work allowed to validation of three DNA markers in sunflower inbred lines for high oleic acid traits. According to results, showing the insertion site which is linked to the Pervenets mutation by the insertion specific PCR primers is more reliable than the SSR marker for selection of the high oleic type sunflower genotypes.

Keywords: *Helianthus annuus, INDEL markers, Marker-assisted selection, SSR, Oleic acid composition*

INTRODUCTION

Vegetable oils are necessary mainly for food and industry. They are mainly composed of triacylglycerol carrying saturated, monounsaturated and polyunsaturated fatty acids. Sunflower oil which is one of the most important oil in the world contains high level of unsaturated fatty acids [linoleic acid (48-74%); oleic acid (14-40%)] and also saturated fatty acids [palmitic acid (4-9%); stearic acid (1-7%)] (Singchai et al., 2013; Nagarathna et al., 2011). Sunflower oil used for both human and non-food purposes in the world. It is desirable for human consumption because of its favorable fatty acid composition (Baydar and Erbas 2005). Oleic type sunflower production and high oleic sunflower oil consumption started rapidly both for healthy frying oil, and also non-food purposes in recent years (Vannozzi 2006). High oleic type vegetable oils also have longer shelf life than standard vegetable oils (Kaya et al., 2012). High oleic type vegetable oil containing diets have been reported to be most effective for preventing cardiovascular diseases (Delplanque et al., 1997; Broun et al., 1999). Classic sunflower varieties are low oleic type (LO) whereas new varieties that are qualified as high oleic (HO) have been developed. Increase of oleic acid content has become one of the major goals to improve vegetable oil quality (Lacombe et al., 2004). In order to reach this aim, Sunflower

lines and hybrids which have high oleic acid content in their seeds have been obtained by selection programs from HO (High oleic) Pervenet mutant by chemical mutagenesis (Soldatov 1976). The phenotypic determination by fatty acid analysis does not allow rapid and early determination of HO genotypes and also cannot provide differentiation of homozygotes from heterozygotes for the mutation (Bilgen et al., 2018). The use of molecular markers has become popular tool for the genetic and breeding studies because of being rapid, cheaper and simple when appropriate markers were developed (Varshney et al., 2005). Therefore, marker assisted selection (MAS) analysis is necessary at genomic level allowing rapid and earlier determination of homozygous HO genotypes for sunflower breeding studies. The aims of study are characterization of sunflower genotypes with high oleic acid content by different DNA markers and evaluate the effectiveness of three markers developed by Berville et al. (2009) and Schuppert et al., (2006).

MATERIAL AND METHODS

Plant materials

For the purpose of screening on high oleic acid genotypes, around 250 F_4 (K2-AD-SN-26) sunflower individuals obtained from a cross between high oleic acid and low oleic acid lines were used. All genotypes were screened by SSR primer and selected 27 genotypes were screened by 3 primer pairs in order to compare effectiveness of different markers. Leaves were collected from the field, labeled with individual number and stored at -80ºC until use.

DNA isolation

Before DNA isolation leave samples were homogenized with Retsch® Model MM300 Mixer Mill. i-genomic Plant DNA Extraction Mini Kit was used for DNA isolation from all samples. Concentration of each DNA was measured with Qubit® 2.0 Fluorometer and the quality of DNA was checked by 1% agarose gel electrophoresis, stained with RedSafe Nucleic Acid Staining Solution and visualized by Gel Imaging System Vilber Lourmat Quantum ST5. Each of the extracted DNA was diluted as 50 ng per µl and was stored at -20 ºC for later uses.

PCR analysis

Genotyping of high oleic (HO) and low oleic (LO) sunflower individuals was performed with 3 primer pairs; SSR (N1-1F/N1-1R), HO PCR specific fragment (N1-3F/N2-1R) and INDEL marker (FAD2-F4/FAD2-R1) that were chosen from the patent obtained by Berville et al. (2009) and Schuppert et al., (2006) (Table 1). Amplified PCR products were controlled by 2% agarose gel electrophoresis, stained with RedSafe Nucleic Acid Staining Solution and visualized by Gel Imaging System Vilber Lourmat Quantum ST5. SSR (N1-1F/N1-1R) fragments were scored in a *Beckman Coulter* GenomeLab™ GeXP Genetic Analysis System and fragment sizes were calculated by its Software (Figure 1).

Table 1. Characteristics of markers used to analyze HO and LO sunflower genotypes

No	Primer type	Primer name	Primer Sequences (5' → 3')
1	SSR	N1-1F N1-1R	TTGGAGTTCGGTTTATTTAT TTAGTAAACGAGCCTGAAC
2	HO PCR specific primer	N1-3F N2-1R	GAGAAGAGGGAGGTGTGAAG AGCGGTTATGGTGAGGTCAG
3	INDEL marker	FAD2-F4 FAD2-R1	GTAACGTCTGCGCGCTTGCAGACATCA GGTTTTGCATGAGGGACTCGATCGAGTG

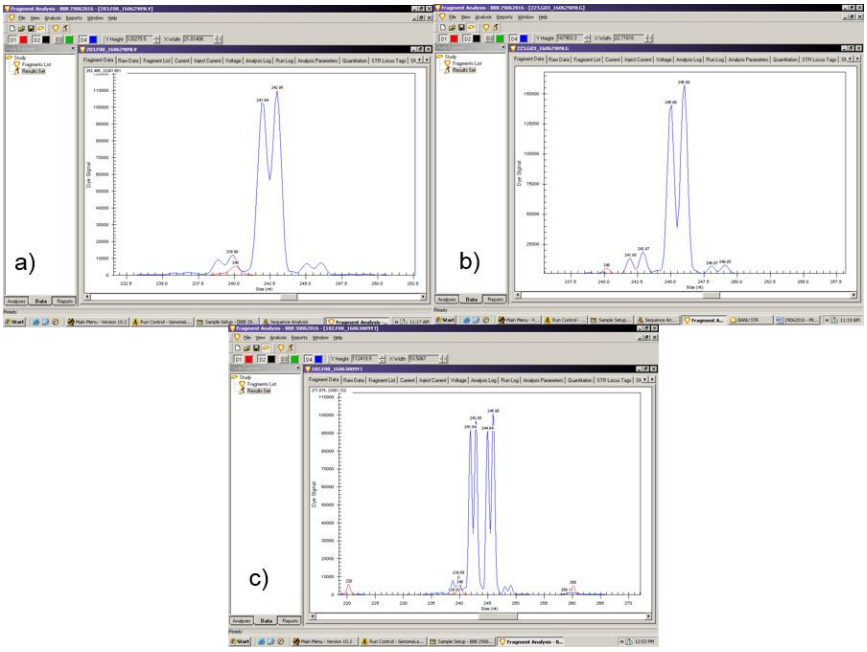

Figure 1. DNA fragment analyses results for studied sunflower genotypes.

RESULTS

The Pervenet mutation was labeled by the polymorphism of the SSR locus located on the Δ12-desaturase gene intron (Berville et al., 2009). Alleles and genotypes of studied sunflower individuals were determined for analyzed SSR (N1-1F/N1-1R) locus. According to DNA fragment analysis for SSR locus 246/246 Homozygous (239 genotypes), 243/243 Homozygous (2 genotypes) and 243/246 Heterozygous genotypes (4 genotypes) were identified. 27 selected sunflower genotypes were also screened with HO PCR specific fragment (N1-3F/N2-1R) in order to confirm HO sunflower genotypes. The Pervenet mutation was labeled by the 870 bp PCR fragment across the 5' insertion point by HO PCR specific fragment (N1-3F/N2-1R) (Berville et al., 2009). The results showed that high oleic containing sunflower individuals (HO genotypes) showed a specific band at about 870 bp length which was absent in low oleic (LO) genotypes (Table 2). The third primer pair, INDEL marker (FAD2-F4/FAD2-R1), were used for screening 27 selected sunflower genotypes again in order to confirm HO sunflower genotypes. The F4/R1 amplicons were approximately 653 bp long in HO genotypes and this specific band was also absent in LO genotypes (Table 2, Figure 2). The results were confirmed by determination of fatty acid composition using gas chromatography in all the studied individuals (Table 2).

Table 2. Marker assisted selection (MAS) and Gas Chromatography (GC) results of selected F4 (K2-AD-SN-26) sunflower genotypes

Sample number	SSR (N1-1F/N1-1R)	HO PCR specific primer (N1-3F/N2-1R) (~870 bp)	INDEL marker (FAD2-F4/FAD2-R1) (~653)	Oleic acid content (%)
10	246	+	+	87.5
13	246	+	+	57.4
15	246	+	+	83.1
25	246	+	+	55.9
28	246	+	+	62.1
37	246	+	+	59.5
49	246	+	+	62.6
58	246	+	+	55.1
67	246	+	+	56.7
70	246	+	+	54.1
78	246	+	+	81.6
80	246	+	+	54.4
84	246	+	+	54.1
87	246	+	+	59.8
110	243/246	+	+	54.9
122	246	+	+	84.7
125	246	+	+	57.9
140	246	+	+	70.2
180	246	+	+	89.4
193	246	+	+	90.8
195	246	+	+	65.9
199	246	+	+	64.4
208	246	+	+	88.1
210	246	+	+	72.5
211	246	+	+	88.2
226	246	+	+	68.1
239	246	+	+	89.7

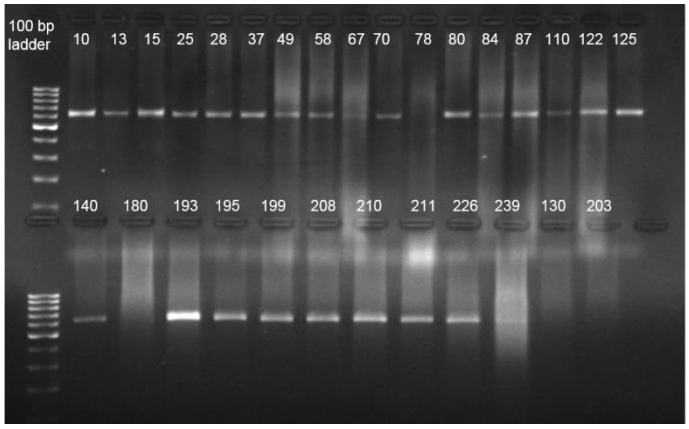

Figure 2. PCR amplification of HO and LO sunflower genotypes with INDEL primer pair (FAD2-F4/FAD2-R1)

DISCUSSION

Various sunflower lines and hybrids have been studied to distinguish HO genotypes from LO genotypes by different researchers and molecular marker types such as RAPD or SSR (Dehmer and Friedt 1998; Schuppert et al., 2006; Berville et al., 2009; Nagarathna et al., 2011; Grandon et al., 2012; Singchai et al., 2013). Dehmer and Friedt (1998) were used RAPD markers (OP-AC10 and OP-F15) in order to differentiate HO and LO genotypes. Nagarathna et al., (2011) studied around 350 sunflower genotypes including RHA-lines, cms lines, inbreds and germplasm lines to screening on high oleic acid. In Nagarathna et al., (2011) For the purpose of genotyping the sunflower lines for high oleic content, HO PCR specific fragment (N1-3F/N2-1R) were chosen and also the seeds were used for the determination of fatty acids (linoleic acid, oleic acid, palmitic acid and stearic acid) using gas chromatography. They reported that the genotypes having a specific band (at 800 to 900 bp) showed high oleic content. Singchai et al., (2013) studied the developed lines that used as the representative of low and high oleic acid sunflowers for genotyping. They screened thirty seven SSR primers including 34 primers of ORS set, 2 primers of HA set and N1-3F/N2-1R primer to identify DNA samples from two lines (high and low oleic acid contents). Out of the 37 SSR primers screened for polymorphism, 10 SSR primers including N1-3F/N2-1R generated differentiating bands between the high and low oleic content lines. With the 10 SSR markers they studied, Singchai et al., (2013) reported that it is possible to identify the genetic markers linked to high oleic acid trait which may be useful for further sunflower breeding program. Dimitrijevic et al., (2017) studied F13-R5 and F4-R1 (Schuppert et al., 2006), and Fsp-b-R1 primer (Lacombe et al., 2009) in order to MAS analysis of F1 and F2 generations that were obtained from crosses between standard linoleic and high oleic parents. They concluded that F4-R1 primer pair was very effective to select HO genotypes. The MAS and GC analysis results of our study were compatible with other studies in the literature.

CONCLUSIONS

Our study has shown that studied 3 primer pairs were effectively select HO sunflower genotypes from LO genotypes. However, 246 bp SSR band can be found in some LO sunflower varieties. So, the SSR marker is able to indicate the Pervenets mutation but not in all the sunflower varieties. Pervenets mutation results from an insertion of a DNA sequence into a region at the downstream of the Δ12-desaturase gene. The HO PCR specific fragment and INDEL marker showed the presence of the inserted sequence and they are able to detect HO varieties more confidently than the SSR marker.

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DEVELOPMENT OF ASSISTED REPRODUCTION TECHNOLOGIES FOR THE ENDANGERED ALBANIAN WATER FROG (PELOPHYLAX SHQIPERICUS): FROM GAMETE RELEASE TO FROGLETS

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ABSTRACT

The Albanian water frog, *Pelophylax shqipericus*, is a species of true frog (family Ranidae) and is native to Albania and Montenegro. This frog is an endangered species and its populations are currently in decline. Significant threats to its habitat are presented by pollution and by drainage of wetlands, and a more direct threat is the aggressive collection of the species for commercial purposes. Assisted Reproductive Technologies (ART) encompasses a range of techniques that manipulate reproductive endocrinology, gametes, and embryos, for the purpose of enhancing reproductive success. The aim of this study is the stabilization of the protocol to be used in the *in-vitro* fertilization the Albanian water frog and the recognition of some of the factors that play a crucial role in the success of this technique. This study investigates the activation and in-vitro fertilization of eggs of *Pelophylax shqipericus* obtained by hormonally induced ovulation. Also, here we show that the egg jelly structure is one of the major factors in the achievement of fertilization in the water frog *P. shqipericus*. Definition of morphology, sperm concentration and fertilization of eggs through the technique of direct spray with the extract of macerated testicles increases significantly the success of the technique. Finally, applied *in-vitro* fertilization protocol showed a 27% success rate. By using the *in-vitro* fertilization technique (IVF), we can give a contribution to the protection and conservation of the critically endangered Albanian water frog. The transfer of this technology and production of endangered amphibians is a conservation milestone that can be applied to other captive breeding programs.

Keywords: *Pelophylax shqipericus, In-vitro fertilization, Assisted reproductive technologies, Conservation*

INTRODUCTION

Declines in amphibian populations is a global phenomenon in conservation biology, and it can bear significant implications for the functioning of many terrestrial ecosystems, and may signify important implications for human welfare. Amphibian species world-wide are declining at an alarming rate due to habitat destruction, environmental pollution, ultraviolet exposure, and pathogenic agents (Daszak et al.,1999; Morell, 2001; Kiesecker et al., 2001). In response to this crisis, in the last decade development of assisted reproduction technologies (ART) has gained considerable interest. Although amphibian ART is a relatively new field of study, several recent advancements in protocol development have led to significant conservation achievements such as the production of endangered tadpoles from frozen-thawed sperm or the release of thousands of tadpoles produced by in-vitro fertilization (Kouba et al., 2009, 2013; Kouba and Vance, 2009). When applied effectively, ART could result in increased breeding

efficiency, reduced costs, halt loss of genetic diversity, reduce mate-pairing issues, and possibly even reject extinction as the only scenario for critically endangered species.

Anurans are characterised by external fertilisation, which is the ancestral condition in amphibians (Wells, 2007). Understanding the factors that affect fertilisation success, and how to manipulate these factors to enhance species propagation, is therefore a critical research priority in conservation biology. Reproductive success for amphibians requires that spermiation, ovulation, oviposition, fertilization, embryonic development, and metamorphosis were accomplished (Whitaker, 2001). The hormone hCG induces spermiation in a wide variety of toads and frogs (McKinnell et al., 1976; Easley et al., 1979; Clulow et al., 1999; Iimori et al., 2005; Browne et al., 2006a, 2006b; Pozzi et al., 2006) and is commonly used in the commercial breeding of Xenopus laevis (Schultz and Dawson, 2003). The sperm-egg interactions occur in the egg-jelly at fertilization and the egg-jelly prevents excess sperm from reaching the egg-surface after the egg is spawned into water. Sperm are prepared for fertilization before they reach the egg surface by a series of interactions with the egg extracellular matrix (Katagirl, 1987; Hedrick and Nishihara, 1991).

Pelophylax shqipericus, the Albanian water frog, is an endangered species and known populations are currently in decline. Significant threats to its habitat are presented by pollution and by drainage of wetlands, and a more direct threat is the aggressive collection of the species for commercial purposes (Szabolcs et al., 2017). To address the problem of extinction of some important amphibian species in Albania, assisted reproductive technology has been applied successfully (Turani and Aliko, 2015; Turani et al., 2018). In an effort to conserve declining populations of amphibians in Albania, the development of protocols for the IVF is of great interest. The present study aimed to advance our understanding of how to optimize the fertilization rate and to maximize fertilization success in the endangered Albanian water frog *Pelophylax shqipericus.*

MATERIAL AND METHODS

Animals

All frogs used in the experiment were captured during the breeding season in May 2017. *Pelophylax shqipericus* were obtained from a pond near Scadar Lake (42^{o}10N 19^{o}19E/42.167^{o}N 19.317^{o}E) in the north-western part, Albania. Between the capture procedure and the start of the experiment, frogs were kept in boxes (40x37x60 cm) separated by sex, on a 12-h/12-h light/dark cycle at 18°C-20°C. Animal maintenance and experimental procedures were in accordance with the Guide for Use and Care of Laboratory Animals (European Communities Council Directive 86/609/EEC) and national and institutional guidelines for animal welfare (Act No. 10465, 29/9/2011: "On the Veterinary Service in the Republic of Albania").

Experimental design

In-vitro fertilization were carried out by following the procedure described by Berger et al. (1994). Two days before the experiment, females and males were weighed, and their snout-vent lengths (SVL) were measured to the nearest 0.1 mm. Both sexes were subcutaneously injected with approximately 100 ml/10 g bodyweight of the human hormone hCG (Silla et al., 2012) in a concentration of 1 mg/100 ml isotonic saline solution. In females, this induces ovulation within 48 h; in males, it has a positive effect on sperm motility. Thereafter, animals were kept individually in covered plastic containers (20x11.5x7.5 cm) fitted with a moist paper towel. All artificial fertilization experiments were conducted at room temperature (21^{0}C).

Gamete collection and quality assessment

After acclimatization in the laboratory, male frogs were double-pithed, and their testes removed. Both testes were dissected, weighed to the nearest milligram and temporarily stored in 13 ml Holtfreter's solution (0.059 M, NaCl; 0.00067M KCl; 0.00076M CaCl2; 0.0024M NaHCO3). Testes were crushed in 5-15 ml of chilled (13ºC) Holtfreter's solution, thoroughly carved up with pincers, and the sperm released into a 50 mm Petri dish with 1 ml Holtfreter's solution. Testes crushes have long been used to efficiently obtain high numbers of mature sperm in amphibian embryology (Rugh, 1948), and this method of sperm collection has been widely used in amphibian fertilization and sperm quality studies (e.g. Browne et al., 1998; Edwards et al., 2004; Hettyey and Roberts, 2006). The sperm concentration was standardized by dilution with Holtfreter's solution to approximately 8 x106 sperm/ml and was controlled (deter-mined by hemocytometry). Sperm were stored on ice until use. Eggs were gently squeezed from the gravid females and the mucous capsules of five selected eggs from each female were dissected from the ovum and vitelline membrane. The oocytes pass through the oviduct where the jelly layers required for fertilization are acquired (Caputo et al., 2001). The capsules and jelly were macerated with filtered tap water in Eppendorph tubes, vortexed for 1 min, centrifuged and placed on ice. The eggs are allowed to develop at the room temperature, in 100 mm Petri plates moistened with antibiotic solution with 25 μg/ml amphotericin B, 10 U/ml penicillin and 10 μg/ml streptomycin.

Sperm morphology

To determine the morphology of *Pelophylax shqipericus* sperm, two to three drops of the single-male sperm suspensions used for the in-vitro fertilization were pipetted onto microscope slides and allowed to dry for later sperm-size measurements. Five slides of the seven males were examined under a KN-100TC, Kyowa, Tokyo fitted with a YCU-300F 3CCD camera that relayed images to a PC running ImageJ software, which was used to make all measurements. We measured the flagellum and head length (μ microns) of 100 sperm cells and calculated the total sperm length and the tail-to-head length ratio.

Sperm survival

For measuring sperm survival, sperm suspensions were prepared by crushing the dissected testes into petri dishes with 0.5 ml aged tap water. To filter out larger tissue pieces that might hinder subsequent sperm counting, we washed the sperm suspension through a filter (hole diameter: 100 mm) into an Eppendorf tube with aged tap water and diluted it to 0.5 ml. Sperm suspension (5 ml) was pipetted from Eppendorf tubes onto microscope slides after 5 min (t0), and again after 3, 8, 24, 48h. To reliably distinguish between living and dead spermatozoa we pipetted 5 ml of 1% neutral red solution onto each sperm solution (Romeis, 1948).

In vitro fertilization (IVF) experiments were carried out by using the sperm prepared as above and eggs were placed in 60 x 15-mm Petri dishes and covered with 5 ml of Holtfreter's solution (20-30 oocytes per Petri dish). By using the pipete the sperm were placed directly on top of each egg. Alternatively, by using a syringe 28G, 13 mm long, drops the sperm are injected under the egg jelly coat. Pre-incubation of sperm with egg jelly was accomplished by vigorously vortexing the jelly from one egg in 100 μl of Holtfreter's solution. Sperm was then incubated for 10 min in the jelly/buffer supernatant and then injected under the jelly coat.

The yield of fertilization is estimated as a percentage of 3-6 of the larval stages formed after 6-8 hours after the application of spermatozoids on the egg. The counting of formed embryos and unfertilized eggs was done under a stereomicroscope. Fertilization success is evident after 20–50 min when the black animal hemisphere of fertilized eggs rotates to the top. At this time, we added enough aged water to cover the eggs completely. To calculate the fertilization success, we first counted the total number of eggs in the petri dish and then, after the first

cleavage was visible (usually after 3 h), all fertilized and unfertilized eggs. The eggs were evaluated as successful fertilized when they reach neural stage (stage 14 according to Gosner) (Gosner, 1960). After this stage, the eggs were transferred from Petri dishes into plastic containers (20 x 11.5 x 7.5 cm) that were filled with aged water to a height of 2 cm. Water in the containers was changed periodically during development of the eggs.

RESULTS

We were able to obtain an in-vitro fertilization efficiency of 27% by simply mincing the testes and adding them directly over the eggs.

Table 1. *In vitro* fertilization of *P. shqipericus* eggs

Fertilization protocol	**Number tested eggs**	**Cleaved embryos (%)**
1. Sperm solution injected under the jelly coat	37	0
2. Sperm pre-incubated in jelly buffer and then injected under the jelly coat	45	8 (17.7)
3. Testes minced directly over eggs	30	8 (27)
TOTAL	112	16 (14.3)

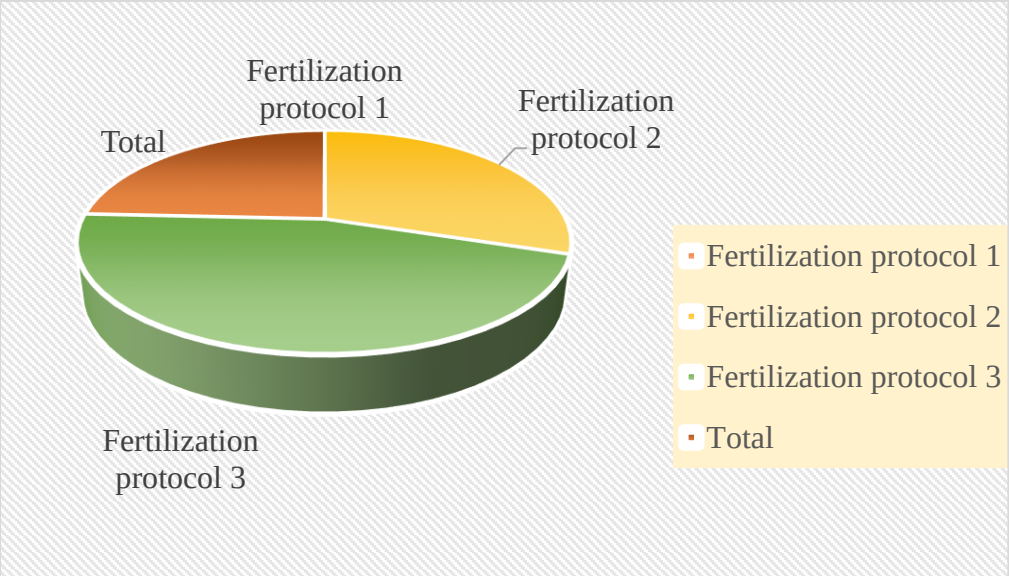

Figure 1. *In vitro* fertilization rate of *Pelophylax shqipericus*

Sperm concentration may play a role in fertilization efficiency. We also examined the possibility that a component of the egg jelly coat may be important for sperm capacitance. To test this, we incubated sperm with Holtfreter's solution and jelly, or Holtfreter's solution alone, and injected this under the jelly coat of eggs. When we injected the sperm with Holtfreter's solution alone, under the jelly coat of eggs, no fertilization happened at all. None of 37 fertilized eggs were developed to embryo, thus the fertility rate was 0%, while pre-incubation of sperm with an extract of the jelly coat in Holtfreter's solution resulted in a fertilization yield of 17.7%. This procedure boosted egg fertilizability to near the levels of fertilization by directly spray

with the extract of macerated testicles (27%), and suggests that interactions between sperm and the jelly coat may play a role in sperm capacitance and subsequent fertilization (Fig.1).

A total of 112 eggs was fertilized and put immediately under culture conditions for further maturation. Furthermore, 16 of 112 eggs (14.3%) developed to two-cell stage following culture in Holtfreter's solution modified with antibiotics. All of two-cells stage embryos reached the larval stage. Healthy frogs were raised and released into the wild.

Sperm morphology, measured by the total sperm length and the ratio between the sperm tail and head length. The average length of the head resulted 3.6 x 10^4µ; the average length of the tail resulted 6.5 x 10^4µ. So, the total sperm length resulted 10.1 x 10^4µ. The ratio between the sperm tail and head length resulted 3:1 x 10^4µ (Fig. 2).

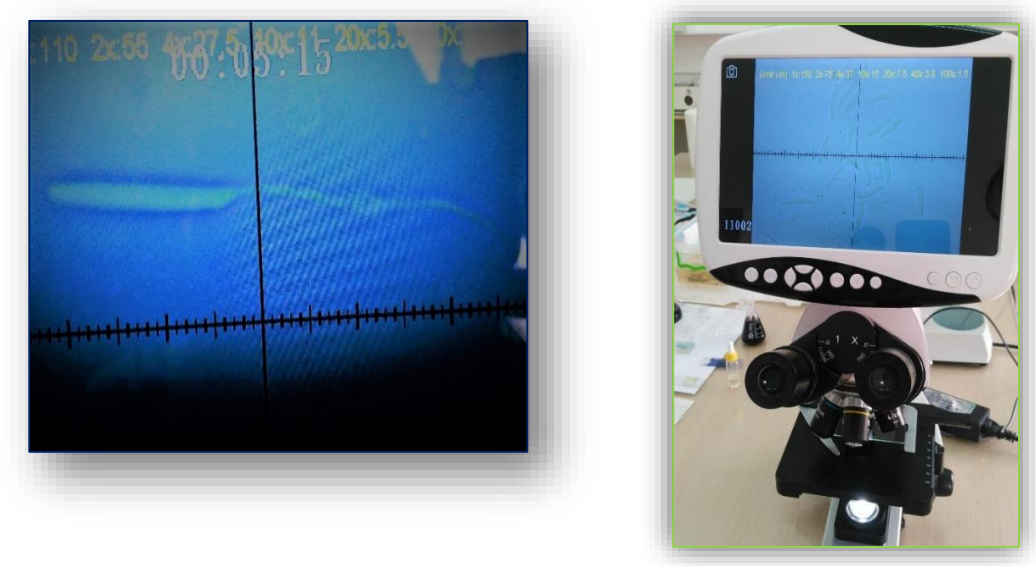

Figure 2. Spermatozoon of *Pelophylax shqipericus*

Figure 3 shows the decrease in sperm survival over time, expressed as the percentage of vital sperm at 3, 8, 24 and >48 h, relative to the percentage that was vital immediately after preparing the suspension (t_0=100%). Sperm survival declines exponentially.

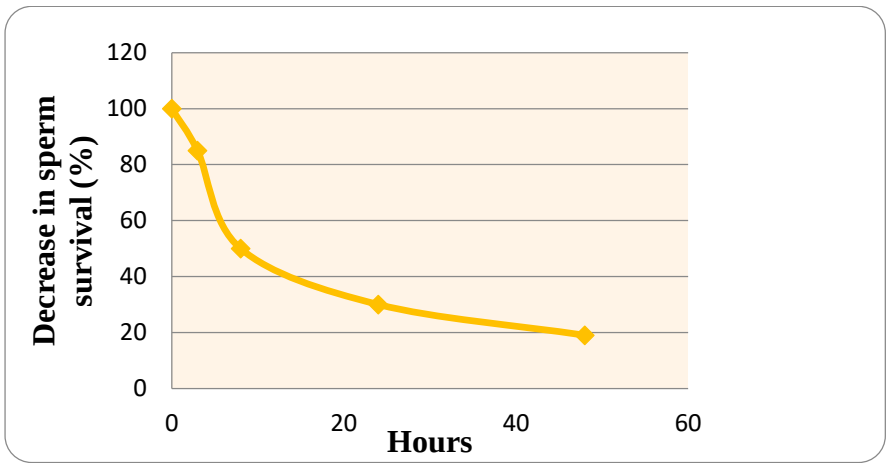

Figure 3. Sperm survival rate of *Pelophylax shqipericus*

DISCUSSION

The data obtained in our laboratory showed that: the protocol of in-vitro fertilisation of frog eggs by direct spray with the extract of macerated testicles was successfully. The average yield performed in our laboratory was 27% of all fertilized eggs. This can be considered a successful performance if taken into account that the efficiency of fertilization for the other techniques of fertilization (sperm diluted solution pipetted on eggs) is approximately 8% (Elinson, 1987; Ueda et al., 2002).

Using in-vitro fertilization of Albanian water frog, *Pelophylax shqipericus*, we found that incubation of sperm with an extract of the jelly coat eggs seems to have a higher relevance in the in-vitro fertilization efficiency. The egg-jelly fluid induced a greater proportion of sperm to become motile, and a faster swimming speed of spermatozoids. Proteins from the jelly coats of anuran eggs have been identified as essentials for fertilization (Barbieri, 1976) influencing the chemoattraction of sperm to eggs (Olson et al., 2001) and their binding to the vitelline envelope (Olson and Chandler, 1999). Components of egg jelly layers are necessary for the fertilization of the egg by chemoattracting sperm. Components of egg jelly layers are necessary for the fertilization of the egg by incoming sperm. Other possible roles of the jelly coat include protection from mechanical stresses (Thomas and Bolton, 1999; Thomas et al., 1999, Simons et al., 2009), prevention of polyspermy (Schuel, 1984), and increasing the effective diameter of the egg for higher sperm-egg collision frequency (Farley and Levitan, 2001; Podolsky, 2001; Podolsky, 2002).

Also, when sperm manipulation was done in very low temperatures (nearly t=4ºC), and when for its dilution Holtfreter's solution diluted to 10% and not concentrated were used. The dilution of sperm seems to reduce its fertilization ability. Incubating the gametes in any buffer solution with an osmolality higher than 50 mOsmol/kg will inhibit fertilization (Edwards et al., 2004), for aquatic anurans are known to achieve optimal rates of fertilization at low osmolarities (0-7mOsmol/kg) (Edwards et al. 2004). It is likely that this inhibition to fertilization is due to inactivation of sperm motility at higher osmolarities (Browne et al., 1998; Kouba et al., 2003; Edwards et al., 2004). Typically, fertilization takes place with sperm concentrations ranging from 10^4 to 10^6 spermatozoa per mL (Wolf and Hedrick, 1971; Browne et al., 1998; Edwards et al., 2004).

Our data demonstrated that longevity of sperm of *P. shqipericus* is highest for up to 3h, giving us the chance to have time for preparations in the laboratories until successful performing of fertilization (Fig. 2).

Conclusions

In vitro fertilization success depends on many factors, starting from egg and sperm release, quality of gametes, and composition of media used for fertilization and development of embryos. So, to realise successfully the *in vitro* fertilization technique for reproduction of Albanian water frog, all the above-mentioned events must be accomplished. Successfully control of sperm-egg interaction, as a crucial step of in vitro fertilization is the main step toward success. Also, sperm morphology and sperm concentration are very important in fertilization success, thus providing an optimal *in-vitro* fertilization protocol.

Finally, we can say that our results demonstrate efficient IVF in a fresh water endemic frog and help lay the foundation for future research and conservation possibilities for *Pelophylax shqipericus.*

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USE OF FACTOR ANALYSIS TO EVALUATE THE WATER QUALITY OF DAM LAKES LOCATED IN ERGENE RIVER BASIN (THRACE REGION, TURKEY)

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ABSTRACT

Altınyazı, Karaidemir, Kayalıköy, Kırklareli, Sultanköy and Süloğlu Dam Lakes are located in Ergene River Basin in the Thrace part of Marmara Region, where has very large agricultural lands because of contained rich soil and much freshwater resources. They were constructed by DSİ (State Water Works) in order to provide irrigation and drinking water and flood protection. The aim of this study was to evaluate the water quality of these reservoirs by a statistical approach by using Pearson Correlation Index (PCI) and Factor Analysis (FA). For this purpose, total of 16 limnologic parameters (temperature, dissolved oxygen, oxygen saturation, pH, electrical conductivity, total dissolved solids, salinity, turbidity, nitrate, nitrite, phosphate, sulfate, fluorine, biological oxygen demand, chemical oxygen demand, fecal coliform) were measured in selected total of 15 stations in spring season of 2018. According to the results of PCI, significant relations were recorded between the investigated parameters at the 0.01 and 0.05 significance levels. According to the results of FA, 3 factors explained 85% of the total variance.

Keywords: *Dam Lakes, Ergene River Basin, Factor analysis*

INTRODUCTION

Multistatistical techniques, which may help the interpretation of complex data matrices to better understand the ecological status of the investigated ecosystems, are being widely used in large numbers of countries in especially water quality assessment studies. Factor Analysis is one of the most convenient multivariate statistical methods that is being used commonly all over the world (Shrestha and Kazama, 2007; Akın et al., 2010; Tokatlı et al., 2013).

The Ergene River is the most important river basin of the Thrace Region and it is known to be exposed to a great industrial pressure (Tokatlı, 2015; 2017; Tokatlı and Baştatlı, 2016). Altınyazı, Karaidemir, Kayalıköy, Kırklareli, Sultanköy and Süloğlu Dam Lakes, which are located on the Ergene River Basin, are most important reservoirs of Thrace Region and they were constructed by DSİ, on the Basamaklar, Poğaça, Teke, Şeytandere, Manastır and Süloğlu

Streams respectively (http://www2.dsi.gov.tr/). But as many freshwater ecosystems, these reservoirs are being effected from agricultural and domestic pressure.

In the present study, Pearson Correlation Index (PCI) and Factor Analysis (FA) were applied to lymnological data detected from the reservoirs of Ergene River Basin in order to evaluate the water quality.

MATERIAL AND METHOD

Study area and collection of samples

Altınyazı, Karaidemir, Kayalıköy, Kırklareli, Sultanköy and Süloğlu Dam Lakes and selected stations on the reservoirs are given in Figure 1. Water samples were collected in spring season of 2018 and one water sample was taken from each selected stations on the dam lakes.

Physicochemical analysis

Temperature, dissolved oxygen, oxygen saturation, pH, EC, TDS and salinity parameters were determined by using "Hach Lange HQ40D Multiparameter" device during the field studies; turbidity parameter was determined by using "Hach Lange 2100Q Portable Turbiditymeter" device during the field studies; nitrate, nitrite, phosphate, sulphate, fluorine and COD parameters were determined by using "Hach Lange DR3900 Spectrophotometer" device during the laboratory studies; BOD parameter was determined by using "Hach Lange BOD Trak II" device during the laboratory studies.

Microbiological analysis

Microbiological analysis was carried out using membrane filtration technique. All water samples were filtered with membrane filtration technique and the membrane filter was placed in coliform chromogenic m-FC Agar. All growth mediums were left to incubate for 24 hours at 44.5 ± 0.2 0C and counted by automatic colony counter.

Statistical analysis

Pearson Correlation Index (PCI) was applied to the results in order to determine the relations between the psychochemical parameters by using the SPSS 17 package program. Factor Analysis (FA) was applied to the results in order to determine the effective varifactors on reservoirs of Ergene River Basin according to correlated variables by using the SPSS 17 package program.

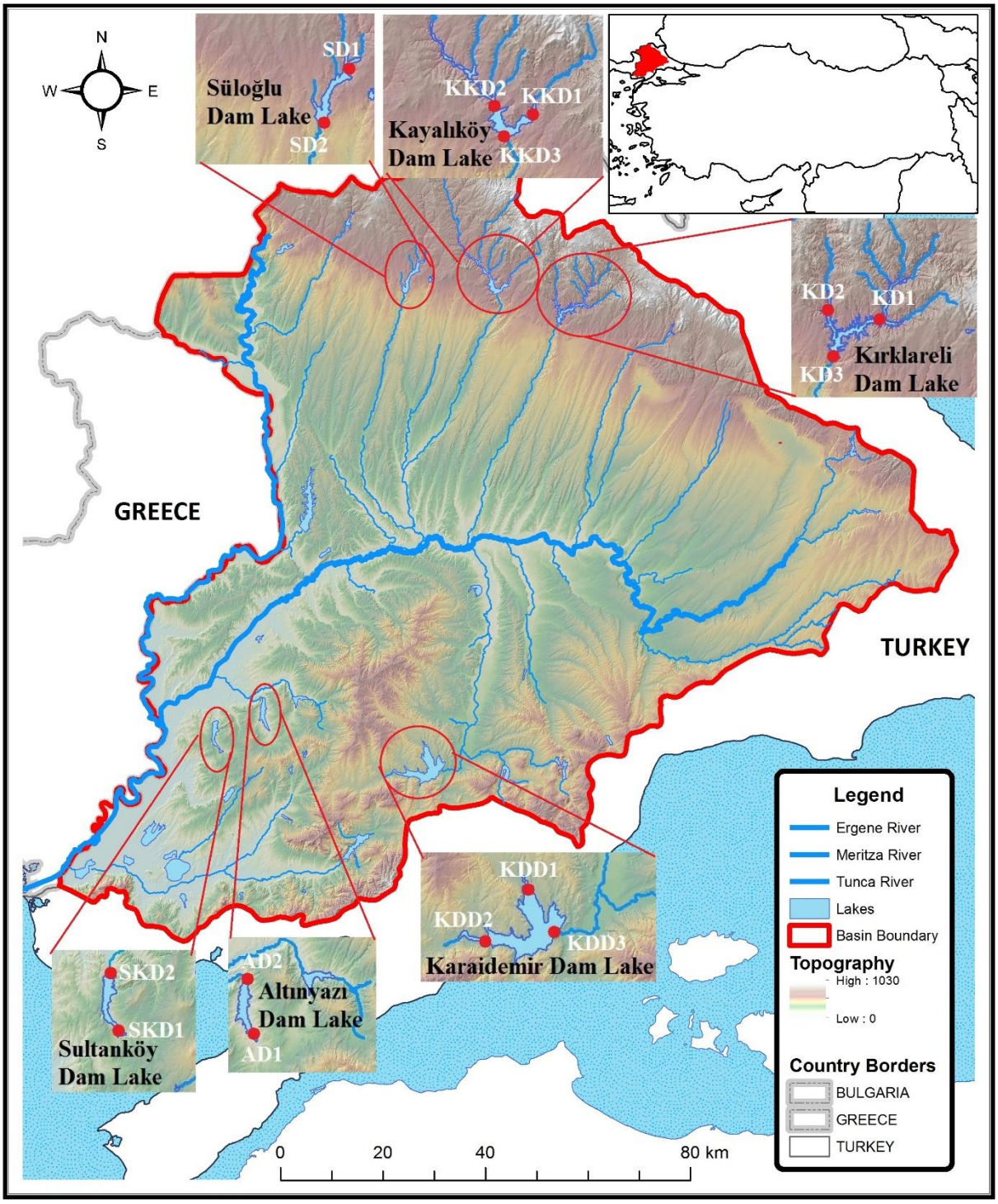

Figure 1. Ergene River Basin, dam lakes and selected stations

RESULTS AND DISCUSSION

Pearson Correlation Index

The relations between the levels of physical and chemical parameters in the reservoirs of Ergene River Basin were determined by using detected data (n = 15 for all parameters) and all detected relations are given in Table 1.

Factor Analysis (FA)

FA was used to determine the effective varifactors on the reservoirs of Ergene River Basin by using correlated variables. Uncorrelated variables were removed from the data set in order to increase the reliability of FA. A total of 11 variables were used to detect the varifactors (n = 15 for all parameters). Result of KMO (Kaiser-Meyer-Olkin) test that presents the measure of sampling adequacy was 0.527 and this value means that, the sampling adequacy was in a good level for the present application (>0.5) (Liu et al., 2003).

Eigenvalues higher than one were taken as criterion for evaluate the principal components that required to explain the sources of variance in the data. According to rotated cumulative percentage variance, 3 factors explained 85% of the total variance (Table 2) and the scree plot of FA is given in Figure 2.

Table 2. Extracted values of various FA parameters

Total Variance Explained									
	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
Component	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	6.288	57.163	57.163	6.288	57.163	57.163	5.052	45.930	45.930
2	1.876	17.058	74.221	1.876	17.058	74.221	2.513	22.841	68.771
3	1.188	10.800	85.021	1.188	10.800	85.021	1.787	16.250	85.021

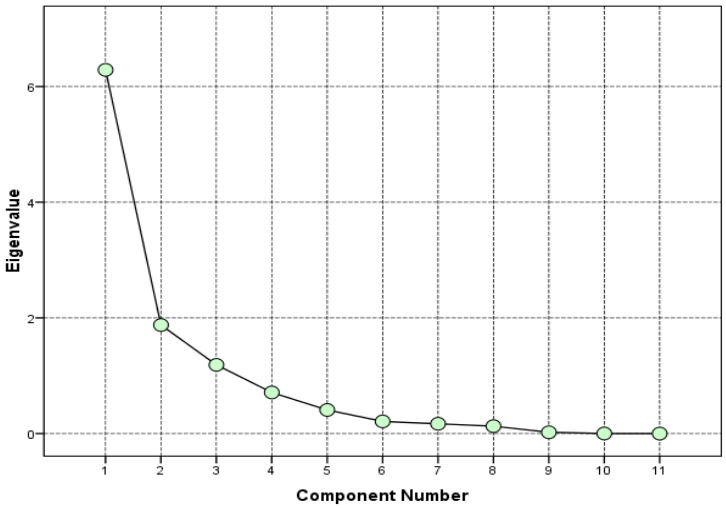

Figure 2. Scree plot of FA

Liu et al. (2003) classified the factor loadings according to loading values as “strong (>0.75)”, “moderate (0.75 – 0.50)” and “weak (0.50 – 0.30)”. The parameter loadings higher than 0.5 calculated after rotation for 3 components are given in Figure 3. Also component plot in rotated space, which shows the related variables of 3 factors, is given in Figure 4.

First factor (F1) explained 45.9% of total variance and it was related to the variables of SO_4, EC, TDS, salinity, F, pH and NO_3 parameters. SO_4, EC, TDS and salinity parameters were strong positively; NO_3 parameter was moderate positively; and pH parameter was moderate negatively loaded with this factor (Figure 3, 4).

Table 1. Pearson Correlation Index coefficients

	Temp	**DO**	**O2sat**	**pH**	**EC**	**TDS**	**Sal**	**Tur**	**NO_3**	**NO_2**	**PO_4**	**SO_4**	**F**	**COD**	**BOD**	**FC**
Temp	1															
DO	-.128	1														
O2sat	.130	.963**	1													
pH	.200	.083	.190	1												
EC	-.546*	.320	.145	-.632*	1											
TDS	-.579*	.298	.115	-.638*	.998**	1										
Sal	-.591*	.289	.103	-.639*	.997**	.999**	1									
Tur	.621*	-.358	-.213	-.049	-.434	-.461	-.470	1								
NO_3	-.542*	.202	.025	-.355	.643**	.634*	.626*	-.226	1							
NO_2	-.523*	.668**	.498	-.404	.765**	.746**	.742**	-.245	.620*	1						
PO_4	-.043	-.052	-.119	-.593*	.211	.199	.188	.431	.413	.407	1					
SO_4	-.439	.123	-.025	-.605*	.948**	.941**	.937**	-.250	.670**	.650**	.222	1				
F	-.314	.406	.297	-.428	.763**	.735**	.729**	-.096	.670**	.751**	.191	.821**	1			
COD	.006	.329	.286	-.311	.143	.123	.122	.255	-.040	.455	.352	.089	.354	1		
BOD	.346	.412	.510	.123	-.099	-.135	-.149	.457	-.086	.201	.020	-.090	.194	.213	1	
FC	.526*	-.336	-.160	.246	-.420	-.428	-.422	.410	-.535*	-.511	-.238	-.278	-.166	-.022	-.082	1

Temp: Temperature; DO: Dissolved oxygen; O2sat: Oxygen saturation; Sal: Salinity; Tur: Turbidity; FC: Fecal coliform

*: Correlation is significant at the 0.05 level ($p<0.05$); **: Correlation is significant at the 0,01 level ($p<0.01$)

Second factor (F2) explained 22.8% of total variance and it was related to the variables of NO_3, fecal coliform, temperature and turbidity parameters. Fecal coliform and temperature parameters were strong positively; turbidity parameter was moderate positively; and NO_3 parameter was moderate negatively loaded with this factor (Figure 3, 4).

Third factor (F3) explained 16.2% of total variance and it was related to the variables of pH, turbidity and pH parameters. PO_4 parameter was strong positively; turbidity parameter was moderate positively; and pH parameter was moderate negatively loaded with this factor (Figure 3, 4).

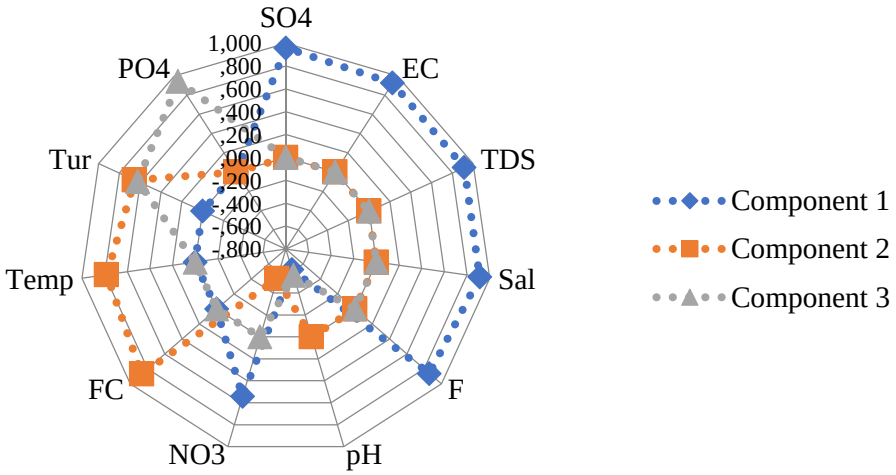

Figure 3. Rotated component matrix

As it is known that one of the most widely used statistical techniques is Factor Analysis, which provides valuable and easy explaining data. In a study performed in Xiangjiang watershed in China, FA was used to evaluate the quality of some aquatic habitats. According to data observed, FA reduced the data sets in 4 latent factors for 3 different sites accounting for 71.62%, 71.77% and 72.01% of the total variance (Zhang et al., 2009). In another study performed in Uluabat Lake in Turkey, FA was used to assess the water quality. According to data observed, 3 factors explained 77.35% of total variance (Iscen et al., 2007).

Agricultural applications could significantly raise the concentrations of nitrogenous compounds in close aquatic ecosystems to the agricultural lands (Wetzel, 201; Manahan, 2011). According to results of FA, "F1 Factor", which was explained 45.9% of total variance and strong positively related to the variables of SO_4, EC, TDS, salinity, F and NO_3 parameters, was determined as the most effective component for the reservoirs of Ergene River Basin.

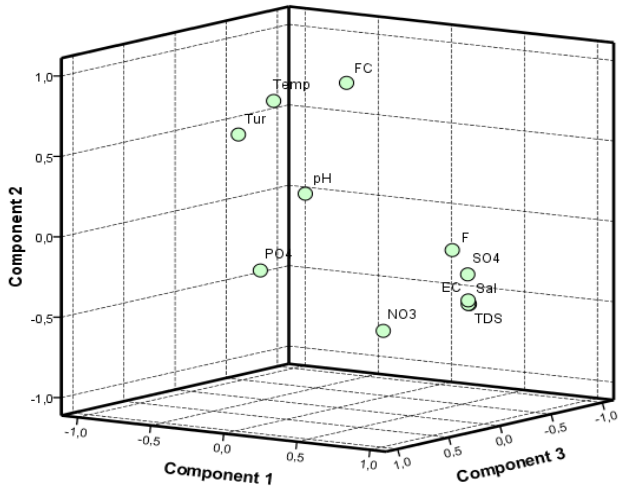

Figure 4. Component plot in rotated space

CONCLUSION

In this study, Factor Analysis was used to evaluate the water quality of the reservoirs of Ergene River Basin by using a large number of physical, chemical and biological data. Factor Analysis helped to identify the effective factors on the system and 3 effective factors were determined that were explained 85% of the total variance. In conclusion, multistatistical evaluation is necessary for a sophisticated environmental evaluation especially in water quality assessment studies, because of obtained large numbers of different parameters and difficulty of the interpretations of all parameters.

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USE OF PRINCIPLE COMPONENT ANALYSIS TO EVALUATE THE GROUNDWATER QUALITY OF VILLAGES LOCATED IN ERGENE RIVER BASIN

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Abstract

Ergene River is located on the Thrace part of Marmara Region and it is known as one of the most contaminated aquatic habitats of Turkey. This study was carried out to evaluate the groundwater quality of settlement areas located in the Ergene River Basin by using some statistical methods. Water samples were collected from 30 villages in summer season of 2018. Some physical and chemical water quality parameters including dissolved oxygen, oxygen saturation, pH, electrical conductivity (EC), total dissolved solids (TDS), salinity, turbidity, nitrite (NO_2), nitrate (NO_3), phosphate (PO_4), sulphate (SO_4) and chemical oxygen demand (COD) were determined and the results were evaluated by using by using Pearson Correlation Index (PCI) and Principle Component Analysis (PCA). According to the results of PCI, significant relations were recorded between the investigated parameters at the 0.01 and 0.05 significance levels. According to the results of PCA, 2 factors named as "Ionic Factor" and "Agricultural Factor" explained 77.7% of the total variance.

Keywords: *Ergene River Basin, Groundwater quality, Principle Component Analysis*

INTRODUCTION

Freshwater resources are among the most significant and adversely affected components of environment. Pollution caused by agricultural activities in especially rural areas decreases the quality of these limited freshwater (Çiçek et al., 2013; Tokatlı et al., 2014; Köse et al., 2015). Statistical techniques may help the interpretation of complex data matrices to better understand the ecological status of the investigated ecosystems. Principle Component Analysis is one of the most convenient statistical methods that is being used commonly in many countries in water quality assessment studies (Shrestha and Kazama, 2007; Akın et al., 2010; Tokatlı et al., 2013). The aim of this study was to evaluate the groundwater quality of settlement areas located in the Ergene River Basin by a statistical approach using Pearson Correlation Index (PCI) and Principle Component Analysis (PCI).

MATERIAL AND METHOD

Study area and collection of samples

In this study, groundwater samples were collected in summer season of 2018 from 30 stations from the drill fountains of the villages located in the Ergene River Basin. Groundwater with a volume of three wells was purged before sampling. Drinking water samples were then collected at the outflow of drill pump in polyethylene bottles. Coordinate information and

locations of selected stations are given in Table 1 and map of investigated area is given in Figure 1.

Physicochemical analysis

Dissolved oxygen, oxygen saturation, pH, EC, TDS and salinity parameters were determined by using "Hach Lange HQ40D Multiparameter" device during the field studies; turbidity parameter was determined by using "Hach Lange 2100Q Portable Turbiditymeter" device during the field studies; nitrate, nitrite, phosphate, sulphate, fluorine and COD parameters were determined by using "Hach Lange DR3900 Spectrophotometer" device during the laboratory studies.

Statistical analysis

Pearson Correlation Index (PCI) was applied to the results in order to determine the relations between the psychochemical parameters by using the SPSS 17 package program. Principle Component Analysis (PCA) was applied to the results in order to determine the effective varifactors according to correlated variables by using the SPSS 17 package program.

Table 1. Location properties of villages

Locality	**Coordinates**		**Locality**	**Coordinates**	
	North	South		North	South
Muratlı	41.17275	27.49570	Karakavak	41.32615	27.07046
Sarılar	41.14440	27.66180	Kadriye	41.34883	26.99870
Çorlu	41.15593	27.81326	Çerkezmüsellim	41.27186	27.02568
Velimeşe	41.24793	27.88046	Hayrabolu	41.21345	27.10629
Çerkezköy	41.28212	28.00176	Pehlivanköy	41.34710	26.92391
Saray	41.44099	27.92175	Danişment	41.30453	26.90137
Karlı	41.36929	27.86502	Çöpköy	41.21846	26.82429
Marmaracık	41.20692	27.75227	Bayramlı	41.30688	26.82262
Vakıflar	41.26342	27.64992	Uzunköprü	41.26693	26.68699
Karamusul	41.30349	27.44734	Salarlı	41.22682	26.62626
Müsellim	41.34041	27.37037	Kurtbey	41.14380	26.57977
Düğüncübaşı	41.33248	27.27715	Yenicegörece	41.13088	26.46713
Lüleburgaz	41.40263	27.36572	Meriç	41.19106	26.41824
Babaeski	41.43123	27.09134	Adasarhanlı	41.08398	26.35818
Alpullu	41.37195	27.14307	İpsala	40.92896	26.39274

Figure 1. Study area and selected stations

RESULTS AND DISCUSSION

Pearson Correlation Index

The relations between the levels of physical and chemical parameters in the groundwater of Ergene River Basin were determined by using detected data (n = 30 for all parameters) and all detected relations are given in Table 2.

According to results of PCI, the relations between oxygen saturation – dissolved oxygen (+); EC – TDS (+), salinity (+), SO_4 (+), F (+) and COD (-); TDS – salinity (+), SO_4 (+), F (+) and COD (-); salinity – SO_4 (+), F (+) and COD (-); SO_4 – F (+) and COD (-) levels were directly proportional at the 0.01 significance level.

Principle Component Analysis (PCA)

PCA was used to determine the effective varifactors on the groundwater of Ergene River Basin by using correlated variables. Uncorrelated variables were removed from the data set in order to increase the reliability of PCA. A total of 7 variables were used to detect the varifactors (n = 30 for all parameters). Result of KMO (Kaiser-Meyer-Olkin) test that presents the measure of sampling adequacy was 0.814 and this value means that, the sampling adequacy was in a good level for the present application (>0.5) (Liu et al., 2003).

Eigenvalues higher than one were taken as criterion for evaluate the principal components that required to explain the sources of variance in the data. According to rotated cumulative percentage variance, 2 factors explained 77.7% of the total variance (Table 3) and the scree plot of FA is given in Figure 2.

Table 2. Pearson Correlation Index coefficients

	DO	O2Sat	pH	EC	TDS	Sal	Tur	NO3	NO2	PO4	SO4	F	COD
DO	1												
O2Sat	.995**	1											
pH	-.243	-.258	1										
EC	-.107	-.040	-.186	1									
TDS	-.105	-.042	-.174	.998**	1								
Sal	-.107	-.044	-.173	.998**	1.000**	1							
Tur	-.214	-.200	-.239	.282	.284	.282	1						
NO3	.366*	.370*	-.438*	.362*	.376*	.371*	.110	1					
NO2	-.246	-.215	-.323	.161	.148	.149	-.014	.074	1				
PO4	.119	.140	-.063	-.032	-.049	-.047	-.090	.020	-.050	1			
SO4	-.032	.031	-.343	.783**	.771**	.771**	.251	.261	.154	.046	1		
F	-.189	-.147	.322	.623**	.615**	.617**	.099	-.141	-.049	-.004	.537**	1	
COD	.009	-.046	.280	-.599**	-.600**	-.599**	-.420*	-.406*	-.212	-.040	-.486**	-.201	1

DO: Dissolved oxygen; O2sat: Oxygen saturation; Sal: Salinity; Tur: Turbidity

*: Correlation is significant at the 0.05 level ($p<0.05$); **: Correlation is significant at the 0,01 level ($p<0.01$)

Table 3. Extracted values of various FA parameters

Total Variance Explained									
Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	4.264	60.910	60.910	4.264	60.910	60.910	4.107	58.675	58.675
2	1.181	16.870	77.780	1.181	16.870	77.780	1.337	19.106	77.780

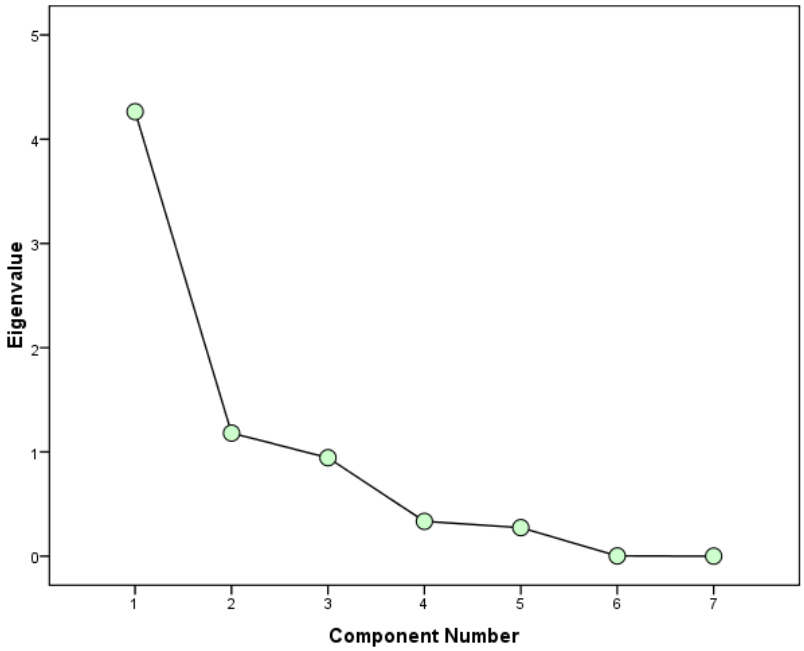

Figure 2. Scree plot of FA

Liu et al. (2003) classified the factor loadings according to loading values as "strong (>0.75)", "moderate (0.75 – 0.50)" and "weak (0.50 – 0.30)". The parameter loadings higher than 0.5 calculated after rotation for 3 components are given in Figure 3. Also component plot in rotated space, which shows the related variables of 2 factors, is given in Figure 4.

First factor (F1), named as "Ionic Factor", explained 58.6% of total EC, salinity, TDS, sulphate and fluorine parameters. All the parameters were strong positively loaded with this factor (Figure 3, 4). Second factor (F2), "Agricultural Factor", explained 19.1% of total variance and it was related to the variables of nitrate and nitrite parameters. Nitrate parameter was strong and nitrite parameter was moderate positively loaded with this factor (Figure 3, 4).

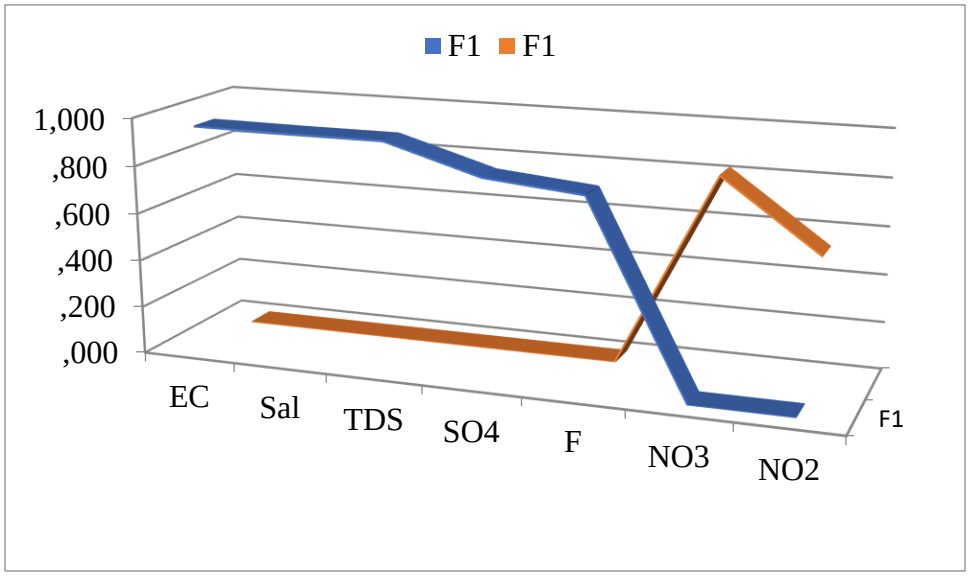

Figure 3. Rotated component matrix

EC is affected by the presence of dissolved solids. TDS is defined as the quantity of dissolved material in water. Also salinity is defined as the total of all salts dissolved in water. EC, TDS, and salinity parameters in water are closely related and these parameters may indicate general water quality. Discharges to groundwater may change the EC, TDS and salinity levels. Sewage water and especially irrigation practices are known as significantly effective factors on these parameters (Wetzel, 2001; Manahan, 2011). In this study, significant relations were determined among EC, TDS and salinity parameters ($p<0.01$) and according to the results of PCA, first factor (F1), which was related to the variables of EC, TDS and salinity, was the most effective factor on the groundwater quality in Ergene River Basin.

It is known that fertilizers used in agricultural activities increase the level of nitrogen compounds in water and soil especially in rural areas (Wetzel, 2001; Manahan, 2011). The main sources of nitrogen compounds in groundwater is nitrogen rich fertilizers in general that is commonly used around the basin (Self and Waskom, 2013; Tokatlı, 2014). According to the results of PCA, "Agricultural Factor" (F2) was defined as an effective factor on the groundwater quality of the basin, which was related to the variables of nitrate and nitrite.

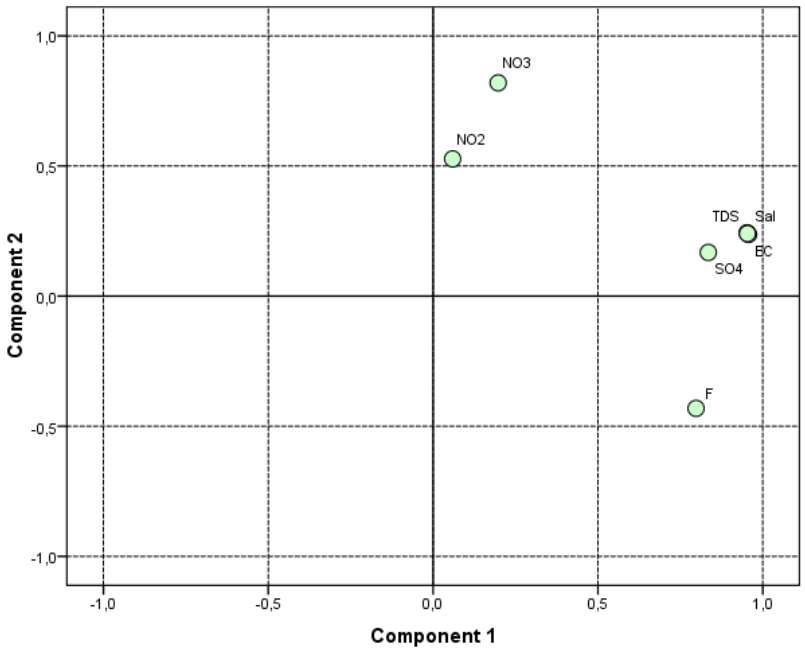

Figure 4. Component plot in rotated space

CONCLUSION

In this study, Principle Component Analysis (PCA) was used to evaluate the groundwater quality of the groundwater of Ergene River Basin by using a large number of physical and chemical data. PCA helped to identify the effective factors on the groundwater of the system and 2 effective factors were determined that were explained 77.7% of the total variance. In conclusion, multistatistical evaluation is necessary for a sophisticated environmental evaluation especially in

groundwater quality assessment studies, because of obtained large numbers of different parameters.

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COMPARISON OF ORGANIC POLLUTION BETWEEN TWO MAIN BRANCHES OF DRIN RIVER

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ABSTRACT

The objective of this paper is to compare data on pollution from organochlorinated pesticides and polychlorinated biphenyls (PCB) in waters of the two most important branches of Drin River, one passing through Kosovo and the other originating from Ohrid Lake and passing through north-eastern Albania. The organochlorinated pesticides and polychlorinated biphenyls are the widest class of organic pollutions and the most problematic in environment. The method applied to detect pesticides was sampling water in 10- 25 cm of depth in two specific locations, one at the end of each of the branches of the river before their conjuction. Samples of surface waters were taken during 2012 – 2015. The liquid – liquid (L/L) water extraction and gas chromatography (GC) with micro electron-capture detector (μECD) were applied for pesticide residue analyse. The quantified pesticides were α-HCH, β-HCH, HCB, Lindan, Heptachlor, 2,4-DDE, 4,4-DDE, DDT, DDD representing organoclorinated pesticides and PCB 28, PCB 101, PCB118, PCB 153, PCB 138 for polychlorinated biphenyls. The most polluted branch resulted to be Black Drin.
Keywords: *Drin River, Organochlorinated pesticides, polychlorinated biphenyls.*

INTRODUCTION

Drin, the longest river in Albania, is formed near the Albanian border from two main branches: White Drin which springs and passes through Kosovo and Black Drin which originates from the Ohrid lake and passes in western part of FYROM and in north-western Albania.

Aim of study

The aim of this study is to compare pollution from organochlorinated pesticides (OCP) and polychlorinated biphenyls (PCB) in these two branches of the river. A number of compounds were chosen for the evaluation of the level of organic pollution, namely: α-HCH, β-HCH, HCB, Lindan, Heptachlor, 2,4-DDE, 4,4-DDE, DDT, DDD as representatives of organoclorinated pesticids and PCB-28, PCB-52, PCB-101, PCB-118, PCB- 138, PCB-153 and PCB-180 as representatives of polychlorinated biphenyls.

MATERIAL AND METHODS

Study area

One sample point at the end of each river branch before their union was chosen: Morine for White Drin and Zall Rec for Black Drin. Samples of surfice waters were taken each month during the period October 2012 to September 2015.

Sampling technique

Samples were collected using grab sampler bottle UWITEC, transferred in 1 litter glass bottles and stored in 4°C prior to chemical analyses.

Extraction and measurement

Water samples were processed by Liquid – Liquid extraction using 1 L separator funnel. Samples were spiked with 10 µl PCB 29 as internal standard in 2.5 ng μl^{-1} concentration and extracted with 40 ml n-hexane. The organic phase (n-hexane) was dried with 10g Na_2SO_4. The extracts were cleaned in a glass column filled with deactivated 5% water fluorosil (100-200 mesh or 0.075-0.150 mm). The column was rinsed out with 8 ml mixture of 4:1 n-hexane and DCM. The extract was evaporated till 1 ml extract by Rotary Evaporator (Laborota 4000, Heidolph) and N2 evaporator (Thermo Scientific). The 1ml extracts were transferred in the chromatographic vials. Gas chromatographic analyses were performed with Agilent 7890 gas chromatograph equipped with a micro^{63}Ni electron-capture detector and a split/splitless injector and auto-injector. The column used was a HP-5 [low/mid polarity, 5% (phenyl methyl siloxane)] (30 m x 0.32 mm I.D., 0.25µm film). The split/splitless injector and detector temperatures were set at 280^{0}C and 300^{o}C respectively. Nitrogen (N_2) at 3.5 ml/min was used as carrier gas and 29 ml min^{-1} for make-up. The initial oven temperature was kept at 60^{0}C for 15 min, increased to 200^{o}C at 20^{o}C/min, held for 10min and then increased to 250^{o}C at 4^{o}C/min for 20min. Temperature was finally increased to 300^{o}C at 10^{o}C/min and held for 7 min. Injection volume was 1µl. The organochlorinated pesticides and polychlorinated biphenyls quantification was performed by internal standard method. The system was calibrated with a standard mixture containing organochlorinated pesticides and polychlorinated biphenyls. Values of each component are expressed in ng/l and mean concentration during the year for each component was calculated.

RESULTS

Mean concentrations of each component for the two sample point are presented in the tables and graphs below.

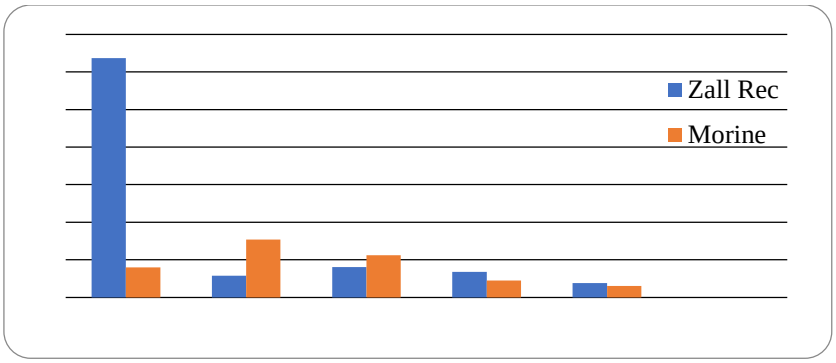
Table 1. Mean concentration of PCBs (ng/l).

	PCB-28	PCB-101	PCB-118	PCB-153	PCB-138	PCB-52	PCB-180
Zall Rec	63.69	5.79	8.03	6.82	3.81	Nd*	Nd*
Morine	7.97	15.37	11.18	4.5	3.07	Nd*	Nd*

Nd = not detected.

PCB-52 and PCB 180 were not detectable in any in sample in either sample points. In an other study on the same time period, Nuro and Marku have found these two PCB in considerable levels in sediment samples near the delta of Drin river (Nuro and Marku, 2013).

PCB-28 concentration were 10 fold higher in Black Drin compared to White Drin. PCB-153 and PCB-138 mean concentration were only slighty higher in Black Drin, while PCB-101 and PCB-118 were slightly higher in White Drin. However, in all the samples, the concentration of every studied PCB was lower than the EU limit.

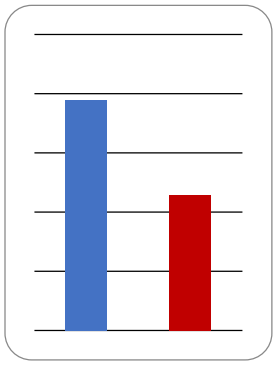


Graph 1. Comparison of PCBs.

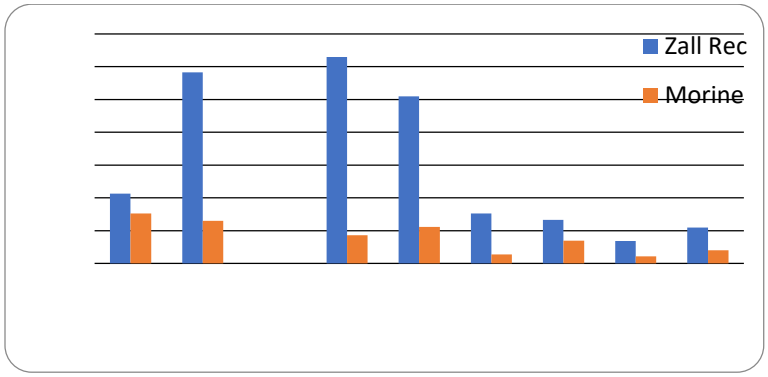
Table 2. Mean concentration of OCPs (ng/l).

	α-HCH	**β-HCH**	**HCB**	**Lindan**	**Heptaclor**	**2.4-DDE**	**4.4-DDE**	**DDT**	**DDD**
Zall Rec	106.52	291.42	1948.42	314.69	254.83	76.28	66.61	34.12	54.66
Morine	76.38	65.16	1146.27	43.07	55.96	13.68	34.67	10.72	20.2

The values of OCP mean concentrations vary on the range of ten to a few hundreds ng/l for α-HCH, β-HCH, Lindan, Heptaclor, DDE, DDT and DDD. Only HCB values range is in thousands ng/l. For this reason, we have separately represented HCB from the other OCPs. Figure 2 shows the comparison of HCB mean concentrations in the two sampling points while figure 3 the comparison of all the other OCPs.



Graph 2. Comparison of HCB.



Graph 3.Comparison of OCPs except for HCB.

Black Drin results more polluted than White Drin for each OCP. The difference is more obvious for β-HCH, Lindan, Heptaclor and 2,4-DDE which mean concentrations in Black Drin are a multitude of those in White Drin. Also the mean concentrations of HCB, 4,4-DDE, DDT and DDD in this river branch are almost twice those in the other branch. A slightly higher but considerable difference of 30 ng/l was found for α-HCH in Black Drin.

A study concerning similar substances in for Ohrid Lake where Black Drin originates, has reported levels 12 – 24 ng/l for Lindan and 19 – 32 ng/l for HCH. These are very low compared to our results in Zall Rec, the end point of this river branch, respectively in the range of 300 ng/l for Lindan and 400 ng/l for total HCH. Slightly higher levels are detected in our study even for 4.4-DDE, DDT and DDD compared with Velianovskaet al. (Veljanoska-Sarafiloska et al., 2011) This

discrepancy may be a testimony of agricultural pollution in north-eastern Albania where this river branch passes and colletcts smaller branches. Pollution from municipal, industrial, and agrochemical sources remains a major threat to Balkan freshwater ecosystems. Mining mainly affect Bulgarian and Albanian rivers, industrial pollution is important in Bulgaria, FYROM and Bosnia and Herzegovina, agricultural pollution is widespread in Greece, Bulgaria and Albania, while municipal waste water pollution prevails in all countries except Greece (Lazarov et al, 2005).

The sea, rivers and lakes have become the environmental reservoirs for all possible organic pollutants (Chee et al., 1996). The organochlorine pesticides group includes DDT (dichlorodiphenyltrichloroethane), methoxychlor, aldrin, dieldrin, chlordane, toxaphene, endrin, heptachlor, and lindane (gamma isomer of benzene hexachloride (BHC)). These are trade names for closely related hydrocarbon compounds to which several chlorine atoms have been joined (Hung and Thiemann, (2002). Due to their environmental persistence, these pollutants can cause contamination of surface water and underground water (Jones and de Voogt, 1999). Persistent organic pollutants (POPs), including organochlorine pesticides (OCPs), are of global concern because of their toxicity, resistance to degradation, potential for long-term transport and their tendency to accumulate in fatty tissues (lipophilicity), the latter of which renders them likely to bioaccumulate through food chain (Skoulikidis, 2009).

CONCLUSIONS

It results that the overall level of pollution is higher in Black Drin. Levels of PCB are within EU norms in both river branches, but OCP levels are above them. HCB is the most problematic substance. Other pollutants with high levels are Lindan, HCH and Heptaclor.

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A COMPARATIVE STUDY THE ANTIOXIDANT PROPERTIES OF DIFFERENT CITRUS JUICES

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ABSTRACT

Citrus fruits are important sources of beneficial phytochemicals, such as vitamins A, C and E, mineral elements, flavonoids, coumarins, limonoids and carotenoids. Epidemiological studies have shown that citrus species possess significant biological functions on human health, including antioxidative, anticarcinogenic, antiatherosclerotic, antimutagenic, and angiogenesis inhibitory activities. In this study, total phenolic, total flavonoid, total carotenoid and vitamin C contents of the juices of seven citrus varieties were determined grown in Antalya, Turkey. Additionally, antioxidant activities were also investigated using DPPH method. Total phenolic contents of the citrus juices varied from 18.21 to 52.44 mg gallic acid equivalent/100 mL and flavonoid contents varied from 2.77 to 10.64 mg catechin equivalent/100 mL. Total carotenoid contents changed between 1.27 and 1.86 mg/mL and vitamin C contents were from 46.06 to 86.01 mg/100mL. IC_{50} values of the citrus juices ranged between 52.88 and 122.84 µl. Significant differences were statistically observed among citrus varieties in terms of investigated parameters.

Keywords: *Citrus, Antioxidant activity, Phenolic, Flavonoid*

INTRODUCTION

The citrus species, belonging to the family *Rutaceae*, are the most popular agricultural product in the world (Turner and Burri, 2013). Turkey is one of the main citrus producers among the World countries and ranks 9th in the World (Faostat, 2016). Total citrus fruit production of Turkey was 4.796.726 tonnes in 2017. Orange, mandarin, lemon and grapefruit are the main citrus species produced in Turkey. The Mediterranean and Aegean Regions are the major citrus growing areas of Turkey. The West Mediterranean Region is the second largest citrus area of Turkey. Antalya province, located in this region, accounts for 14% of Turkey's total citrus production (TUİK, 2017).

Citrus fruits are important source of bioactive compounds, including ascorbic acid, carotenoids, flavonoids, phenolic compounds, dietary fiber, vitamins and minerals which are beneficial aspects to human health. Epidemiological evidences have suggested that these compounds possess important biological activities such as antioxidant, anti-inflammation, anti-mutagenicity, anti-carcinogenicity and anti-aging (Zou et al., 2016). The bioactive components and antioxidant properties of citrus fruits have been studied by many authors in recent years. The type and amount of bioactive compounds present in citrus fruits can be affected by many factors such as cultivar, fruit part, maturity state, cultural practices, processing, geographical and ecological conditions (Gorinstein et al., 2001; Abeysinghe et al., 2007; Alvarez et al., 2012; Sicari et al., 2016; Yoo et al., 2016). The antioxidant activity of citrus fruits is mainly due to phenolics and vitamin C (Abeysinghe et al., 2007; Xu et al., 2008). Flavonoids are major phenolic compounds in citrus fruits. The main flavonoid groups in citrus juice are flavanones, flavones and flavonols (Hunlun et

al., 2017). Among the flavonoids, naringin, neohesperidin, neoeriocitrin, hesperidin, narirutin and didymin are found in bergamot, orange, mandarin, grapefruit and bitter orange juices (Tripoli et al., 2007). In addition to flavonoids, citrus juices contain significant amount of phenolic acids, including ferulic, sinapik, cafeic, chlorogenic, *p*-coumaric and *o*-coumaric acids (Wang et al., 2007; Xu et al., 2008). Phenolic compounds have been reported to possess a wide range of biological actions, such as a key enzymes in mitochondria, protection against coronary hearth diseases, anti-inflammatory, anti-tumor, antioxidative and antimicrobial activities (Harborne and Williams, 2000; Morton et al., 2000). Vitamin C is the most commonly vitamin found in citrus fruits (Zou et al., 2016), and it shows antioxidant activity through scavenging reactive oxygen species and protecting against oxidation of biological molecules (Tripoli et al., 2007; Sdiri et al., 2012). Carotenoids are pigments responsible for the characteristic color of citrus juices and peels. More than a hundred individual carotenoids have been identified in citrus fruits. Carotenoids of citrus juices play an important role in the prevention of chronic diseases such as certain types of cancer, cataract and cardiovascular diseases, because of their antioxidant properties (Fanciullino et al., 2006). The aim of this study was to determine the total phenolic, flavonoid, carotenoid and vitamin C contents and antioxidant activities of seven citrus varieties grown in Antalya.

MATERIAL AND METHODS

Seven citrus varieties were selected for this study, including two mandarins (Yerli Apireno, Klemantin Fino), two grapefruits (Marsh Seedless, Star Ruby), one sour orange (Common Sour Orange), one lemon (Interdonato) and one bergamot variety (Yerli A-41). The fruits were collected from Batı Akdeniz Agricultural Research Institute (BATEM) citrus orchard in Antalya. All varieties were harvested at maturity stage. Citrus fruit juices were obtained by hand squeezing method. All analyses were carried out in four replicates. Fifteen fruits were used for each replication.

Total Phenolic content

Total phenolic content of juice samples were determined using the Folin-Ciocalteu reagent method as described Spanos and Wrolstad (1990). The absorbance was measured against the blank solution at 765 nm using a Shimadzu UV-1800 spectrophotometer (Shimadzu Inc., Kyoto, Japan). The results were expressed as mg gallic acid equivalent (GAE)/100 mL fresh juice.

Total Flavonoid content

Total flavonoid content in samples were quantified using a modified colorimetric method described by Zhishen et al. (1999). The absorbance was detected at 510 nm using a Shimadzu UV-1800 spectrophotometer (Shimadzu Inc., Kyoto, Japan) using catechin as the standard. The results were expressed as mg catechin equivalent (CE)/100 mL fresh juice.

Total carotenoid content

The method of Wang et al. (2008) was used for total carotenoid determination. Absorbance was measured at 450 nm by Shimadzu UV-1800 spectrophotometer (Shimadzu Inc., Kyoto, Japan). Total carotenoid content was expressed as mg β-carotene equivalents/ mL fresh juice.

Vitamin C

The content of vitamin C in juices was analyzed with the HPLC method (Sdiri et al., 2012). Each juice sample was extracted with 3% metaphosphoric acid solution. The mixture was centrifuged at 6500 rpm for 10 min at 4°C and the supernatant were filtered through a 0.45 μm membrane filter before injection. The separation was performed on ODS-3-C-18 Column (250x4.6 i.d.) using 2% potassium dihydrogen phosphate (pH 2.3) as the mobile phase at a flow rate of 0.6 mL/min at 25° C column temperature and 243 nm. Vitamin C contents were expressed as mg/100mL.

Antioxidant activity

The antioxidant activity of the citrus juices was evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging method (Brand-Williams et al., 1995) with some modifications. Five concentrations of juice samples were mixed 0.6 mL DPPH radical solution (1mM). The volume of mixtures was completed with 6 mL of pure methanol. After shaking, the mixtures were incubated at room temperature in the dark for 15 min. The absorbance of mixtures was measured by a spectrophotometer at 517 nm against blank without samples). The results were expressed as IC_{50} value.

Statistical analysis

SAS software program were used for statistical assessment. All experiments were carried out in four replicates. The results were expressed as means ± standard deviation (SD). All data were subjected to analysis of variance (ANOVA). The significant difference of means were compared by Duncan's multiple-range test at the level of $P < 0.05$.

RESULTS AND DISCUSSION

Total Phenolic and flavonoid content

The contents of total phenolic and flavonoid in the juices of seven citrus varieties are shown in Table 1. There were observed significant differences in the contents of total phenolic and flavonoid in juice samples ($P<0.05$). Star Ruby had the highest total phenolic content (52.44 mg GAE/100 mL), followed by Yerli Apireno (44.29 mg GAE/100 mL), Marsh Seedless (40.76 mg GAE/100 mL) and Common Sour Orange (40.52 mg GAE/100 mL). Our values were lower than those reported in the literature (Ersus and Çam, 2007; Xu et al., 2008; Kelebek, 2010; Sicari et al., 2016). The total flavonoid content of the juice samples ranged from 2.77 to 10.6 mg CE/100 mL. The highest total flavonoid content was found in bergamot (Yerli A-41) juice. The lowest values for total phenolic (18.21 mg GAE/100 mL) and total flavonoid (2.77 mg CE/100 mL) were detected in lemon (Interdonato) juice. The variation in phenolic and flavonoid content of citrus fruits depends on genetic properties, degree of maturity, climatic conditions and processing (Gil-Izquierdo et al., 2002; Germana et al., 2004; Nogata et al. 2006; Xu et al., 2008; Sun et al., 2015).

Table 1. Total phenolic and flavonoid content of citrus varieties*

Varieties	**Species**	**Total phenolic content (mg GAE/100 mL)**	**Total flavonoid content (mg CE/100 mL)**
Klemantin Fino	Mandarin	39.99±2.72 b	3.76±0.03 d
Yerli Apireno	Mandarin	44.29±5.68 b	8.42±1.30 b
Star Ruby	Grapefruit	52.44±1.49 a	8.05±0.53 b
Marsh Seedless	Grapefruit	40.76±1.47 b	4.95±0.11 c
Interdonato	Lemon	18.21±2.70 d	2.77±0.19 e
Common Sour Orange	Sour Orange	40.52±2.76 b	3.92±0.11 d
Yerli A-41	Bergamot	30.37±2.15 c	10.64±0.23 a

*Means with different letters within the same column represent significant differences at *P<0.05*

Total carotenoid, Vitamin C and Antioxidant Activity

Total carotenoid, vitamin C and IC_{50} values of citrus juices are presented in Table 1. A statistical significant difference (P <0.05) was found among the samples for examined parameters. The highest total carotenoid value was detected in Yerli Apireno (1.86 mg/ml), followed by Common Sour Orange (1.76 mg/mL) and Marsh Seedless (1.66 mg/mL). Bergamot (Yerli A-41) had the lowest total carotenoid content (1.27 mg/mL). Total carotenoid contents in juices from different citrus varieties were higher than those reported by Xu et al. (2008). These differences may be due to the geographic factors, cultivar, methods of extraction and analyses. Vitamin C values of citrus juices were varied from 46.06 mg/100 mL to 86.01 mg/100 mL. The highest vitamin C content was found in Klemantin Fino, while the lowest content was found in Interdonato in this study.

Table 2. Total carotenoid, Vitamin C and IC_{50} values of citrus varieties*

Varieties	**Species**	**Total carotenoid content (mg/mL)**	**Vitamin C (mg/100 mL)**	**IC_{50} (µL)**
Klemantin Fino	Mandarin	1.37±0.03 f	86.01±6.22 a	52.88±0.02 g
Yerli Apireno	Mandarin	1.86±0.03 a	65.64±5.89 c	84.54±0.02 b
Star Ruby	Grapefruit	1.47±0.03 e	76.44±2.63 b	82.73±0.02 d
Marsh Seedless	Grapefruit	1.66±0.03 c	72.89±2.58 b	112.84±0.02 a
Interdonato	Lemon	1.57±0.03 d	46.06±1.41 d	84.33±0.01 c
Common Sour Orange	Sour Orange	1.76±0.03 b	74.49±2.21 b	66.16±0.01 f
Yerli A-41	Bergamot	1.27±0.03 g	73.27±1.67 b	67.26±0.01 e

*Means with different letters within the same column represent significant differences at *P<0.05*

Our values for mandarins, grapefruits, lemon and sour orange were higher than reported by Ersus and Çam (2007), Xu et al. (2008), and Sdiri et al. (2012). The content of vitamin C in bergamot was lower than the values reported by Sicari et al. (2016). Genotypic factors, climatic conditions, cultural practices, harvest time and processing methods affect the vitamin C content of fruits and vegetables (Lee and Kader, 2000).

The antioxidant activities of citrus juices were determined by DPPH free radical scavenging method in this study and were expressed as IC_{50}. This value is expresses the amount of antioxidant needed to decrease the radical concentration by 50%. So, lower IC_{50} value means a higher antioxidant activity of the sample (Kelebek, 2010). IC_{50} values for citrus juices ranked 52.88-122.84 µL. The highest IC_{50} value was recorded in Marsh Seedless and the lowest in Klemantin Fino. Among the citrus varieties, Klemantin Fino had the highest antioxidant activity, followed by Common Sour Orange and Yerli A-41.

CONCLUSIONS

Total phenolic, flavonoid, carotenoid, vitamin C and antioxidant activity of juices obtained from seven citrus varieties were evaluated in this study. Significant differences were statistically observed among citrus varieties in terms of investigated parameters. The antioxidant properties of citrus juices were influenced by the type of varieties. Our results suggest that citrus fruits are a potential antioxidant sources and can be provide important information for breeding studies, consumers and processing industry. In addition, citrus varieties studied in this work can be potentially for food and nutraceutical formulations. Further studies on the individual bioactive components of citrus varieties are necessary to evaluate their potential health benefits.

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DETERMINATION OF OCHRATOXIN A IN SUN AND MICROWAVE DRIED PLUMS

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ABSTRACT

Drying is a food preservation method in which water is removed from the material. By reducing the value of water activity by the drying process, it provides suitable conditions to prevent especially microbiological activities in food products. With the drying process, storage conditions are easier and are generally preferred due to economic gain. The environmental condition during ripening, harvesting drying and storage of plum seem favourable for mold growth and mycotoxin production in fruits. Ochratoxin A (OTA) is a toxic secondary metabolite, naturally produced by species mold. As far as humans concerned, the International Agency for Research on Cancer classified OTA as a possible carcinogen to humans. In this study, plum samples which were dried by using 2 different drying methods and were stored at room temperature for two years. Samples were dried in the sun and two different microwave power and analyzed in terms of their OTA content by HPLC-FLD after pre-separation using immunoaffinity columns in 2 replicates in 2 parallels. The HPLC system was an Agilent 1260 Infinity system with an autosampler using a fluorescence detector. The wavelengths for excitation and emission were 333 nm and 460 nm, respectively. The chromatographic column was 5 µm ODS C18, 250 x 4.6 mm column. The mobile phase used for OTA analysis was a mixture of water, acetonitrile, and acetic acid (49.5:49.5:1 %; v/v/v). The flow rate was 0.8 mL/ min, and the column temperature was maintained at 25 ^{o}C. The injection volume was 100 µL. As a result, OTA was not detected in both sun and microwave dried plums. It is believed that careful selection of raw materials during the drying and fulfilling recommended production and storage requirements may prevent the emergence of OTA.

Keywords: *OTA, Plum, Drying, Microwave*

INTRODUCTION

Plum is rich fruit in antioxidants and fibre and contributes significantly to human nutrition (Atıcı, 2013). The plum, a kind of temperate climate fruit, has many uses according to different consumption habits of the societies. Drying is a food preservation method in which water is removed from the material. It is applied to prevent microbial and chemical deterioration and to increase the shelf life (Kart, 2017). Unwanted changes in water activity by drying process are brought to suitable conditions to prevent especially microbiological activities in food products (Kartal, 2011). Together with the drying process, storage conditions are easier and are generally preferred due to economic gain. Important part of agricultural products can be stored dried (Er, 2011). As dried fruits contain essential amino acids, vitamins, minerals and rich dietary fibre that are useful for protecting health, they have become an increasingly attractive snack food (Asghar et al., 2017). Foods are dried either by using solar heat or by using heat from other sources. These

two separate applications are named "drying in the sun" and "artificial drying" (Cemeroğlu, 2011). It is not possible to control hygienic conditions in sun-dried products. In addition, the dried product is contaminated by various insects, birds and similar in the open area. In the artificial drying process, the product is more hygienic in terms of microbiological and controlled drying process can be performed (Cemeroğlu, 2011; Ayan, 2010). The plum is dried by artificial drying methods with hot air. And heat transfer is carried out by transport and transmission mechanisms. Depending on this, drying is faster than drying in the sun, but it is not fast enough. Slow drying causes some problems. In particular, fermentation occurs in the core zone and in the quality losses during the drying of the whole plum. Because of these problems encountered in the dried plums, whole dried plums are usually divided into two (Kart, 2017).

Mycotoxins are secondary metabolites produced by toxigenic fungi during the field and/or during storage, showing various effects on human health. Levels may change according to the season, growth areas and storage conditions. They are also very stable in different conditions and therefore are difficult to be eliminated from the food chain. This is food security worldwide and causes losses in food production that affect international trade (Perrin et al., 2015; Wang et al., 2018; Wang et al., 2018b). Numerous studies have shown that mycotoxins can cause DNA damage and are harmful to human and animal health even at low concentrations (Li et al., 2017). The most important mycotoxins emerging from dry plums are AF (B1, B2, G1 and G2) and OTA (Özer et al., 2012). Ochratoxin is a mycotoxin produced by some Aspergillus and Penicillium species. Ochratoxin A is the most common and most toxic type. Ochratoxin (OTA); It is a colorless, crystalline compound that gives fluorescence under UV light (Ringot et al., 2006). The most common and most toxic type for food is OTA (Tosun et al., 2006). It has nephrotoxic, carcinogenic, genotoxic, immunosuppressive, teratogenic, mutagenic and hepatotoxic effects on human health (Ringot et al., 2006). The exposure of people to OTA is most likely due to the low-level contamination of different foods in a wide range (Cabanes and Bragulat, 2018). In terms of the amount of OTA contained in foodstuffs, limit values have been determined by both national and international organizations (Gökmen, 2016). In the European Union, the maximum amount of OTA that foodstuffs may contain is determined by the Regulation (EC) No: 1881/2006; limit values determined according to Turkish Food Codex Contamination Regulation are used for domestic market products. According to this regulation; there is no limit on dry plum; but the maximum amount of OTA that can be found in dried grapes is 10 ppb (10 µg/kg) (Anon, 2018).

The stages of drying the fruit vary according to the product, but they can be sorted in general as elimination, washing, sizing, peeling, slicing-division and pitting. Drying may be terminated by drying in grapes 15-20%, 16-20% in plums, 15-18% in apricots, 25-28% in peaches and 18-20% in apples (Drusch and Ragab, 2003). Dried fruit due to inadequate drying or inadequate storage conditions are products sensitive to mold contamination and mycotoxin formation. Mycotoxin formation in dried products can occur at any stage of pre-harvest, harvest, drying, packaging and storage. The risk of mycotoxin formation is significantly reduced when the water activity value of the product is reduced to a level that prevents mold growth (Gürhayta and Çağındı, 2015). measures to protect crops from mold formation and mycotoxin formation must be started before harvest. Storage is the riskiest step in mycosis and mycotoxin synthesis (Drusch and Ragab, 2003). Many research on dried fruit is concerned with fruits that grow in hot climates such as figs and grapes. However, the available data on dried dates, prunes, apricots are very limited. To identify the true mycotoxin risk in dry fruits other than dried figs and grapes, these crops should be better studied using increasing sample numbers and standardized methodology (Özer et al., 2012). In this study, aiming to determine the result of Ochratoxin A of plum samples dried by using 2 different

drying methods including sun and microwave (2 different microwave powers) and stored at room temperature for two years.

MATERIALS AND METHODS

Material

In this research, damson plum (*Prunus domestica* subsp. Insititia) which was obtained from İzmir was used. Plum samples were brought to the laboratory of Manisa Celal Bayar University Department of Food Engineering where experiments were carried out and kept at +4 °C and 80-90% relative humidity.

Methods

Preparation of samples

Plums that are not suitable for drying are subjected to the sorting process. It is very important that the plums are the same size for uniform drying. For this purpose, the extracted plums were sized. Plums were dipped for 1 min with 1% NaOH solution at 55 °C. After the dipping process, the plums were washed under running water for 1 min. After washing, in order to remove excess water on the plums, they were left in the filter for 5 minutes. Plums were cut in half with a knife and pitted. The samples were prepared in 2 replicates in 2 parallels.

Natural drying on the sun

The plums, divided in two, were placed one day with the cut surface facing up, and the next day with the cut surface facing down. Plums were kept in the sun between August 30, 2016, and September 20, 2016, between 09: 00-21: 00 hours and taken to the laboratory environment to minimize humidity change during evening/night hours. Among these dates, the highest temperature average measured in Manisa was recorded as 33.7 °C and the lowest temperature average was recorded as 19.4 °C. Whether the plums reached the final dry matter content (82%) was determined by monitoring their weight throughout the drying period. The dried plums were allowed to cool at room temperature for a while. It is then wrapped in aluminium foil placed in the refrigerator bag and filled into capped glass jars and stored until deep-freezing to be analyzed. The samples were coded as SD.

Drying with microwave (MW)

In the MW drying process, a kitchen type microwave oven (AR 245, Arzum, Turkey) was used. Samples were dried in the microwave at 2 different power levels (720 W (P80) and 900 W (P100)). All the drying operations were carried out with 400 g of fresh plums. The drying process was applied for 20 s while applying power and waiting for 20 s. Whether the plums reached the final dry matter content (82%) was determined by monitoring their weight throughout the drying period. Dried plums were kept in the room for 5 minutes, then wrapped in aluminium foil, which was transferred to the refrigerator bag for hot packaging, and filled into capped glass jars and stored until deep-freezing.

Drying time

The drying period of the plum samples was carried out following the reduction of the weight of the samples during the drying process. Approximately 400 g of fresh plums were placed in each drying cycle, with a known dry matter starting ratio and the sample weight was monitored at 5 min

intervals. The weight of the product has been determined by the dry matter test to reach the final product content of 82% (18% moisture content). When the product reached this weight, the drying process was terminated and the drying time was recorded.

Determination of water activity

The water activity determination in the dried plum samples was carried out using a measuring set comprising a water activity measuring probe connected to a data logger (Testo 400, Germany). For this purpose, the dried plums were cut into small pieces and the water activity value was read after placing approximately 3 g of the sample in the measuring chamber and waiting until the relative humidity in the container reached the equilibrium (Kart, 2017).

Analysis of Ochratoxin A

For the analysis of ochratoxin AOAC method 2000.03, Shundo et al., 2009 method was used and some modifications were made.

Extraction

A 10 g of the sample was mixed and stirred at medium speed for 3 minutes on a stirrer (Waring, USA) with the addition of 2.5 g of NaCl and 100 mL of MeOH: acetonitrile: water (4:4:2%; v: v: v) (HPLC purity). The mixture was passed through a filter paper (Whatman No. 4), and 10 mL of this filtrate was taken and mixed with 40 mL of phosphate buffered salt solution. The mixture was centrifuged at 4000 rev/min speed and, at 4 °C. Then, the supernatant was filtered with a 1.5 μm microfiber filter to make the immunoaffinity suitable for passage through the colon.

Immunoaffinity column

Immunoaffinity columns (Ochratest, Vicam, USA) were attached onto a vacuum manifold at a rate of 2–3 mL/min was passed through the column so as to provide a total of 10 mL of filtrate. Thus, OTA is retained by the antibody. After all of the sample was passed through the column, the column was washed by passing 10 mL of PBS solution through the column at the same flow rate and then passing 10 mL of ultrapure water. For extraction OTA from column, 1.5 mL of MeOH (HPLC grade) and 1.5 mL of ultrapure water were passed through the column respectively to provide solvent aeration of OTA.

HPLC system

Table 1 gives the chromatographic conditions for the analysis of OTA.

Table 1. Chromatographic conditions for analysis of OTA

HPLC system:	Agilent 1260 Infinity model
Detector	Fluorescence detector
	Ext 333 nm
	Em 460 nm
Column	Termo Scientific C18 column (4,6 mm x 250 mm; 5 μm)
Flow rate	0,8 mL/min.
Injection volume	100 μL
Column temperature	25 °C
Mobile phase:	Water: acetonitrile: acetic acid (49.5:49.5:1%; v:v:v)

A 40 µL of a 1 mL standard mixture (50 µg / mL OTA) containing OTA (Spelco, Sigma-Aldrich, USA) was transferred to a 10 mL balloon bag, and a second-stage standard 0,2 µg/mL OTA) was obtained by adding 960 µL of methanol over this. In order to prepare the third step standard, the second step was completed by taking 100 µL of a standard ball from the standard and injecting 10 mL with the mobil phase. Then, 5 standard series were prepared using the third stage standard (0.002 µg/mL OTA).

RESULTS AND DISCUSSION

The drying time values of the plum samples were subjected to microwave drying process on the sun and at two different powers (720 W (P80) and 900 W (P100)) are given in Table 2. It has been determined that 4 days and 14 hours for half-dried plums on the sun. Microwave drying is made on the highest power value half plum drying time for the drying operation 26 min 27 s P100, P80 was determined to be 27 min 7 h for drying.

Table 2. Drying time of plums

Drying method	Time
SD	4 days 14 h
P100	26 min. 27 s
P80	27 min. 7 s

As a result, drying in the microwave seems to significantly reduce the drying time compared to drying in the sun. The increase in microwave power applied to the specimens caused a decrease in drying time. Michalska et al., 2016 different microwave power from the microwave vacuum drying time in the study from 32 to 120 minutes and dried plum convection-microwave pre-drying the final drying times were determined as 394 to 664 min. Baysal et al., 2015 study conducted in the microwave drying process apple slices were calculated at 20 minutes.

The water activity values of the plum samples subjected to microwave drying at the sun and at two different powers (720 and 900 W) are given in Table 3.

It was noted that the water activity value of fresh plum being used was reduced to 0.60-0.68 in dried samples with both methods and figures. It is seen that these values are at a level which can provide the product safely in terms of microbiology (Özay 1993).

Table 3. Water activity values of dried plums

Drying method	a_w
SD	0.60 ±0.02
P100	0.66 ±0.07
P80	0.65 ±0.09

Water activity is an important physicochemical property in food technology. Unlike moisture value, it determines physical, chemical and microbiological stability in food quality. In fresh fruit with high water content, the water activity values of 0.97-0.99 are dried down to 0.60 aw. It has been found that the water activity values in dry fruits vary between 0,505 and 0,694 (Özay, 1993). Rodriguez et al., 2015, found that fresh water activity values in the range of 0,966±0,002. After drying, water activity values varied between 0.441 and 0.845 depending on the experimental

conditions (Rodriguez et al., 2015). Iamanaka et al., 2005 found that the a_w value of dried plums was in the range of 0.712-0.863 and the mean was 0.796. The water activity values obtained for the drying types are close to these values and are compatible with our work. OTA was not found on dried plums both in the sun and in the microwave. It is considered that raw materials are carefully selected during the drying process and prevent OTA from emerging if the recommended production and storage requirements are met. Iamanaka et al., 2005, reported that Ochratoxin A value was below the detection limit of <0.1 mg/kg in 19 samples and OTA value was 0.1-5.0 mg/kg in 1 sample using 20 plum samples. Heshmati et al., 2017 reported that OTA levels in all dried fruit species (mulberry, dates, figs and apricots) were under EU regulation (10 mg/kg) and in the MOE> 10000 levels below the toxicologically non-toxic level for consumers. Özer et al. (2012) studies on mycotoxin contamination of plums indicate that OTA formation is the major mycotoxin problem in these fruits.

CONCLUSIONS

In this study, plum samples which were dried by using 2 different drying methods and were stored at room temperature for two years. Mycotoxins are one of the most important risk factors of agricultural product quality and safety. Studies on the risk assessment of mycotoxins are drawing more and more attention on a global scale. OTA formation in dried fruit is a threat to human health. Consumers often do not consume visible moldy or rotten fresh fruit. However, when rotten or moldy raw materials are used to produce dried fruit, mycotoxins can form at intensive levels. Therefore, strong fruits should be used in dry fruit production, the product should be dried quickly and stored under dry conditions. Today, due to taste and nutritional values, especially in European countries, prunes consumption is increasing in countries outside production areas. Because dried fruits are often consumed directly, the consumer must be aware of the quality and safety of these products. As a result of the work done, OTA was not found in dried plums on both sun and microwave. It has also been observed that microwave technology could be an alternative drying method in plum drying.

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THE INFLUENCE OF CULTURE MEDIUM ON IN VITRO PROPAGATION ON KALANCHOE BLOSSFELDIANA

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ABSTRACT

Our research aimed at developing a protocol for breeding *Kalanchoe blossfeldiana in vitro* for the purpose of obtaining a large number of plants free of viruses as well as a good rate of multiplication. Three concentrations of sodium hypochlorite (10%, 15%, 20%) solutions were used for disinfection. To initiate the cultivation of uninodal segments we used three growing mediums: the Murashige & Skooq initiation, Murashige and Skooq with IBA 0,1 mg/l and BAP 0,1 mg/l, Murashige & Skooq with BA 0,1 mg/l, IBA 0,5 mg/l and 30 g/l sucrose content and the multiplication crops were made in Gamborg B5 with IBA 0,01 mg/l, BA 0,1 mg/l and sucrose 20 g/l. Out of the three different concentrations of disinfection, the best results were obtained by using 10 % sodium hypochlorite. For testing the culture mediums we took into account the average number of leaves and roots; the best results were obtained on the Murashige and Skooq culture medium, while the Gamborg B5 culture medium showed significant differences. The same applies to the average number of roots analyzed is on the MS culture mediums. Based on the results obtained, we can recommend for the purpose of *in vitro* propagation of *Kalanchoe blossfeldiana* species both Murashige & Skooq with BA 0,1 mg/l, IBA 0,5 mg/l and 30 g/l sucrose and rooting on MS without hormones.

Keywords: *Kalanchoe, In vitro, Explant, Culture medium*

INTRODUCTION

Kalanchoe is a perennial and herbaceous plant, which is part of the Crassulaceae family. The genus Kalanchoe includes approximately 200 species of succulents originating in South Africa and Madagascar. The word Kalanchoe derives from “Kalan Cauhuy” which can be translated to "the drops and increases". The genus Kalanchoe was described for the first time in 1973, by botanist Michel Adanson (1727-1806), who was of Scottish-French origin. Among Kalanchoe species, the most cultivated are *Kalanchoe hybrida Hort.* or sunflower of coral and *Kalanchoe blossfeldiana* (Flaming over Katy, Panda Plant), originating from Northern Madagascar, hereinafter referred to as such in honor of the German botanist Robert Blossfeld (1882-1945) who, in 1932, introduced a culture in Potsdam, Germany. The plant’s small, white

flowers, which can be star-shaped or involute, are grouped into inflorescence umbels. The colors range from vivid shades of red, pink, orange, yellow or white, violet. Kalanchoe can be propagated mainly through young shoot cuttings. Propagation can also be achieved via seeds. If the plant is cultivated outdoors, it must be protected from freezing. (Toma, 2014) Being a slow-growing plant, it is extremely important to develop a system of very fast-growing tissues. One of the most extensive farming techniques of tissue culture is micropropagation, as it is very efficient, mainly because of the possibility to multiply virus free plants and because it may be a better alternative to multiplying by seeds and cuttings. In 1990, Dickens C.V.S and J. Van Staden at the University of Pietermaritzbug Natal in South Africa, published the study "The *in vitro* flowering of *Kalanchoe blossfeldiana Poellniz*. The effects of growth regulators and gallic acid". For this study explants from *Kalanchoe blossfeldiana* were used. These were subsequently cloned and multiplied by vegetative means. *In vitro* they have been grown in an environment which is composed of mineral salts, sucrose and agar, having a pH of 5.8, and under long day conditions (18 h of light from fluorescent tubes and glow plugs) at a temperature of 25 ºC. Going further, in 1992 a study by researchers Maria Ioannou and N. Ioannou is published at the Institute for Agricultural Research in Nicosia, Cyprus, on *in vitro* propagation of leaf segments (both basal and apex types) of *Kalanchoe blossfeldiana*. For this experiment leaves of healthy Kalanchoe were used, reared outdoors, which were washed with water and disinfected with a combination of soap and water as well as a solution with a concentration of 15% chlorine, for 15 minutes, and then were rinsed three times with distilled water. The results obtained demonstrated that the *in vitro* production of plants of *Kalanchoe blossfeldiana* fragments of leaves is a viable propagation method.

MATERIAL AND METHODS

Kalanchoe is a plant that propagates quite easily through stem and leaf cuttings, but this method is slow and inadequate, and thus, it's *in vitro* multiplication has become essential (Stanica, 2004). Plants resulting from *in vitro* multiplication process show better development of stems and their branches as well as shorter internodes, which are some of the preferred commercial characteristics. (Peticila A., 2015) All the plant material used in this study was taken from plants of *Kalanchoe blossfeldiana*, produced and grown in a greenhouse in the Netherlands. Uniondale segments were used as explants, and the research was carried out within the Micro-Specimen Laboratory within the Research Center for Food and Agricultural Products of the University of Agricultural Sciences and Veterinary Medicine in Bucharest.

Preparation of explants

After the separation from the mother plant, the uninodal segments were washed with running water for 5 minutes, followed by a fungicide disinfection, followed by successive rinses with sterile distilled water. The sterilization processes was carried out by submerging the explants in a 70% ethanol solution, followed by two rinses with sterilized distilled water for 5 minutes. Finally, the uninodal segments were disinfected with 0.4% NaOCl, then rinsed 3 times, for 5 minutes each, in sterile distilled water. Three such disinfection protocols were used as show in the Table 1.

Table 1. The desinfection protocols used

Protocol options	Ingredients	Time
Option 1	Distilled water (rinsing)	5 minutes
	Fungicide 4.5g / l	3 minutes
	Distilled water (rinsing)	2x5 minutes
	Ethanol 70%	3 seconds
	Distilled sterile water (rinsing)	5 minutes
	NaOCl 0.4% per 100gr (10% commercial bleach)	5 minutes
	Distilled sterile water (rinsing)	3x5 minutes
Option 2	Distilled water (rinsing)	5 minutes
	Fungicide 4.5g / l	5 minutes
	Distilled water (rinsing)	2x5 minutes
	Ethanol 70%	20 seconds
	Distilled sterile water (rinsing)	5 minutes
	NaOCl 0.4% per 100gr (15% commercial bleach)	15 minutes
	Distilled sterile water (rinsing)	3x5 minutes
Option 3	Distilled water (rinsing)	5 minutes
	Fungicide 4.5g / l	5 minutes
	Distilled water (rinsing)	2x5 minutes
	Ethanol 70%	20 seconds
	Distilled sterile water (rinsing)	5 minutes
	NaOCl 0.4% per 100gr (20% commercial bleach)	15 minutes
	Distilled sterile water (rinsing)	3x5 minutes

Culture medium used

During our research, the *in vitro* multiplication procedure of *Kalanchoe blossfeldiana* consisted of three stages: Stage 1 - initiation of *in vitro* culture for the formation of new plant organs, stage

2 - replication in a multiplication medium of the newly formed individual plantlets (obtained in the previous step) for stem growth and root formation, and step 3 - *in vivo* transfer of plants and their acclimatization. The basic culture medium used in our research contains inorganic salts MS (Murashige and Skoog) supplemented with Fe chelate, vitamins, inositol, sucrose and agar. The composition of the medium is detailed in the table below. In the culture initiation medium (MS +, MS -), BA and IBA were added in varying proportions, and both added growth hormone mediums and no growth hormone mediums were used to stimulate root formation and development, in order to better test their role in the roots formation and growth process. Also, a B5 (Gamborg) medium was used in the multiplication step (Table 2). The pH of the medium was adjusted to 5.7, 5.8 and 5.9 (by alkalinisation with either 1N NaOH - to lower the pH or HCl - to increase the pH).

Table 2. The culture medium used

MS(-) Medium		MS(+) Medium		MS modified Medium		B5 Medium	
Microelements MS 100X	1ml	Microelements MS 100X	1ml	Microelements MS 100X	1ml	Microelements MS 100X	1ml
Macroelements MS 10X	100ml	Macroelements MS 10X	100ml	Macroelements MS 10X	100 ml	Macroelements MS 10X	100ml
Iron Chelate FeNaEDTA 200x	5ml	Iron Chelate FeNaEDTA 200x	5ml	Iron Chelate FeNaEDTA 200x	5ml	Iron Chelate FeNaEDTA 200x	5ml
Vitamins MS 100X	10ml	Vitamins MS 100X	10ml	Vitamins MS 100X	10ml	Vitamins MS 100X	10ml
Nicotinic acid Thiamine HCl Pyridoxine HCl		Nicotinic acid Thiamine HCl Pyridoxine HCl		Nicotinic acid Thiamine HCl Pyridoxine HCl		Nicotinic acid Thiamine HCl Pyridoxine HCl	
Inositol 100X	10ml	Inositol 100X	10ml	Inositol 100X	10ml	Inositol 100X	10ml
		Indol-3-butiric acid IBA 0.1mg/l BA benzyladenine 0.1mg/l		benzyladenine BA 0.1 mg/l Indol-3-butiric acid IBA 0.5mg/l		Indol-3-butiric acid IBA 0.01mg/l BA benzyladenine 0.1mg/l	
Sucrose	25g	Sucrose	25g	Sucrose	30g	Sucrose	20g
Agar	7g	Agar	7g	Agar	7g	Agar	7g
Distilled water up to 1 liter		Distilled water up to 1 liter		Distilled water up to 1 liter		Distilled water up to 1 liter	

Culture initiation

The uniondale segments of *Kalanchoe blossfeldiana* were subjected to the disinfection protocol described above, then they were transferred to tubes containing the culture medium necessary for plant regeneration. The tubes were labeled and stored in the growth room for 20 days at a temperature of 22°C and a humidity of 30-40% under white fluorescent light for 9 hours of darkness and 15 hours of light. The explants were monitored weekly to observe the effects of disinfection and possible developments of the new plant (measurements of leaves, number of buds,

mean leaf size and number of roots were taken). Disinfection efficiency was recorded 19 days after inoculation by visual determinations of contamination.

Culture multiplication

In the second stage of our research, the explants (that were inoculated during the *in vitro* culture initiation) were multiplied in new culture mediums, both MS + and MS-. In view of the fact that *Kalanchoe blossfeldiana* shoots become semi-lignified when mature, a B5 medium (Gamborg), which is a medium dedicated to the micropropagation of woody plants, was used in order to observe its effects on the development of stems and shoots. Two multiplications of the viable explants were made, their evolution being followed for 9 weeks. Throughout this period, measurements and observations were made regarding the length and number of shoots, the number of buds, the number of leaves and their average size, the number of roots and the number of resulted plants, in order to be interpreted and analyzed. The plants were prepared for *in vivo* transfer and acclimatization after approximately 12 weeks since the initiation of culture took place.

Acclimatization

The plants with already formed and developed roots were removed from the tubes or jars when their width (stem and leaves) was 1.5 to 4 cm (approximately 12 weeks from inoculation), and when the roots were viable. After the roots were washed with running water in order to remove the agar and sucrose, the plants were transferred to a substrate consisting of a mixture (previously sterilized for 20 minutes at 121 ° C) of peat and perlite in seed-starting trays.

RESULTS

We started our research started with 60 explants of *Kalanchoe blossfeldiana*, represented by uninodal segments of approximately 0.5 cm, which were subjected, before inoculation, to the disinfection protocol described above. 10 uninodal segments were inoculated in MS + medium and 10 similar segments were inoculated in MS – medium - for each of the options represented by the disinfectant solution. The first observations on explant evolution occurred 19 days after inoculation. One of the 20 inoculated explants had an infection, while the rest of the plants (leaves, buds and even roots) showed growth. **Table 3**.

Table 3. Evolution of explants according to the disinfection protocol used

Desinfection substance and concentration	Culture medium	Number of viable explants
NaOCl 10%	MS +	9
	MS -	10
NaOCl 15%	MS+	2
	MS -	6
NaOCl 20%	MS +	0
	MS-	0

In the following period, approximately 10 days later, observations and measurements were made on the inoculated plants on the two types of mediums (MS + and MS -), observing the development of their organs (buds, leaves, roots) as well as their number and size. As a result of the measurements, the largest growths were recorded in the case of explants inoculated on the MS – culture medium - (in terms of both leaf number and root development - only 20%).

After 20 days, the first plant multiplication after the initial inoculation is made. The 19 remaining plants show both leaf and root growth, two of the explants growing roots within the first 10 days of inoculation. Following the multiplication, 34 new explants resulted. 17 of these were inoculated on MS – medium without growth hormones, while the remaining 17 were inoculated on the B5 medium (Gamborg), the medium dedicated to the multiplication of woody plants, in order to test its influence on the growth and development of the organs of the plant, to which IBA (0.1mg / l) and BA (0.1mg / l) were added.

Table 4 shows the development of the number of plants inoculated within the first experiment, throughout these 22 days.

Table 4. Evolution of explants subjected to the first disinfection option (NaOcl 10%) 11.10.2017

Date	Culture medium	Type of explant	Primordia formation
30.10.2017	MS +	9 US	8
	MS -	11 US	11
15.11.2017	MS+	8 US	8
	MS-	11US	11
20.11.2017	MS+	8 US	8
	MS-	11 US	11

US = Uninodale segments

19 days after inoculation, the plants that were placed in the MS – culture medium had a total number of leaves of 23 (with a total average size of 16.5 mm), the number of buds were 14 and the total number of the roots was 10, while the explants on MS + medium had a total number of leaves of 53 (with a total average size of 20.5 mm), the number of buds were 14 and the total number of roots were 8.

DISCUSSION

During the next 15 days, there was an increase in both the number of leaves and their size (especially for MS + cultivar plants) and roots, especially for those inoculated on the MS – medium, as follows: in 15 days, the total number of plant leaves on the MS + culture medium increased from 53 to 81 (and the average size from 20.5 mm to 26.5 mm), the number of buds remained unchanged. Regarding the roots, it was noted that only 2 out of the 8 explants developed roots, their total number remaining 8, the same as at the October 30 measurement. In contrast, the uninodal segments that were inoculated on the MS – culture medium - had better root development, with all plants showing root primordia in a total of 26 and a total number of buds of 19. In terms of total number of leaves, this recorded a significant increase from 23 to 54.

The last observations and measurements before multiplication were done for 45 days. During this stage, the 11 plants on MS – developed a total number of 73 leaves (with a total average size

of 25.7mm), the number of roots increased to 39, and bud number remained unchanged while for the plants inoculated on the MS + medium, there was a small increase in the number of leaves (from 81 to 90), having a total average size of 26.5 mm, while the number of roots remained relatively small, 12 compared to the previous measurement when they were only 8. Following this experiment, attention was directed to the 34 plants from the first experiment, which were subjected to new measurements at a 10-day interval.

18 days after inoculation, we noticed that out of the 17 explants in the MS – cultured medium, only two had roots (the total number of roots was 3), and 5 showed stagnation (lack of new development). For the remaining 10 plants, the formation of new leaves was noted, their total number being 54 (with a total average size of 22 mm). As far as the B5 plants are concerned, 2 developed roots (their total number being 3), 7 registered stagnation, and the remaining 11 had a total number of leaves of 17 (16.7 mm total average). The next measurement after 10 days showed that the number of plants inoculated on the MS medium – which developed roots reached 9 with a total number of roots of 63), and the number of plants that have registered stagnation was reduced to 2, thus 15 plants presented leaf system development (the total number of leaves increased to 71 with a total mean size of 37.2 mm). For the B5 plants, it was noted that the number of those that showed stagnation remained the same (7), while the number of rooted plants increased by 6 (total number of roots - 49). The last measurement was done after 20 days. As a result, it was decided to acclimatize (in vivo) the plants that were quite developed in terms of shoots and leaves, as well as roots. Thus, the seven plants both on the MS – and B5 mediums and on the B5-medium, developed roots (in a total of 89).

DS5% for three mean mediums 3.8 - 3.9

From the above data, resulting from the monofactorial experience, where one of the factors is the culture medium and the other from the dynamics of the explant development and analyzed with the Duncan test, we concluded that there are significant differences between the MS and B5 culture mediums in favor of MS and between the two MS mediums, MS + was significant for the average number of leaves while MS- was significant for the average number of roots (Table 5).

Table 5. Results of Duncan test

Medium	Average no of leaves	Average no of roots
MS-	14.3 a	13.7 a
MS+	15.0 a	12.0 a
B5	10.3 b	6.3 b

After 30 days a new acclimatization process was done for the rest of the plants remaining in the culture medium, and it was noted that the acclimatization was done successfully for all plants, the acclimatization rate being 100%.

CONCLUSIONS

The most effective disinfection protocol was the one employing a 10% NaOCl (0.4%) solution with a 5 minute disinfection time (95% of the plants remained viable, while the infection rate was

only 5%), the uninodal segments used turned out to be quite sensitive to a higher concentration of disinfectant.

The best results (the longest shoot length, the largest number of leaves, the largest number of roots and the longest roots) were obtained for plants inoculated in the culture medium without growth hormones, which could be explained by the fact that *Kalanchoe blossfeldiana* naturally produces a sufficient level of auxins and cytokinins, so their addition to the culture medium did not significantly influence plant development.

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EFFECT OF WATER HARVESTING TECHNIQUES ON SOIL PROPERTIES IN THE SOUTH OMDURMAN AREA- SUDAN

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ABSTRACT

This study was conducted at Khartoum New International Airport, South Omdurman area, Khartoum State, Sudan. A complete randomized block design was followed to study the effect of Holes and Crescents water harvesting techniques on the soil moisture content soil sample were taken prior and immediately after rains and at three weeks intervals. The results indicated that the Holes and Crescents water harvesting techniques affected positively some soil physical properties especially at the upper soil layer (0 – 30 cm) which was subjected to excavation by a loader. These soil properties included porosity, field capacity and infiltration rate as they have direct influence on the soil moisture content. The Holes water harvesting techniques showed an increase of 15% in soil moisture content resulting in better improvement of the soil physical properties as compared to the Crescents water harvesting techniques, hence the farmer techniques recommended for adoption.

Keywords *Holes, Crescents, Water harvesting technique, Infiltration rate, Field capacity, Moisture content*

INTRODUCTION

One of the major critical problems of agriculture is water conservation, especially in rainfed areas. Sound soil and water conservation is based on full integration of engineering, plant and soil sciences. It is essential to develop sound practice that will permit the entrapment and storage in soil profile a greater percentage of available precipitation as by water harvesting techniques which provide an entirely new potential source of water. Water has an essential role in sustaining life and development especially in arid and semiarid regions. According to the United Nations (2003), water resources are envisaged decline steadily because of population growth, pollution and frequent climate changes due to the problem of global warming. Hence, the water crisis is getting more attention among all countries specially the developing ones. Therefore, new strategies and techniques to deal with water problems are highly needed. Water harvesting and spreading techniques succeeded in providing feasible solutions for improving the living conditions of millions of people facing serious domestic water supply problems. Water harvesting goes back in the old history. It is an old and ancient method for collecting water, practiced by man since life existed on this planet. Much of the early history of rain water harvesting has its origin in many

parts of the world. The old civilizations developed in western Asia and in central and northern Africa gave strong evidences that they had known water harvesting. In Europe, especially in small islands with no significant river systems, rain water is the only source of water. The island of Gibraltar has one of the largest rain water collection systems in existence. (UNEP, 1983) and (UCWR, 2003). In Sudan the central clay plains, east of the Nile, the use of earth bunds (Terases) are familiar to intercept sheet-flow runoff, following heavy storms, from adjacent catchments. Sixty and seventy percent of runoff is concentrated in the flood period from June to September (Kutsch, 1982; Ahmed and Eldaw, 2003).

This study was conducted with a view to attaining the following objectives:

1. To compare different water harvesting techniques on the basis of soil moisture content (SMC).
2. To examine the viability of improving the physical condition of the soil.

MATERIALS AND METHODS

The study area:

Site location:

The study was conducted at Khartoum New International Airport (KNIA) in the south western direction of Omdurman, Khartoum State at Latitude 15˚ 13́ N and Longitude 32˚ 19́ E, at a distance of 50 km South of Khartoum center and 20 km west of the White Nile River.

Meteorological and Soil data:

Rainfall:

The rainy season normally falls between July and September each year and the annual average rainfall is about 150 mm. The effective rainy season starts in late June, increases in July and reaches its peak in August.

Topography:

The topography of the study area is generally fairly flat but few isolated ridges and sand dunes may be observed in the western part of the site and the ground surface slopes gently to the east.

Vegetation:

Harrison and Jackson (1955) stated that vegetation of the study area is dominated by *Acacia tortilis* and *Maeruacrassifolia* desert scrub. Generally, the natural grazing area in Khartoum state is estimated as 40% of total area. The annual grasses and herbs form about 75% of the natural vegetation cover, while the perennial grasses and shrubs/trees form 5% and 20%, respectively. In summary the degradation of the study area is characterized by the disappearance of trees cover mainly due to conflict between people needs and woodland preservation. Thus, the whole state has badly denuded of its natural tree cover.

Climate:

The climate is hot, dry, dusty during the summer season and dry, cold during the winter.

Soil:

The area is covered by a light brown and very thin gravely sand layer (about 10mm thick), and few angular to sub-angular, 20 to 60mm sized fragments of the ferruginous sandstone. The

southern part of the site is covered by sandy gravel probably formed due to the weathering of Nubian Group rocks which are outcropping in some places in the area. Runoff usually occurs during heavy rains.

Experimental treatments:

These included two water harvesting techniques which were constructed before the onset of the rainy season; each treatment was represented by a block which included the plant species. The Holes and Crescents types of water harvesting techniques were used; mainly because the site is known to be extremely rough terrain.

(a) Holes (Deep pits) technique:

Each Hole was 2.5 m in width, 4 m in length and 50 cm deep. The distance between holes in the row was 10 meters while the distance between rows was also 10 meters. The slope direction was made from the upper side to trap the sheet flow run-off after rain storms.

(b) Crescents or curved terraces technique:

Each Crescent was 30 meters in diameter and 50 cm deep. The crescents were laid 15 m apart.

Soil mechanical and physical properties:

Soil mechanical and physical analysis was performed at the Soil Science Department, laboratory of the Faculty of Agriculture, University of Khartoum.

Soil class and bulk density:

Soil class was determined using the hydrometer method proposed by Bouyoucus (1951). Samples were taken from six locations in each plot. From each location 3 samples were taken at depths of 30, 60 and 90cm below the soil surface.

The mean bulk density in gm/cm^3 for each depth was determined using the following equation:

Bulk density (g/cm^3) = Dry soil weight (g) / Soil volume (cm^3)

Bulk Density =m/v (g/cm^3)
Where:
m = mass of sample in g
v = volume of sample in cc (ml)

Infiltration rate:

Steady state infiltration rates were measured for each treatment (plot) using the double ring infiltrometer following the procedure described by Michael (1978).

The double ring infiltrmeter was made of 0.25 cm thick metal sheet and consisted of two concentric cylinders, 28 cm height with diameter of 28 cm for the inner ring and 55 cm for the outer one. The infiltrometer was pressed firmly in the soil and hammered gently with the help of a wooden plate until it was driven to a depth of 10 cm in the soil. A filter paper was then placed at the bottom of the inner cylinder to prevent disturbing the surface of the soil, then water was poured gently into the inner cylinder. The space between the inner and the outer cylinders was filled immediately with water after filling the inner one to prevent the horizontal water movement. Readings of the depth of the pond water in the inner cylinder was taken every 5 minutes then the rate of water intake over time was measured as described by Michael (1978).

Soil moisture content determination:

This parameter was determined gravimetrically, soil samples were augured from different locations at 0.3 m increment from the soil surface to a depth of 0.9 m. The samples were oven dried at 105^{o}C for 24 hours then weighed to determine moisture content on dry basis.

$$\text{Moisture content \%} = \frac{\text{wt of wet sample} - \text{wt of oven dry sample}}{\text{Wt of oven dry sample}} \times 100$$

Where:

wt = the sample weight in gm.

Equipment:

The following equipment was used in the experiments:

1- A Loader was used to construct the rain water harvesting structures

2- Auger for soil sampling.

3- Sample containers for the determination of the moisture content of the soil and plastic bags to keep the soil samples.

4- An oven for drying the soil moisture.

5- Measuring tools (metering tape, a sensitive balance.

6- Double ring infiltrometer.

Statistical analysis

Data for each trial were analyzed as Complete Randomized Block Design (C.R.B.D) by standard analysis of variance techniques. Mean significant ($p \leq 0.05$) treatments were separated using Duncan's Multiple Range Test procedure (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Soil particles distribution

Figure 1 and Table 1 show that the amount of sand particles was greater in the upper 30 cm depth than in the lower depths (30-60 cm) and (60-90 cm) in both treatments. The soil texture was sandy clay for the upper part for both soil types, while it was clayey for depth (60-90 cm) for both soil treatments, this might be due to eroded slope of the upper terraces where the sand and some gravel were exposed as a result of erosion. The soil is sandy clay in the upper zone (30-60 cm) with more sand in the top 30 cm. The clay content increase with depth more sand and gravel are seen above soil surface mainly due to a washing proves of clay down the slope.

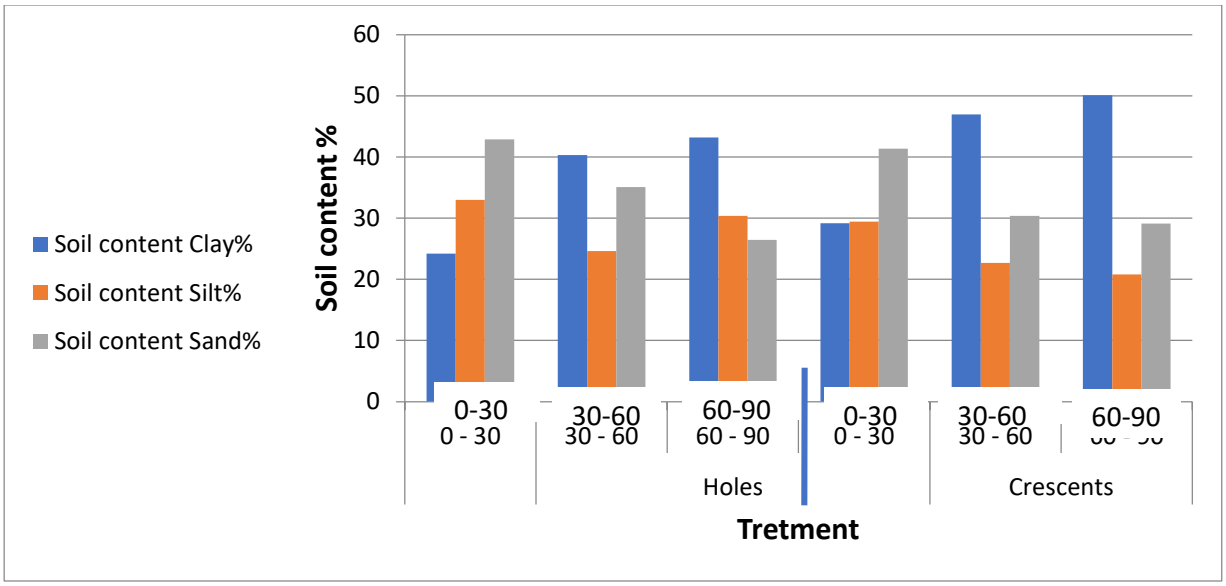

Figure 1. Soil mechanical analysis for different depth and treatments

Soil bulk density

Figure 2 and Table 2 show that the bulk density in g/cm^3 for each 30 cm increment from the soil surface down to the depth of 90 cm for both the Holes (T1) and Crescents (T2) water harvesting techniques. It becomes apparent that the bulk density was found to increase with increasing depth, with an average of 1.64 g/cm^3 at all locations. This result may be attributed to the fact that the soil bulk density increases with increase in the clay content, a fact which is supported by the findings of salah (1991).

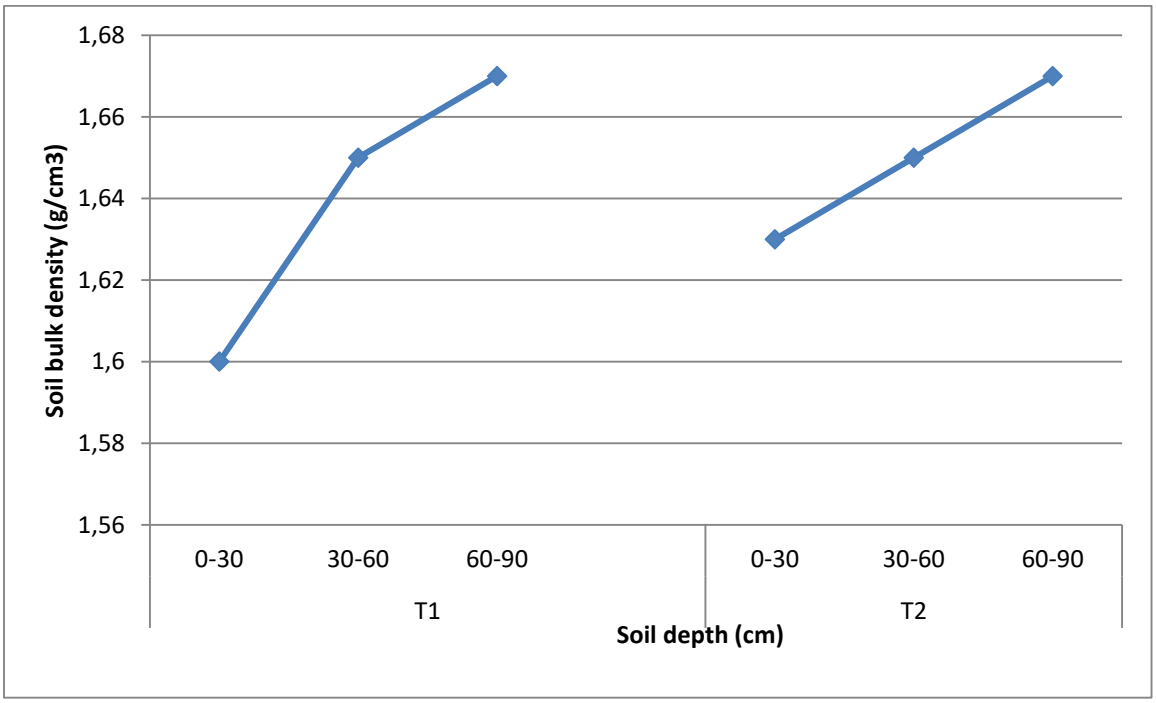

Figure 2. Soil bulk density (g/cm^3) of the treatment

Infiltration rate

The infiltration rate (mm/h) and the accumulative infiltration rate (mm) for the Holes and Crescents water harvesting techniques treatments can be depicted in Figure 3 and Table 3. In both cases the infiltration rate (mm/h) and the accumulative infiltration (mm) were plotted against elapsed cm (min). The infiltration rate started at about 300 mm/h then dropped down gradually for about 30 min before reaching relatively steady state in moderate conditions.

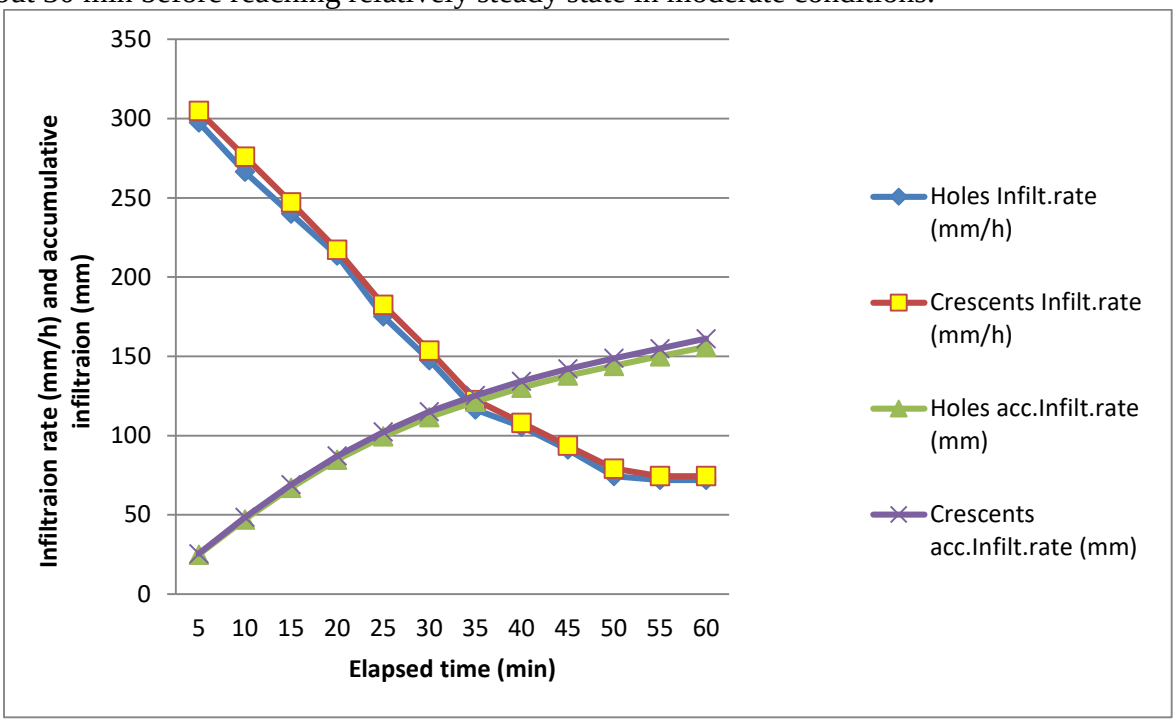

Figure 3. Infiltration rate (mm/h) and accumulative infiltration (mm) versus elapsed time (min) for the site

Soil moisture content

The moisture contents of the soil before and after rain at different depths are shown in Fig (4) and Table (4). The 1st reading before the rain showed a significant difference ($p \leq 0.05$) for depth (60-90), while the 2nd and the 3th readings for depth (0-30) and (60-90), respectively after the rain showed that a significant difference ($p \leq 0.05$) existed among the treatments. There was no significant difference ($p \geq 0.05$) for 4th and 5th, readings at all depths.

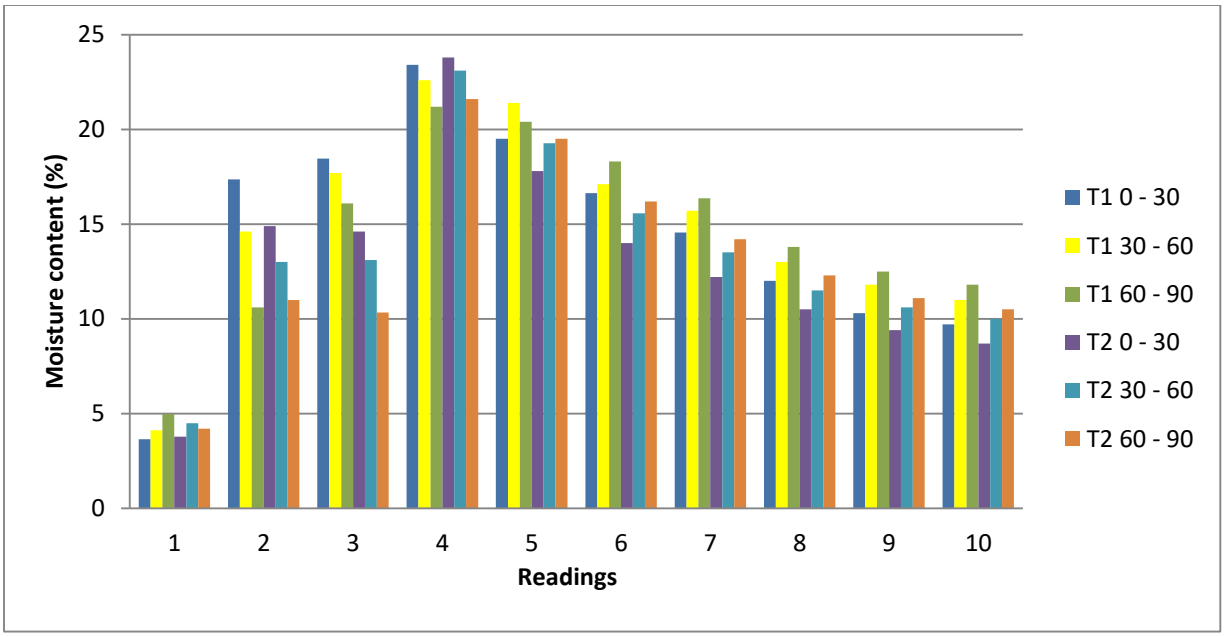

Figure 4. Soil moisture content (SMC) measurement (wt %)

Table 1. Soil mechanical analysis for the location, different depth and treatments.

Treatment	Depth	R1	R2	R3	Means
Holes	0 - 30	1.56	1.63	1.61	1.6
	30 - 60	1.62	1.68	1.65	1.65
	60 - 90	1.66	1.70	1.66	1.67
Crescents	0 - 30	1.63	1.58	1.61	1.63
	30 - 60	1.66	1.61	1.67	1.65
	60 - 90	1.66	1.65	1.71	1.67

Table 2. The bulk density in g/cm3 for the location, different depths and treatments

Treatment	Depth	Soil content			Soil texture
		Clay%	Silt%	Sand%	
Holes	0 - 30	24.17	32.96	42.87	Sand
	30 - 60	40.32	24.6	35.08	Clay
	60 - 90	43.2	30.38	26.42	Clay
Crescents	0 - 30	29.18	29.45	41.37	Sand
	30 - 60	46.96	22.68	30.36	Clay
	60 - 90	50.09	20.78	29.13	Clay

Table 3. The values of infiltration rate (mm/h) and accumulative infiltration (mm)

Time (min)	Holes		Crescents	
	Infilt. Rate (mm/h)	Acc. infilt. values (mm)	Infilt. Rate (mm/h)	Acc. infilt. Values (mm)
5	297.6	24.8	304.8	25.4
10	266.4	47	276	48.4
15	240	67	247.2	69
20	213.6	84.8	217.2	87.1
25	175.2	99.4	182.4	102.3
30	147.6	111.7	153.6	115.1
35	116.4	121.4	122.4	125.3
40	105.6	130.2	108	134.3
45	91.2	137.8	93.6	142.1
50	74.4	144	79.2	148.7
55	72	150	74.4	154.9
60	72	156	74.4	161.1

Table 3. Average moisture content before and after rains

Readings		1		2		3		4		5	
Treatment	Depth	Bef	Aft	Bef	Aft	Bef	Aft	Bef	Aft	Bef	Aft
		MC %	MC %	MC %	MC %	MC %	MC %	MC %	MC %	MC %	MC %
Holes	0 - 30	3.65	17.36	18.5	23.4	16.5	19.5	12.0	14.6	9.7	10.3
	30 - 60	4.12	14.6	17.7	22.6	17.1	21.4	13.0	15.7	11.0	11.8
	60 - 90	4.98	10.2	16.1	21.2	18.3	20.4	13.8	16.4	11.8	12.5
Crescents	0 - 30	3.78	14.9	14.6	23.8	14.0	17.8	10.5	12.2	8.7	9.4
	30 - 60	4.49	13.0	13.1	23.1	15.6	19.3	11.5	13.5	10.0	10.6
	60 - 90	4.20	11.0	10.3	21.6	16.2	19.5	12.3	14.2	10.5	11.1

CONCLUSIONS

The following conclusions can be drawn from the results of this study. The Holes and Crescents water harvesting techniques improved soil moisture content significantly. Higher values of moisture content were recorded for the Holes type of water harvesting technique as compared to the Crescents type. From the results obtained and conclusions drawn the following recommendation can be made. Further research should be conducted to investigate the performance of different indigenous tree species under more water harvesting techniques to enable selecting the water harvesting techniques and the species most appropriate to the environmental conditions of the area.

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THE ABILITY OF CERTAIN SOIL BACTERIA ON REMEDIATION OF ETHALFLURALIN HERBICIDE IN SUBMERGED CULTURE CONDITIONS VIA TURBIDITY

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ABSTRACT

The aim of this study is investigate the removal efficiency of certain soil bacteria on ethalfluralin biodegradation with Ethalfluralin, chemical oxygen demand (COD), Biochemical Oxygen Demand $(BOD)_5$, Total Organic Carbon (TOC) and reveal the population dynamics of these bacteria during biodegradation process under agitated culture conditions via turbidity and number of colony forming unit (CFU). Bacteria were firstly isolated from agricultural soil in agar media taken from Thrace region of Turkey. These cultures were used in experimental studies. For determination of Ethalfluralin active ingredient, EPA Method 8081B was used. COD experiments were done according to standard methods 5220C closed reflux titrimetric method, BOD_5 measurement was carried out in 24-hour intervals according to the Standard Method 5210B (5 day BOD_5 test) and for total organic carbon analyses, standard method 5310B High temperature combustion method was used according to APHA, (1998) and decreasing of the ethalfluralin followed about five days. Turbidity experiments were performed by Photolab 6600 UV-VIS Spectrophotometer. As a result of the study, best removal performance observed in *Bacillus thuringiensis* and *Bacillus muralis* as 88% and 82% for Ethalfluralin while 83% and 80% in COD at 5 days, 81% and 73% in BOD_5 and 74% and 61% in TOC parameters. The lowest performance was seen on Fusarium fujikuroispecies for Ethalfluralin, COD, BOD_5 and TOC as 41%, 53%, 47% and 43% respectively on same time period. The performance for *Micrococcus luteus* and *Micrococcus yunnanensis* species occurred between 60% and 70% for these parameters.

Keywords: *Bacteria, Ethalfluralin, Biodegradation, Removal efficiency*

INTRODUCTION

The soil ecosystem is a theater of biological processes with complex links and regulated by its microbiota (Radivojević et al., 2014). To manage weeds, common methods such as cultural, mechanical, biological, and chemical methods are utilized (Abeysekara, 2011). Weed control using herbicides is the most popular method among farmers and it allows inexpensive weed control and provides a cost-effective method in the cultivation of agricultural crops (Juraimi et al., 2013).

Pesticide contamination of the surface and ground waters was well documented (Laabs et al. 2007), although an effective way to eliminate environmental contaminants is yet to be discovered. However, certain significant progress was made in soil bioremediation technologies including microbe-induced, chemical reductive and oxidative technologies during recent years (Guimarães et al., 2010).

Pesticides are the most extensively used chemical compounds in agricultural activities (Socorro et al., 2015). Over the past decade, interest on the presence and distribution of

pesticides and other organic pollutants has been increased in environmental waters with lower impact (Malik et al. 2007). Pesticides are chemicals that used for control certain forms of plants (Krieger, 2010). Consideration of the human health emergency due to these chemicals introduce multiple challenges such as modes of actions to pests except humans (Manikkam et al., 2012). It is significiant to eliminate pesticides from environment since their opposite effects. The most preferred methods for eliminate pesticides are photocatalytic degradation, coagulation, fenton oxidation and ozonation. But their application time process, costs and ineffectiveness of large area are disadvantages (Tay et al., 2009). Previous studies on microbial degradation of these chemicals revealed that certain bacterial species were able to degrade herbicides (Erguven and Yildirim, 2016). Microorganisms could degrade herbicides by using them as energy and carbon sources (Garbisu and Alkorta, 2003). The results of the bioremediation studies in water have revealed that this process corralated with the pesticide reduction. This method is a low cost, effective and one of the alternative process that does not result toxic products (Massiha et al., 2011). Scientists researched microorganisms to degrade pesticides, especially through white-rot fungi (Fan et al., 2013). Bioremediation time is also very short and microorganisms can deifficultly survive in the target field) (Maruyama et al., 2006).

Dinitroaniline pesticides are selective types of herbicides that used for control of a broad-leafed weeds of important agro-economically crops. Ethalfluralin (N-ethyl-α,α,α-trifluoro-N-(2-methylallyl)-2,6-dinitro-p-toluidine) was used for the protection of crops such as cotton, soya beans, tomatoes, peanuts and sunflowers. This herbicide degrades in surface water but does not hydrolyze. Ethalfluralin is generally applied to the surface of the field by spraying after planting the crops. The effectiveness of these type of herbicies is dependent on transfer of the herbicide into the soil by irrigation, rainfall or mechanical incorporation (Prostko et al., 2001).

Ethalfluralin is one of the selective herbicide of Semi-Volatile Organic Compound (SVOC) which has a vapor pressure of $1.2 \times 10{-}2$ Pa (at $8.9 \times 10{-}5$ mm of Hg) at 25 °C, and a Henry's low constant of 18 Pa m3 mol−1. Ethalfluralin can be volatilize from aqueous solutions. In the European Union and the United States, ethalfluralin is employed in several countries for over 30 years.

The present paper aimed to provide a comparison of the removal performance of five soil bacteria against herbicide ethalfluralin in agitated culture media with most important environmental parameters and reveal the baseline of bioremediation activities. One of the main objectives of the present study was to provide recommedations for scientists who attempt to rehabilitate the receiving environments polluted by the abovementioned types of pesticides. Furthermore, it also aimed to provide recommendations for farmers who use ethalfluralin or other dinitroaniline group herbicide for rehabilitation of their agricultural fields after cultivation.

MATERIAL AND METHODS

Bacteria

Bacillus thuringiensis, Bacillus muralis, Micrococcus luteus, Micrococcus yunnanensis and *Fusarium fujikuroi* used in this study. These bacterial strains are currently available in our culture collections. The strains were grown in petri dishes on plate count agars at 4°C in refrigerator.

Chemicals

Ethalfluralin, plate count agar (PCA) and sabouraud broth (SDB) were obtained from sigma aldrich (Turkey) with a cas and lot number of 55283-68-6, 70152 and S3306 respectively.

Submerged cultures preparation

Submerged culture medium of *Bacillus thuringiensis, Bacillus muralis, Micrococcus luteus, Micrococcus yunnanensis* and *Fusarium fujikuroi* were cultured at 20˚C on PCA slants in glass tube with diameter of 10 mm. After three days of incubation, conidial suspensions were prepared and used for the preparation of inoculum. 1 ml of the bacterial suspension (includes approximately 2 x 107CFU/ml) was transferred into a 100 ml flask containing 99 ml of SDB and agitated on a rotary shaker at 130 rpm for 5 days at 20˚C in dark. After incubation finished, these flasks homogenized and homogenized mycelial cultures were used as inoculum for bioremediation studies under submerged culture medium. 1 ml of homogenized mycelial culture was transferred into 100 ml flasks containing 99 ml of 10000 mg/l of Ethalfluralin (application concentration for farmers) on an agitated incubator for 7 days at 20 ˚C in triplicate. After incubation, all flasks filtered for removing bacterial biomass and this filtrate was used for determinate Ethalfluralin, COD, BOD_5 and TOC reduction rates for five days. These filtrates were also used for determinating the beginning time of bioremediation process on 24 hours period.

Bioremediation Studies

On 24 hours intervals, each part of the samples are used for Ethalfluralin, COD, TOC and BOD_5 experiments. For ethalfluralin analysis, EPA, 8081 method was used. In this method, Perkin Elmer Clarus 500GC-ECD was used. All samples were spiked with internal and surrogate standards for determine the recovery rate. For the carrier gas, high purity helium (99.99%) was used at a constant flow rate of 1.5 mL/min. As the surrogate standard, tetrachlorom- xylene was used. Tetrachlorom- xylene was spiked to the sample prior to extraction. Quintozene was as an internal standard and spiked just before capping the chromatography vials. Average recovery rate was 93%. The limits of detection (LOD) values were calculated for each congener as average blank concentrations plus three times the standard deviations. In case of weight losses, the new concentration was recalculated according to (Kmellar et al., 2011). Any sample concentrations that fall below the LOD value were ignored. For each set of analysis, blank samples were corrected and all results were blank corrected. Chloroform was obtained from Merck (Augsburg, Germany). All utilized chemicals were of GC grade. Methanol was used as a working standard solution and were prepared with analytical standards for GC calibration in the range of 0.1–100 mg/l. For the COD experiments, closed reflux titrimetric method used identified in the Standard Method 5220C and decreasing of the substrate followed day by day. BOD_5 experiments were performed with Standard Method 5210B (5 day BOD_5) test and the TOC test was performed in line with the method of burning at a high temperature identified in the Standard Method 5310A with TEKMAR - DOHRMANN - Apollo 9000 device (A.P.H. Association, 1998) Turbidity measurements taken from ethalfluralin media were performed according to Harry et al. (1990) at 650 nm with Photolab 6600 UV-VIS Spectrophotometer device.

RESULTS

The performance of the removal of *Bacillus thuringiensis, Bacillus muralis, Micrococcus luteus, Micrococcus yunnanensis* and *Fusarium fujikuroi* with Ethalfluralin, COD, TOC and BOD_5 and the bioremediation process baseline including the colony count and turbidity are illustrated in Fig. 1-5, respectively.

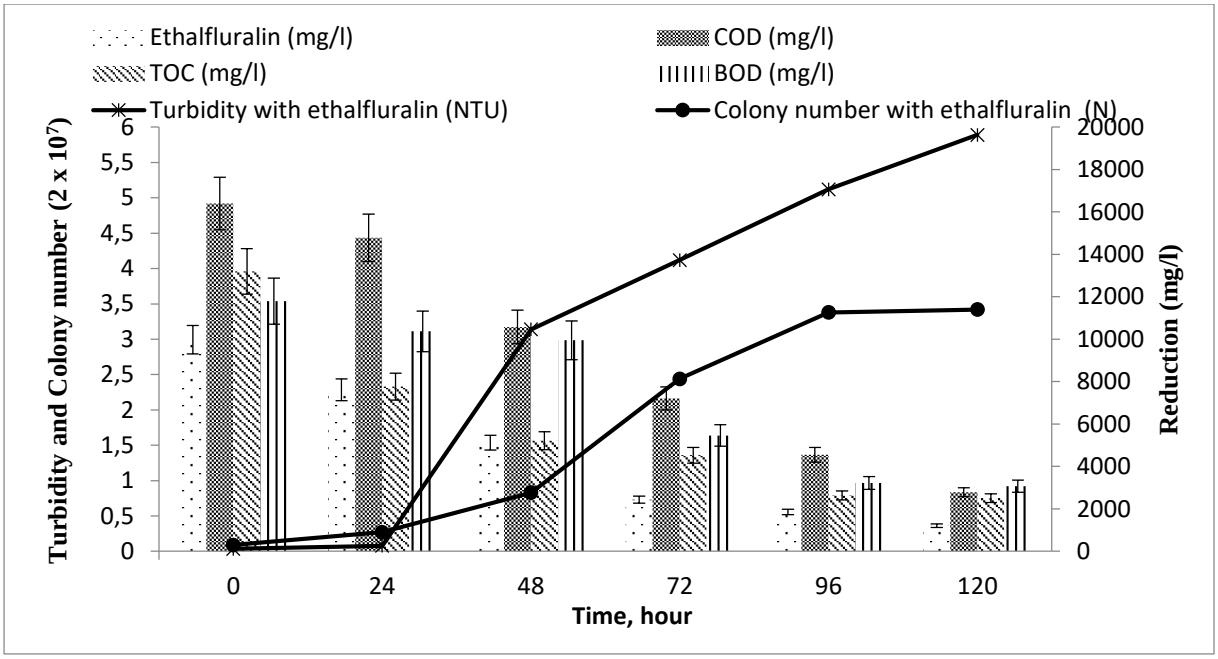

Figure 1. Reduction of ethalfluralin related with turbidity by *Bacillus thuringiensis*

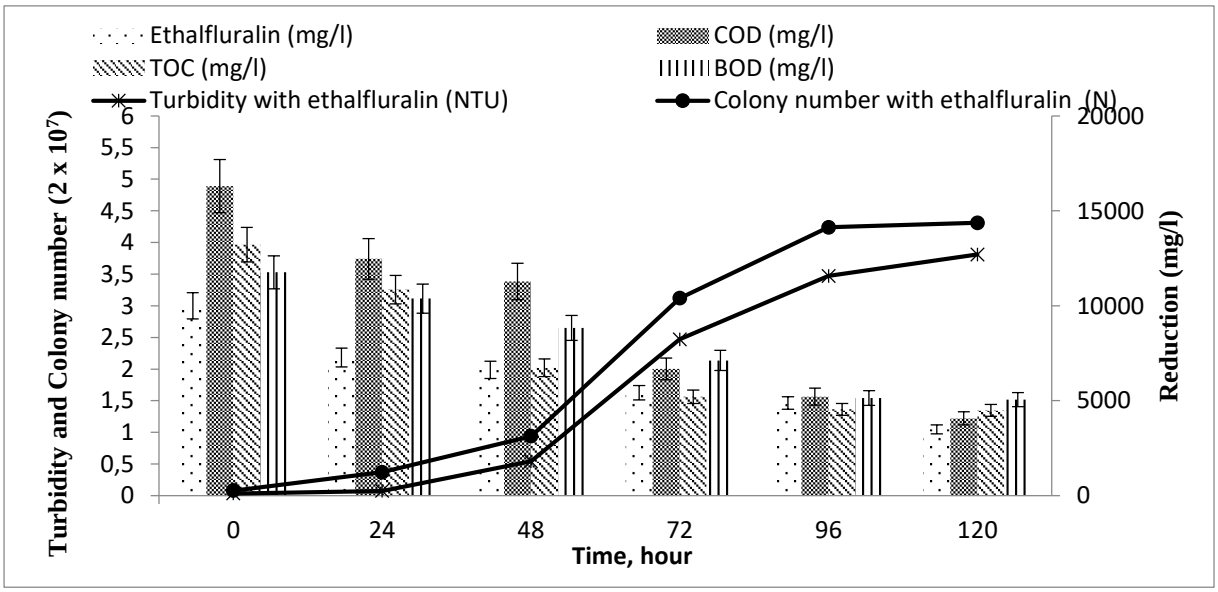

Figure 2. Reduction of ethalfluralin related with turbidity by *Micrococcus yunnanensis*

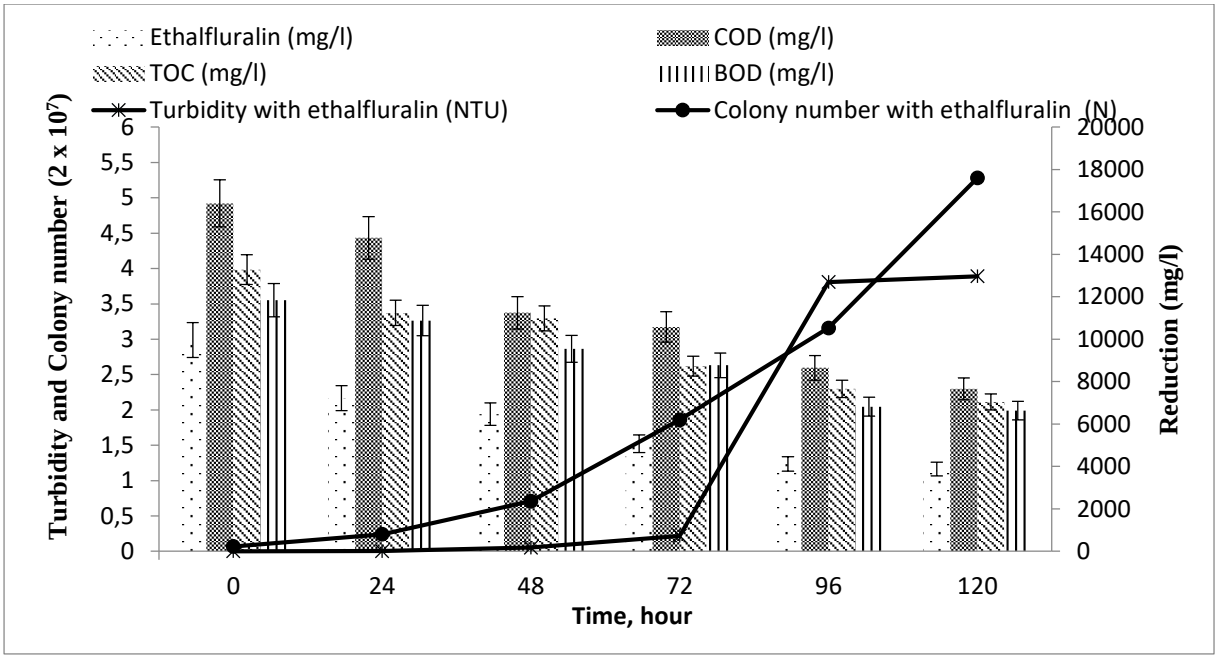

Figure 3. Reduction of ethalfluralin related with turbidity by *Fusarium fujikuroi*

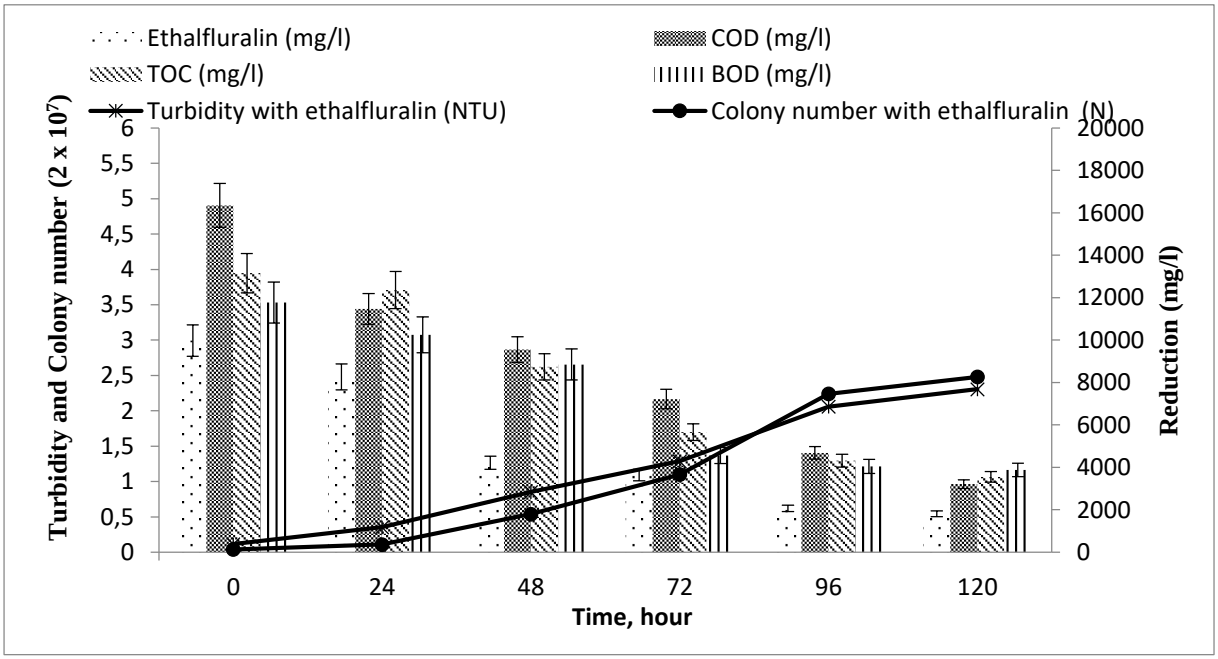

Figure 4. Reduction of ethalfluralin related with turbidity by *Bacillus muralis*

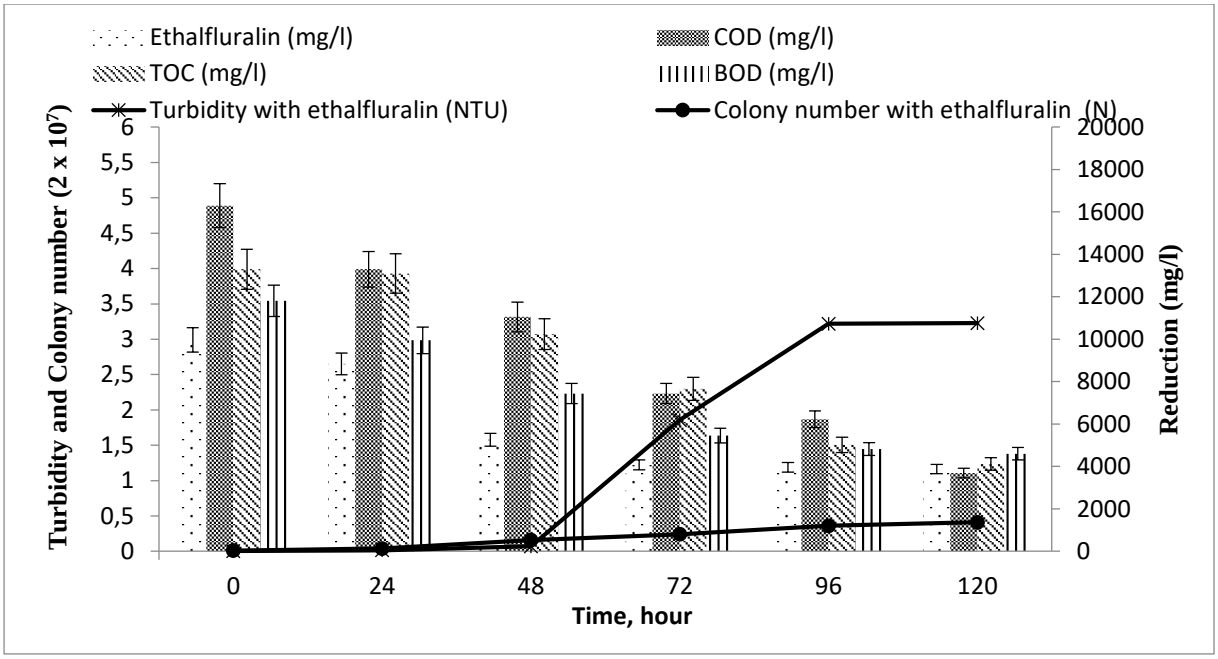

Figure 5. Reduction of ethalfluralin related with turbidity by *Micrococcus luteus*

Differences in bacteria species in the agitated culture media demonstrated the different results on Ethalfluralin, COD, TOC and BOD_5 reduction rates. The COD reduction efficiencies on *Bacillus thuringiensis, Clostridium tetani, Fusarium fujikuroi, Bacillus muralis* and *Micrococcus luteus* species were 83%, 75%, 53%, 80% and 77%, respectively in five days. At the end of the 5th day, there were negligible variations in all three parameters. At the end of 120th hour, The COD which was originally calculated as 16400 mg/l decreased to 2790 mg/l. The poorest removal performance was observed with *Fusarium fujikuroi*, decreasing the COD from 16400 mg/l to 7660 mg/l (Fig. 3.).

DISCUSSION

Based on the study findings, it was observed that as the population increased in all cultures, the turbidity of the agitated culture media increased as well. In studies that employed culture media with and without ethalfluralin, although there were differences between the values of the turbidity caused by the population size and identified cultures, it was impossible to obtain knowledge on these differences based on turbidity, and the number of microorganisms demonstrating the best and the worst removal efficiency, according to the results obtained in Ethalfluralin, COD, TOC and BOD_5 experiments. However, the increase in the population count and the value of turbidity in the media with ethalfluralin started later when compared to those in the blank media without ethalfluralin. This period may extend from hours to days. This is related to the adjustment period of the microorganisms (Lag phase) to the media with ethalfluralin. It also leads to the increase in the number of bacteria that utilize phosphorus and carbon and the value of turbidity caused by the microorganisms, along with the degradation of ethalfluralin. In the current study, it was observed that the rates of Ethalfluralin, COD, TOC and BOD_5 removal were between 88% and 41%. When the results of the monitoring of the bacterial activity in the Ethalfluralin culture medium through turbidity and population increase were examined, it was found that the increase in turbidity was dependent on *Bacillus thuringiensis* and *Bacillus muralis*, which had the best COD removal rate in the Ethalfluralin

medium, particularly after the 24th and 72th hours. Thus, lag phase of *Bacillus thuringiensis* occured within a longer period than that of *Bacillus muralis.*

The population size and the increase in turbidity in *Fusarium fujikuroi* that had the worst removal rate in the Ethalfluralin was observed after the 72th hour in the Ethalfluralin medium. Erguven and Bayhan (2016) conducted a study with fungi in a liquid medium, where Trifluralin was added and microbial activity was monitored with COD analysis. In that study, it was observed that the COD removal rates of *Metacordyceps chlamydosporia* and *Penicillium simplicissimum* were 80% and 59%, respectively. Based on their results, it was observed that *Metacordyceps chlamydosporia* demonstrated the highest COD removal rate (17400mg/l), which was reduced to 3480 mg/l at the end of the 5th day. During the decomposition, *Penicillum simplicissimum* had the lowest rate of COD reduction from 17400 mg/l to 7130 mg/l. In a previous study on biodegradation of herbicide chlorsulfuron, COD removal rates were observed between 70% and 93% (Erguven and Yildirim, 2016). (Yonten et al., 2017) studied the removal efficiency performance of *P. eryngii var. ferulae* for COD and they found *P. eryngii var. ferulae* was a suitable adsorbent for bioremediation of sulfamethazine contaminated soil field. Slight increase in turbidity and colony count could be observed by monitoring the bacterial population in the medium with ethalfluralin particularly after the 24th hour on Bacillus thuringiensis, *Micrococcus yunnanensis* and *Bacillus muralis* (Fig. 1-3), after the 48th hour on Fusarium fujikuroi (Fig. 3.) and after the hour 72th on *Micrococcus luteus* (Fig. 5.). The significant increase in colony count and turbidity demonstrated that the best COD, TOC and BOD_5 removal in the medium with ethalfluralin was observed with *B. Thuringiensis* and *B. muralis.*

CONCLUSIONS

Thus, for bioremediation of ethalfluralin contaminated liquid medium, these types of soil bacteria was an adequate species. The soil includes numerous bacteria that could degrade herbicides. In the medium, pesticide persistence is caused by the physico-chemical properties of organisms that could degrade pesticides.

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EVOLUTION OF THE DIFFERENT ASSESSMENT METHODS ON LEG WEAKNESSES AND LAMENESS OF BROILERS

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ABSTRACT

In this study, firstly, a brief evolution of the chickens was mentioned and then 'leg weakness' and what leg weakness means in the broiler sector were investigated. "Leg weakness" is a vague term used to describe properties of infective and non-infective nature that occur in modern, fast-growing broilers. Modern broilers commercially grown are prone to foot problems, including lameness, footpad dermatitis and hock-burn. Lameness is an extensive term used for some damages of broiler chickens with infective and non-infective source. Lameness is a very big problem in the broiler industry. For the United State in 2002, the costs of lameness were predicted to be between $80 million and $120 million. However, in literature, it has been proven that the lameness strongly correlated with weight, growth rate and activity. The time before the chick reaches, a live weight of 1500 g was reduced from 120 days to 30 days in 80 years. As results of fast growing, severe problems have been occurred in broiler chickens. For example, the animals with severe problems have a reduced feed efficiency and lower growth. The carcass quality of these animals has also been decreased in value. Additional to the welfare problems that have been caused by leg problems, also financial losses has been occurred for the producers. Therefore, the first purpose of this study is created as to review the leg weaknesses of broilers and provides to readers a brief discussion of the factors influencing this problem. The second purpose of this study is to review the lameness and lameness assessment methods for broilers. Additionally, the advantages and disadvantages of these methods are discussed. At the end of this review, brief conclusions can be found with related reference list.

Keywords: *Broiler, Leg Weknesses, Lameness, Chicken, Technology.*

INTRODUCTION

The poultry industry has grown enormously over its fifty-year history until these days and now more than 20 billion broiler chickens are produced every year. The demand for increased growth rate and production due to the economic benefits of higher body weight has led to the differential growth of body parts like accelerated growth of muscle (Verma, 2006).

However this growth is not accompanied by the skeletal development. Lame broilers cannot walk easily and unfortunately they cannot reach the feeder and the drinker when they are hungry or thirsty. Lameness is reducing their life quality. The existence of lameness is strongly correlated with weight and rapid growth of broilers (Su et al., 2000). Furthermore, movement problems may be painful for the broilers. It can decrease the broiler's activity and increase different problems, like hock burns and chest dirtiness (Thapa, 2004). Rapid growth rate

accompanied by adequate nutrition, proper management, optimum lighting and temperature, a disease free environment prevents weakness in broiler chickens. The absence of any of these factors combined with the intrinsic weight bearing characteristics give rise to the different degrees of leg disorders (Verma, 2006). Leg weakness can be classified as infectious, developmental/metabolic and degenerative disorders. Leg weakness leads to high incidence of morbidity than mortality (Verma, 2006). However, the disabled bird experiences pain, does not reach feed and water and dies due to inanition (Webster, 1995; Weeks, 1997).

Therefore, the first purpose of this study is created as to review the leg weaknesses of broilers and provides to readers a brief discussion of the factors influencing this problem. Among the factors that cause the leg weakness include nutritional deficits, mechanically induced trauma, toxins, genetic defects, pathogens infectious diseases, sex, weight and growth rate, age, the efficiency of feed conversion, handling and movement. The second purpose of this study is to review the lameness and lameness assessment methods for broilers.

Lameness

According to the European Commission report lameness is the main cause of bad welfare in broiler chickens (Scahaw, 2000). Lameness is an extensive term used for some damages of broiler chickens with infective and non-infective source (Rousing et al., 2000). Lameness is also a very big problem in the broiler industry. The financial losses due to lameness in the commercial grown broilers are considerable (Cook, 2000). For the United State in 2002, the costs of lameness were predicted to be between $80 million and $120 million (Sullivan, 1994).

Causes of Lameness

Among the factors that cause lameness include infectious diseases, genetics, sex, weight and growth rate, age, the efficiency of feed conversion, nutrition, handling and movement. These factors will be discussed further below. The key factors here of non-infective and non-nutritional bone abnormalities its genetic selection and the management (Bradshaw et al., 2002).

Assessment of Lameness

Traditional methods to determine the gait score as an indicator for lameness include manual scoring of birds' movement and other behaviours in the farm. Nevertheless, it is difficult to score the behaviours of broiler chickens. Compared to traditional methods, fully-automated image analysis techniques have many potential for lameness assessment. Automatic video image technique to analyze the activity as an indicator of lameness in broilers is becoming popular (Aydin et al., 2013) because it is getting cheaper. It is also non-contact method which allows recording more frequent data during the life-span of broiler chickens. There is also no need for huge data storage when data are automatically evaluated in real-time (Aydin et al., 2013). The measurements can also be performed continuously and automatically throughout the life-span of birds with non-invasive and non-intrusive way. It is also do not involve the biosecurity risk of having people visiting different commercial farms to perform visual gait scoring for boiler chickens (Dawkins et al., 2009).

Manual Assessments (Gait Scoring System)

The first manual assessment technique was developed by Kestin et al. (1992) to evaluate the gait problem in birds by visually observing and giving some gait scores for each broiler chicken. In this method, a score is assigned ranging from 0 (no leg problems) to 5 (completely

paralyzed) according to the criteria as follows: 0 (healthy broiler); 1 (the broiler moves fast, but there is a slight walking deficiency); 2 (the broiler moves fast, but significant walking deficiency is observed); 3 (the broiler moves fast, but there is a significant deficiency); 4 (the broiler cannot moves fast and there is a serious difficulty); and 5 (the broiler cannot move anymore).

Bristol Scoring System

The University of Bristol's Gait Scoring Guide is widely used to assess walking ability. This scoring system works on the same principle as the Gait Scoring System. Here, too, each broiler again a score from 0 to 5 according to certain criteria. The score is awarded by experts (Kestin et al., 1992). Although this method is often used because it is so easy to apply, it is still very subjective. It depends on the expertise and experience of the observer. Other studies showed that the repeatability of the visual gait score is not entirely reliable. From other studies even more important are that only the movement scored with this method, and not the pressure exerted by the chick. It will therefore not give objective information on whether the animal is in pain or not (Corr et al., 1998).

Automatic Assessment Systems

Pedobarograph System

The light intensity will be in proportion to the applied pressure (Corr et al., 1998). The gait-analysis was performed using a purpose-built pedobarograaf. When pressure is exerted on the surface, the emulsion side of the photographic paper pressed closer against the glass so that the light is distributed. This can be seen on the bottom of the glass. The glass plate and the career were both covered with polythene-backed protective sheeting (Benckhote, Whatman International Ltd) in order to ensure the birds. For a homogeneous surface under the glass is a mirror placed at an angle of 45 degrees. The divided light is reflected by the mirror and filmed with a closed-circuit camera (Panasonic WV-BP3101B0). The video was recorded on a S-VHS recorder (Panasonic AG-7355), and images warden transferred to a Powermac 8100/110 computer with a Scion LG-3 frame grabber card and analyzed using Scion Image (version 1.57) software (Scion Corporation, Maryland, USA). In this study, there were 12 frames per second. Each pixel is given a value between 1 and 254, depending on the brightness of that point. The system can then be calibrated to relate with applied pressure (Corr et al., 2003).

There were several gait parameters measured and compared between the different groups:

• Speed (m / sec)

• Step frequency (steps / min)

• Step width

• Step length

• Step angle

Video Recordings

In another study, the behaviour of broiler chickens related with lameness was investigated by (Weeks et al., 2000). Comparisons were performed between healthy broilers and lame birds

between 39 and 49 days old. Healthy birds spent 76% of their time as lying and 24% of their time as standing and/or moving. Lame broilers with gait score 3 spent 86% of their time as lying in. Lying events were also increased with the age of broilers (Weeks et al., 2000).

Latency to Lie Test

In broilers, another method for lameness assessment was described by (Weeks et al., 2002). The time duration that birds stayed standing in water was measured and the results were checked with traditional results. There was an important ($P < 0.001$) correlation among the gait scores and LTL of birds. More than 750 birds at the age between 32 and 45 days old were tested in a broiler house. Almost all of the healthy broilers were able to stand for at least 15 minutes and most of the lame broilers sit down in five minutes.

Force Plate Study of Avian Gate

Another method was developed by Corr et al. (2007) to define the ground reaction force of birds. The ground reaction force was tested while the broilers walked on the experiment setup. GRF patterns represented important changes during growth. It was concluded that the force plate is an appropriate study tool for recording the ground reaction force patterns of broilers.

Precision Livestock Farming Approaches to Detect the Lameness of Broilers

Automatic monitoring the activity in broiler chickens is one of the easiest ways to define lameness at broiler houses. A fully automated monitoring system was developed by Aydin et al. (2010) to measure the broilers activity at different gait scores. The activities were obtained by using an automatic video recording system. Then, the images of the birds with six different lameness level were automatically analyzed by the developed algorithm. The results showed that, there was an important correlation among the lameness obtained by expert and activity recorded by image monitoring tool (Aydin et al., 2010). It was also detected that there was an important lower activity of high gait score broilers (GS4&GS5). Therefore, it was concluded that, this technique can be used as indicator of high lameness level (GS4&GS5) in broiler houses. Another study was performed by (Aydin et al., 2013) to define a new technique to estimate the spatial use of broilers by using image analysis. An important relationship was observed for both experiments among the lameness and the movement recorded by the image monitoring tool. The results also showed that there was a strong relationship between the spatial use of broilers with a certain lameness level and activity. Therefore, it was also concluded that the spatial use of broilers can also be a kind of indicator for activity and criteria for lameness assessment (Aydin et al., 2013).

CONCLUSIONS

As explained in this study, leg weakness includes a wide range of abnormalities due to a multitude of etiological causes. It is definitely affect the growth and end-weight of broiler chickens consequently causing a huge economic loss to the farmer. As detailed above, the leg weakness of broiler chickens is influenced by many different factors, which needs to be taken into account when managing broilers. Leg weakness can be prevented by modifying the environment and diet. Also growth rates can be reduced by artificial lighting and restricted feeding. However, proper manage mental practices should be adopted.

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AN ARTIFICIAL NEURAL NETWORKS MODELL FOR PREDICTING BOD OF ISHËM RIVER

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ABSTRACT

Water quality depends on the determination of the many parameters determined by chemical methods. These methods are often tedious and time consuming. Artificial Neural Networks (ANNs) are suitable models for many purposes. Recently, ANNs have been widely used in modeling, control, pattern recognition, signal processing, prediction purposes and so on. Many of the physical and chemical water quality variables (pH, turbidity, TDS, temperature, electrical conductivity, dissolved oxygen, chemical oxygen demand, hardness, chloride, sulphate, phosphate, calcium, magnesium, nitrate, nitrite and amonium) that affect biochemical oxygen demand (BOD) concentrations were collected at 7 sampling sites in the Ishëm River Basin during 2010–2017. In this study, we use a three layer feed-forward model with back-propagation multi-layer perception (MLP) to model the relationship between the water qualities parameters used to predict the BOD. The available data set was partitioned in three subsets: a training set, a validation set and a test set according to station. In order to reach an optimum amount of hidden layer nodes, nodes 2, 3, 5, 7, 9, 10 were tested. Within this range, the ANN architecture having 10 inputs and 1 hidden layer with 5 nodes gives the best choice. The ANN was successfully trained and validated with 80% and 10% of the data sets respectively. Comparison of results shows that the ANN model gives reasonable estimates for the BOD prediction. Performance of the models was evaluated by average absolute relative error (AARE) and root mean square error (RMSE). The correlation coefficient of ANN models for prediction of BOD was 0.925. Sensitivity analysis was also carried out to identify the most significant input-output relationship. Hence, the ANNs were able to show remarkable prediction performance to predicting the BOD in Ishëm River.

Keywords: *Artificial Neural Network model, Multi-layer perceptron, Prediction, Ishëm River, BOD, Water quality parameters.*

INTRODUCTION

Water quality is one of the main characteristics of a river, even when its purpose is other than human water supply. Water quality in the surface waters has started to degenerate as a result of wastewater being let go to the receiving ground and surface water without control.

Biochemical oxygen demand (BOD) is an important parameter for usage conditions of surface waters. The assessment of the water quality is an important part of integrated water

resource management. Among different parameters that are being monitored, BOD in rivers is the only water quality indicator that every EU member state is obliged to monitor on a regular basis. The water quality indicators monitored at the national level are temperature, pH, dissolved oxygen (DO), BOD, chemical oxygen demand (COD), ammonium (NH_4), nitrate (NO_3), nitrite (NO_2) and phosphorus (P), in rivers. BOD is a measure of organic pollution in an aquatic system and it is inversely related to the DO level in water, i.e. high BOD values indicate a low level of DO or even anoxic conditions in water (Singh et al. 2009). Also, it represents the approximate amount of biodegradable organic matter in the water and provides a strong indication of the extent of water pollution (Marcotullio 2007). Therefore, it is established as the most significant water quality parameter needed to identify and implement strategies for the protection of water resources (Basant et al. 2010). In general, the BOD levels depend on the organic waste loads that come from the household wastewater, industries, and silage effluent and manure from agriculture (European Environment Agency EEA 2015).

River water pollution caused by various human activities represents severe stress on human well-being and hinders economic development. It also adversely affects ecosystems, depleting aquatic life in watercourses that used to be abundant in species and populations.

Some water quality parameters (pH, temperature, DO, etc.) can be accurately measured in the field. Performing the test for BOD level is rather time-consuming and requires 5 days in the laboratory to complete the test. The laboratory tests do not take into consideration oxygen consumption by living organisms, but only oxygen required for oxidation of an organic waste load in a given volume of water, thus results can be inaccurate (Verma et al., 2013).

Several water quality traditional models such as mechanistic, statistical and deterministic approaches have been directed toward developing a reliable model that could predict quickly and at low cost water quality parameters such as BOD. Since the most of input data which are needed by most of these models are often difficult to obtain, make these models very expensive and time consuming.

Recent researches have been applied intelligence techniques for water quality modelling. Among these techniques ANN is the suitable approach. ANNs are able to extract a relationship model inputs and outputs to provide quite better accurate predictions with a minimum data requirement by “learning” from available data presented to them. Thus, ANNs have become a powerful tool in predicting different parameters in many difficult problems.

Based on Devolli et al. (2012) Ishëm River has higher levels of pollution compared to other Albanian rivers, due to the great impact of urban and industrial discharges. The content of BOD, COD and ammonium in all sites exceed the maximum permitted levels.

This study aims to analyze and discuss the performances of ANN in prediction of BOD in the Ishëm River.

MATERIAL AND METHODS

Description of the study area

The Ishëm (or Ishmi) is a river in western Albania, which brings water to the area north of the Albanian capital, Tirana. It forms part of a watercourse (Tiranë-Gjole-Ishëm), but only the lower third of the watercourse is known as the Ishëm, the Ishëm proper is formed at the confluence of the rivers Gjole and Zezë, a few km northwest of Fushë-Krujë. It flows into the Adriatic Sea near the town Ishëm, the length of the watercourse is recorded in different sources as between 74 and 79 km. (Çullaj et al., 2005; Pano, 2008; Kolaneci, 2000)

Latitude: 41° 34' 23.99" N

Longitude: 19° 33' 16.19" E

The Ishëm is formed from several rivers which arise to the northeast of Tirana in the Skanderbegg Mountains beyond the Krujë range, the most important of these are:

The Tiranë (Lumi i Tiranës), which has its source to the northeast of Mount Dajt, is the main tributary of the Ishëm (Pano N, 2008). It crosses the mountain range to the north of Mount Dajt, through a narrow canon called Shkalla Tujanit, it then flows west all the way across the Plain of Tirana. The city of Tirana stretches along the southern edge of its broad flood plain, at the western edge of the plain, the river meets its most important tributary, the Lanë, which rises on the western slopes of Mount Dajt and flows through the city centre of Tirana to the south of the Tiranë river in a westerly direction until it meets it. After this, the river continues in a northerly direction.

The Tërkuza meets the Tiranë a little further north. It also has its source to the east of the mountain chain and crosses it through a canyon, called Shkalle e Bovillës, which has been dammed in order to create the Bovilla Reservoir, which has a surface area of around 8,000,000 m^3 and has provided drinking water to the city of Tirana since December 1998. The Tërkuza crosses the Tirana Plain in a northwesterly direction, running past Tirana Airport, before it meets the Tirana River. Once these two rivers join, the river is referred to as the Gjole.

The Zezë (Black river) arises east of Krujë. It also runs through a canyon, called Shkalla e Kryemadhës, and then crosses the plain in a northwesternly direction, passing through Fushë-Krujë, it meet the Gjole a few kilometers after the Tërkuza.

From the point where the Zezë joins the Gjole, the river is known as the Ishëm, it flows in a westerly direction until it reaches the edge of the Tirana Plain, then turns to the northwest and heads for the Adriatic. In this part of its journey it passes through a town with the same name. Shortly before the mouth of the river, it is joined by the Droja River, a stream which arises in the mountains northeast of Krujë, the Ishëm discharges into the Adriatic to the southwest of Laç in the Rodon Bay, which is bounded on the western edge by the Cape of Rodon and forms part of the Drin Gulf.

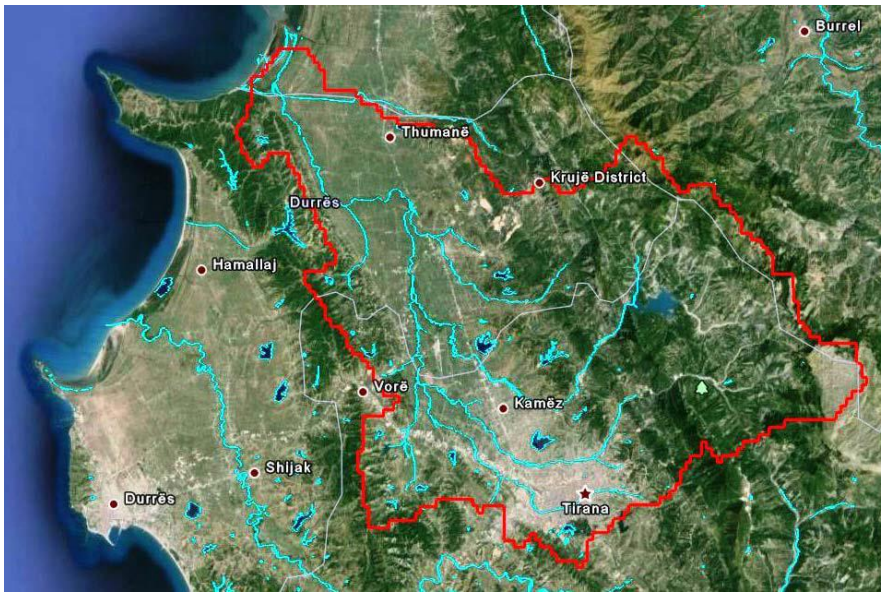

Figure 1. Ishëm River basin

The drainage basin of the Ishëm covers a total area of 673 km^2. The average discharge at the mouth of the river is 20.9 m^3/s. The highest annual discharge is over six times the annual minimum (Pano, 2008).

The water quality dataset

Important pollution sources in Ishëm basin are municipal, industrial wastewater and agricultural run-off. The Ishëm River is also affected by non-point sources of pollution including fertilizers from farm effluents, domestic wastewater discharges and some industries' effluents. Ishëm stream drainage basin has intensive industry activity. The Lana River receives wastewater discharges from Tirana City. And other towns in the basin have no sewage treatment system. Solid wastes of Tirana city is untidy stored in the Lana River Basin. Furthermore, there are intensively daily residence plants around Stream. The Ishëm basin has density agricultural activity. The monitoring network of the Ishëm river water quality on its flow through the observed area hosts 7 monitoring stations.

Water quality at the 7 stations is monitored regularly, monthly or fortnightly, and the dataset for modeling was composed of 10 parameters monitored over a period of 7 years, from 2010 to 2017. The river water flow was not considered since the monitoring data was available at too few stations. The full dataset prepared for the prediction of BOD level in the Ishëm River was comprised of more than 1,100 individual observations.

Artificial neural networks

ANNs are considered as powerful forecasting tools, in comparison to traditional modeling techniques (Awchi, 2014). An ANN is inspired by biological neural system. It is a highly interconnected network composed by many simple processing units called neurons. An ANN normally consists of three layers, an input, a hidden and an output layer. Each layer is composed by neurons which are connected only with neurons of next layer. The strength of this connection is called weight. The determination of these weights is an important step in developing of the ANN model. ANN is able to "learn" from "experience" by looking into previous behavioral relationships. This is carried out by changing the weights. The process of determining unknown weights is called training. A supervised training algorithm requires an external teacher to guide the training process. The primary goal in supervised training is to minimize the error at the output layer by searching for a set of weights that minimize the ANN error between outputs and targets. A supervised training mechanism called back-propagation training algorithm (BP) is normally adopted in most of the applications.

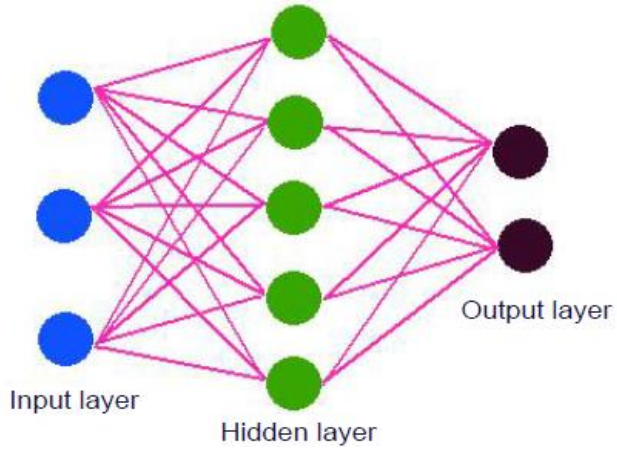

Figure 2. An ANN composed by three layers

We have developed the model by adopting following steps. Firstly, database collection; analysis and preprocessing of the data; training of the neural network. Then we had choice the

architecture, training functions, training algorithms and parameters of the network; testing of the trained network; and using the trained neural network for simulation and prediction.

Training Algorithm

The back-propagation training algorithm is a simple gradient descend technique, and it is widely used in study. But it has some shortcomings such as slow velocity of convergence and local minimum. The Levenberg-Marquardt algorithm (L-M) is a mixture of gradient descend and Gauss-Newton iteration, it is also being used widely recently due to its superior efficiency and high convergence speed (Antcil et al., 2004). It can overcome the weakness caused by BP (Nouir et al., 2007).

Selection of input parameters for the ANN

The selection of input parameters for ANN is a very important aspect for the ANN modeling.

The sensitivity analysis was applied to determine the relative importance of the parameters. The sensitivity analysis is used to determine the effect of changes and to determine the relative importance or effectiveness of a variable on the output. The input variables that do not have a significant effect on the performance of an ANN can be excluded from the input variables, resulting in a more compact network.

The statistical correlations of BOD with their respective inputted parameters were calculated using SPSS Statistics 22 for analyzing and examining the relation among the parameters so as to bring out the relative susceptibility of each parameter.

The correlation coefficients for BOD are chemical oxygen demand (0.763), ammonium (0.725), phosphorus (0.659), pH (0.515), sample point (0.463) at 0.01 level of significant and dissolved oxygen (-0.423), nitrate (0.382), nitrite (0.298) and time (0.257) at 0.05 level of significant had highest correlation while calcium (0.300), and hardness (0.097) had least correlation with biochemical oxygen demand (BOD) in that order.

RESULTS AND DISCUSSION

Before the training of the network both input and output variables were normalized within the range 0.1–0.9. The available data set was partitioned in three subsets: a training set, a validation set and a test set according to station.

In order to reach an optimum amount of hidden layer nodes, 2 – 10 nodes are tested. Within this range, an ANN model, having 10 inputs and 1 hidden layer with 5 nodes and 1000 iteration number, gives the best choice.

The ANN was successfully trained and validated with 80% and 10% of the data set respectively. It is tested with 10% of the data set.

In order to test the robustness of the model, the average absolute relative error (AARE) was used. The AARE gives the performance index in terms of predicting BOD. It also shows the distribution of the prediction errors

$$AARE = \frac{1}{N}\sum_{i=1}^{N}\left|RE\right|$$

in which

$$RE = \frac{d_i - o_i}{d_i} \times 100\%$$

where *RE* is the relative error in forecast expressed as percentage, d_i is the observed BOD for the *i*-th pattern, and o_p is output produced by ANN (the computed BOD) for the *i*-th pattern

and *N* is the total number of the testing patterns. It is clear the smaller the value of AARE, the better the performance is. The performance control of the ANN outputs was evaluated by estimating the determination coefficient (R^2) and RMSE.
Based on the statistical findings, the ANN model with ten inputs (COD, NH_4, DO, P-total, NO_3, NO_2, DO, Sp, time) gives the best estimation.
Several MLP networks were generated and tested for finding the best architecture with various hidden nodes and training algorithms. This is conducted by Alyuda NeuroIntelligence Software.

The Levenberg-Marquardt (L-M) training algorithm was used to adjust the learning procedure. We used momentum and weight decay to improve the network. The results of different MLP and their errors for BOD are shown in Table 1. Average absolute percentage error (AAPE) for each model is calculated. In the trials, the number of neurons in the hidden layer varied between 2 and 10 and number of iterations is set at 3000. As can be seen from Table 1, the model 4 outperforms the others.

Table 1. Different MLP results for BOD

MLP model number	1	2	3	4	5	6	7	8	9
Number of neurons in first hidden layer	2	3	4	5	6	7	8	9	10
Relative error (AAPE)	0.052	0.036	0.031	0.026	0.048	0.064	0.047	0.054	0.052

The architecture of the optimal ANN model for the BOD model is composed of one input layer with ten input variables, one hidden layer with five nodes and one output layer with one output variable. The correlation coefficient (r), RMSE and AARE values computed for the training, validation and test data sets are presented in Table 2.

The scatter plots of actual versus predicted values of obtained using ANN model are shown in Figure 3. The figure reveals that an acceptable agreement between the simulations and observations can be achieved. The correlation coefficient values between the ANN models predicted values and observed data for BOD was 0.925.

Table 2. Performance parameters of the ANN models

ANN-structure	Data set	RMSE	AAPE	R
10-5-1	Training	0.33	0.26	0.952
	Validation	0.42	0.33	0.945
	Test	0.48	0.37	0.925

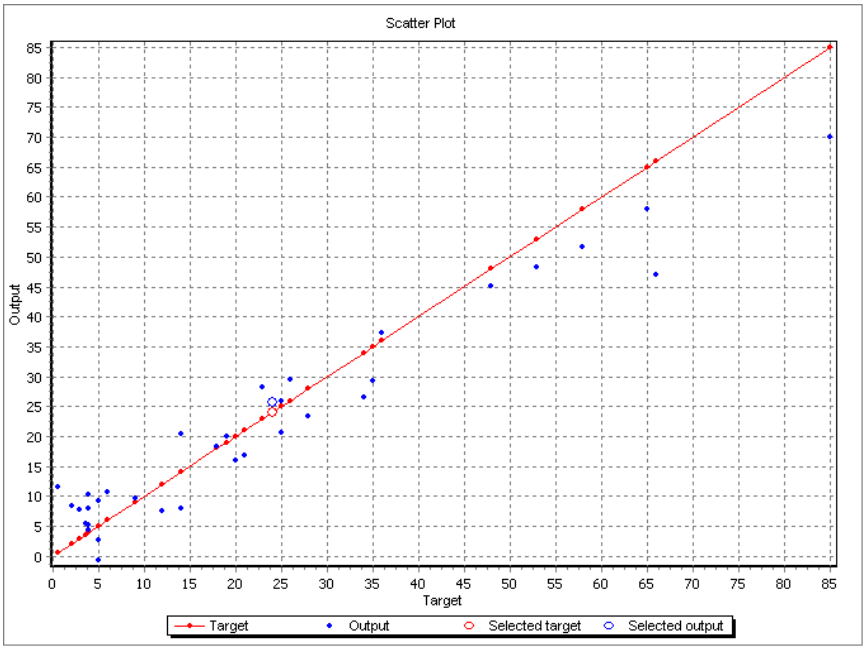

Figure 3. Scatter plots of actual versus predicted values.

CONCLUSIONS

This paper recommends the use of ANN-based water quality parameters prediction model for rivers. In this paper, artificial neural network models were developed for prediction of BOD in the Ishëm River, Albania.

The networks were designed by putting weights between neurons, by using the hyperbolic-tangent function of training. The results for the training and the test data sets were satisfactory. Hence, with the proposed model applications it is possible to manage water quality parameters such BOD in a more cost-effective and easier way.

Comparison of results shows that the ANN model gives reasonable estimates for the BOD prediction. Performance of the models was evaluated by average absolute percentage error (AAPE) and root mean square error (RMSE). The correlation coefficient of ANN models for prediction of BOD was 0.925. Sensitivity analysis was also carried out to identify the most significant input-output relationship. Hence, the ANNs were able to show remarkable prediction performance to predicting the BOD in Ishëm River.

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FOREST GAPS: A REVIEW WITH SPECIAL ATTENTION TO TEMPERATE BEECH FORESTS IN HYRCANIAN REGION

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ABSTRACT

Forest gaps formed by death of individual tree or a few trees can influence on forest structure. In Hyrcanian regions, the natural events and silvicultural systems affect forest dynamics via creating canopy gaps. The main objective of this review was to study the effect of gap size on the natural regeneration and herb-layer diversity within oriental beech (*Fagus orientalis* Lipsky) stands in Hyrcanian regions, northern Iran. The scientific researches in Hyrcanian beech forests showed that the most regeneration of trees was observed in harvest-created and natural gaps of 50-550 m^2 in size and regeneration rate was reduced with increasing gap area. Many factors including light intensity, forest structure, harvesting operation, unsuitable weather condition and seeding cycle can contribute to the quantity of beech trees regeneration within gaps. Also age, crown condition, height of trees in the edge of gaps and age of gaps associated with ecological factors influence on presence, frequency and quality of beech seedlings within beech stand. The studies on plant species diversity in canopy gaps within oriental beech stands indicated that gap size affected on herb-layer diversity values and the gap size of 200-500 m^2 is a suitable gap size for maintaining herb-layer plant diversity and richness values. Generally, it can state to attain the successful regeneration and to conserve herb–layer diversity in beech stands in close-to- nature silviculture, it is essential to consider the gap size of 50-550 m^2 through planning silvicultural operations in Hyrcanian beech forests.

Keywords: *Beech, Canopy gap, Plant diversity, Regeneration, Silviculture*

INTRODUCTION

Forest gap created by death of individual tree or more trees is the principal disturbance in a lot of forest ecosystem in world. (Muscolo et al., 2014). In general, disturbances such as diseases (Sommerfeld et al., 2000; Miller et al., 2007; Vepakamma et al., 2008), storm (Collins and Battaglia, 2002; Muller and Wagner, 2003; Kukkonen et al., 2008), fire (Banal et al., 2007; Zou et al., 2007) and harvesting (Miller et al., 2007; Banal et al., 2007; Toledo-Aceves et al., 2009) causes injuries to trees or even removes them which may finally lead to the creation of a forest gap. The structure and function of forests can be formed by disturbances that produce forest gaps (Huai et al., 2017). Forest dynamics and succession are affected by canopy gap, also microclimate conditions altered within and around gap when the gap is formed (Zhu et al., 2007), and the extend of changes is established via gap area (d'Oliveira and Braz, 2006). The soil and air temperatures, solar radiation, and soil moisture are higher in gaps than the neighboring closed forest (Schliemann and Bockheim 2014). Forest canopy gaps and adjacent closed stands often are differed in regarding to microclimate conditions (Forrester et al., 2012). Plant species composition may be affected by age and size of gaps, also environmental features

including light, soil elements (Qin et al., 2011). The changes in structure, plant species diversity, and regeneration are shaped by gap size and its temporal distribution (Sapkota et al., 2009). The effect of forest gap on growth is related to light and soil nutrients changes, as well as plant capability to respond to these changes (Stan and Daniels, 2014). Forest gap is the main process that determine regeneration improvement in temperate forests and there are a lot of studies about influences of gaps on tree growth (Van Couwenberghe et al., 2010). The aim of this review is to study new and previously published data to provide a clear picture of the role of forest gaps in broad-leaf deciduous forests of Hyrcanian region, northern Iran. The natural phenomena and silvictultural methods were created canopy gaps within forest stands, especially in beech stands. Harvesting methods in forest leads to gap creation with varying sizes (d'Oliveira and Ribas, 2011). The oriental beech (*Fagus orientalis* Lipsky) stands are the most valuable and commercial tree species which were presented in Hyrcanian forests, northern Iran and many studies were conducted about role of canopy gaps within beech stands. In the temperate deciduous forests of Hyrcanian region, different size and shape of gaps were created by various silvicultural methods such as selection method in beech stands. As forest management aims to control the development of forest stands based on management objectives, using different silvicultural methods, to study the role of canopy gaps may help achieve these goals and the collected information on gap dynamics could be valuable for forest management.

MATERIAL AND METHODS

Forest gaps have been studied in the temperate deciduous forests for the past years. This review considers much of this research with a view to assessing the importance of gap disturbance in oriental beech (*Fagus orientalis* Lipsky) forests in Hyrcanian region, northern Iran. This review is constructed on the results of a literature assessment which focused on papers published in the period 2006-2015 using keywords including gap size, regeneration, biodiversity and beech trees. The majority of the articles involved in this review were published in Iranian journals written in Persian and a few in foreign journals. I categorized the selected papers according to gap size (natural and artificial gaps) in beech stands. The artificial gaps were created by single-tree selection and shelterwood systems in oriental beech stands. Beech trees were recognized as gap makers in all stands.

RESULTS AND DISCUSSION

The evolution of any forest is dependent on the establishment of regeneration, in fact, the current status of regeneration of any forest indicates its position in the future (Delfan Abazari et al., 2004). Commercial tree regeneration for sustainable yield is considered an important factor in forest management (Schultze, 2008). One of the major challenges of silviculture and ecology is the understanding of development of plant community structure in forest after disturbance and new seedling growth is the first important incidence after disturbance had occurred (Coates, 2002). Gap size and its shape are the most important factors for tree species regeneration within forest. Based on conducted scientific researches in Hyrcanian beech forests, the most regeneration for this tree was observed in harvest-created and natural gaps of 50-550 m^2 in size and regeneration was reduced in large gap area (Amanzadeh et al., 2006; Shabani et al., 2011; Zolfaghari et al., 2011; Abrari Vajari et al., 2012; Amolikondori et al., 2012; Sefidi et al., 2015; Nasiri et al., 2015). The decreased beech seedlings within large gaps can be contributed to shade tolerant nature of oriental beech, also less competition with other tree species. In the larger gaps within oriental beech forest, we can observe that plants especially, light-adapted herb-layer species grow easily than beech seedlings. Mirdar Harijani et al. (2016) asserted that with increasing gap sizes associated with light and temperature

tensions, beech seedlings decreased. Of course, regeneration rate of beech is correlated to structure (Hahn and Madsen, 2004), light and soil properties (Madsen et al., 2004) in forest gaps. For example, Shabani et al. (2011) declared that there was positive significant correlation between oriental beech seedling density and N%, P, pH and moisture within gaps in beech forest. Several factors such as blackberry, ferns, grazing, unsuitable weather condition, seeding cycle (Amanzade et al., 2006) and harvesting operations can contribute to regeneration quantity of beech trees. Gap size influences the intensity of light and therefore the temperature of air and soil, soil moisture and biological properties of soil which impact on regeneration of trees (Muscolo et al., 2017). In some cases, gap size had no impact on regeneration density of beech seedlings and other tree species (Amolikondori et al., 2012). Age, crown condition, height of trees in the edge of gaps, also age of gaps associated with ecological factors influence on presence, frequency and quality of beech seedlings within forest. It is quite evident that some factors such as light, moisture and nutrients of soil differ from around to inside of forest gaps which effect on plant species with diverse regeneration necessities (Muscolo et al., 2017). In general, it can state to achieve the successful regeneration in beech stand in close-to- nature silviculture it is necessary to consider the gap size of 50-550 m^2 through planning silvicultural operations in hyrcanian beech forests. Since understory plant species account for most plant diversity in temperate forests, it is important for forest managers to determine the factors affecting distribution of these plants (Gracia et al., 2007). The studies on plant species diversity in canopy gaps within oriental beech stands revealed that gap size influenced on herb-layer diversity values (Amanzadeh et al., 2015; Haghverdi et al., 2011; Abrari Vajari et al., 2012; Shabani et al., 2011; Hamrang et al., 2014; Pourbabaei et al., 2013). These researchers suggested the gap size of 200-500 m^2 which is a suitable gap size for maintaining herb-layer plant diversity and richness values. Physical and biological environmental changes occur in the forest after canopy gaps develop (Zhao et al., 2006) and the ground-layer plant species are intensely vulnerable to environmental conditions (Kern et al., 2006). Naaf et al. (2007) stated that the increase in species richness beneath gaps is mainly due to larger space and higher light availability. Creation of gap changes the environmental conditions of understory in a gradient of resources, the irregularity of light in gaps of temperate forest along with soil moisture and nutrient accessibility explain the floristic pattern (Duguid et al., 2013). The variations of light, air and soil temperatures, and soil features in gaps with different sizes might be relative to the impacts of the microsite, which influences the diversity values of plant species. These studies demonstrated significant impacts of gap size (natural and artificial gaps) on plant species diversity and regeneration in oriental beech stands. Higher herb-layer richness and diversity values in large gaps indicate that gap creation could be used as a management approach to increase herbaceous plant richness and structural heterogeneity in Hyrcanian temperate beech forests.

CONCLUSIONS

The findings suggest that canopy gap conditions, especially gap size in beech-dominated deciduous stands may be sufficient to promote the regeneration of beech trees and herbaceous plant diversity values. The applied single-tree selection and shelterwood silvicultural systems in oriental beech stand created gap size at maximum 500 m^2 via felling these trees in overstory strata. The results afford practical evidence to plan a silvicultural system valuable to manage the natural regeneration of beech trees in Hyrcanian forest diminishing the environmental effects. Silvicultural systems in beech stands have the ability to create different gap size, which can be useful for plant diversity and regeneration. This information has significant implications for planning forest management.

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TEST OF CONTAMINATION OF A LICHENIC SPECIES "XANTHORIA PARIETINA" AND A MUSCICOLE SPECIES "HYLOCOMUIM SPLENDENS" BY LEAD/EFFECTS ON SOME PHYSIOLOGICAL PARAMETERS

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Abstract

Air pollution, especially that caused by lead has constantly evolved over time, due to the considerable increase in the number of vehicles on the market. Lead, which is one of the first used metals by mankind, represents a major risk to human health but also to the ecosystem. Living beings are a reflection of the environment in which they evolve and their observations at various levels of the biological organization can provide guidance on the quality and characteristics of this environment. The use of lichens and mosses can provide very precise indications and show as early as possible the natural or the induced changes. That's why, our work is essentially based on the characterization of the accumulating power as well as the impact of the different concentrations of lead on two species, a lichenic species "*Xanthoria parietina*" and muscicole species "*hylocomium splendens*" collected in the region of Bir El Djir "Oran". In order to determine the ability of these plants to accumulate lead and its impact on some physiological parameters (ph, chlorophyll and proline), a contamination in vitro by different concentrations of Pb (NO3) was carried out during a period of 45 days. The obtained results show the presence of lead in the thalli of both species, at levels increasing in parallel with the concentrations to which they were exposed, As well as a disturbance of the cellular metabolism which is interpreted by an increase in ph. Also we have noticed variations of measured physiological parameters; Chlorophyll and proline content that can result from stress, degradation of the fresh material, and disturbance of the photosynthetic process. It is obvious to mention that the two studied species are proven good bio accumulators of lead which makes them excellent bio-indicators for the estimation of pollution especially by metals.

Keywords: *Lichens, Mosses, Lead, Bioaccumulation, Bio-Indication, Physiological Parameters.*

INTRODUCTION

Air pollution and the transfer of harmful quantities of natural or synthetic materials in the atmosphere, the direct or indirect consequence of human activity, is one of the important manifestations of environmental degradation, as it threatens directly the most necessary element for life ‹air›. This type of pollution can have a dramatic effect, if the main problem concerns the health of the populations, the impact on the ecosystem must also be taken in consideration.

The use of bio indicators is based on their integrator character in respect of environments factors. In effects, the livings beings are the reflection of the environment in which they evolves and their observation in various levels of biological organizations can offer indications about the quality and the characteristics of that environment. In bio-monitoring of heavy metal pollution, bio indication techniques occupies an interesting place. The use of lichens and

mosses can offer so precise indication and make obvious as early as possible the natural or caused modifications (Alioua, 2001).

MATERIAL AND METHODS

Presentation of the sampling region

The city of Bir-el Djir (Fig. 1) our sampling region is the main city in the immediate suburbs to the east of Oran outside the borough baptized agricultural in colonial times, the municipality is located 8km from the city center. It is a city that remained essentially agricultural until the end of the years 80, it now hosts a population of 118,000 inhabitants and becomes a major pole of the Oranean agglomeration. It houses several corporate offices with modernist architecture, the new University Hospital 1st November, University education Institutes which USTO is the biggest. The territory of district of Oran is characterized by a relief consisting mainly of mountains, hills and plains, we distinguish in particular the mountain of the Aidour (429m).
According to the National Meteorological Organization 2011 The Oran region has a classical mediterranean climate marked by a summer drought, mild winters, and a bright and clear sky. During the summer months, precipitation becomes rare or even non-existent.
The subtropical anticyclone covers the Oranean region for nearly four months. On the other hand, the area is well watered during the winter. The weak precipitations (420 mm of precipitations) and their frequency (72.9 day by year) are also characteristic of this climate: Minimum annual average temperature is about 12 C°. Maximum annual maximum temperature is about 22 C°

Justification of the choice of the sampling site

After prospecting different region in Oran, we noticed that the lichenic and muscicole species are limited, only the two species *Xanthoria parietina* and *Hylocomium splendens* have taken our attention. Our sampling takes place at the sciences and technology university of Oran ‹‹Mohamed Boudiaf ›› for its proximity and also for the abundance of the species.

Figure 1. Localization of Bir-el Djir city in the district of Oran

Experimental protocol

Sampled biological material

Our study concerned a nitrophile foliated specie of lichens with a lush color developing on different phorophytes, especially on «*Acacia albida*» from where we have chosen it for our experimentation, it is «*Xanthoria parietina»*.

For the mosses our choice concerned the most present specie in the samling site, it is *«Hylocomium splendens».*

Sampling techniques

For lichen thalli, we detached them from their phorophyte using a knife, as for the muscicoles thalli, they were carefully detached from the soil. The samples taken are placed in a well labelled bag, transported to the laboratory for identification.

The essays

Preparation of lead solutions using lead nitrates

We have prepared three solutions with different concentrations of lead, 50, 100 and 350 µg/l, compared to the world reference, the concentration of 50µg/L is the admissible dose in water (Durfor et Baker, 1964).

Contamination essays of two species by lead solution

Once the solutions are prepared, we will proceed to the contamination of the samples in order to test the power of the lichens and the mosses to accumulate the lead.

Analytical techniques

pH measurement

Using a pH meter, we followed the temporal evolution (each week) of the pH of all samples in solutions treated at different concentrations

Chlorophyll dosage

In order to evaluate the effect of the pollution on the photosynthesis and especially on the chlorophyll of lichen talli and mosses, we opted for the dosage of chlorophyll a and b using the method proposed by (Rao and Leblanc, 1965).

Proline dosage

In order to evaluate the effect of the pollution on the proline content in lichens and mosses the calculation of the proline content is determined following the formula proposed by (Mon neveux and Nemmar, 1986).

Lead dosage

The technique of spectrophotometry of atomic absorption (S. A. A) is the most used for the dosage of heavy metals, after mineralization lead measurements were done with solutions of 20 ml of nitric acid of 2%.

Before proceeding lead dosage in samples, first, a calibration curve must be established from the solutions of known concentrations of lead.

The results are directly read on the device if it is preset according to the manufacturer's indications or the calibration curve in lead

The device used is a spectrophotometer (PERKIN- AIMER model 400)

RESULTS

Variations of pH related to the contamination test of the two species

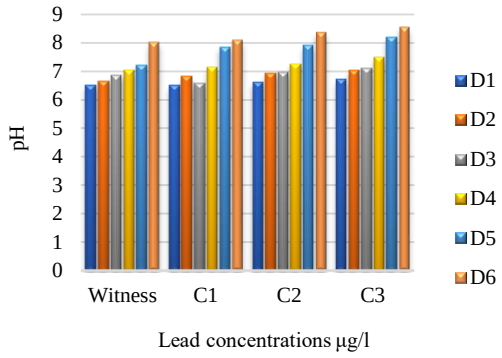

Figure 2.Flucturation of the pH solutions In time for *Xanthoria Parietina.*

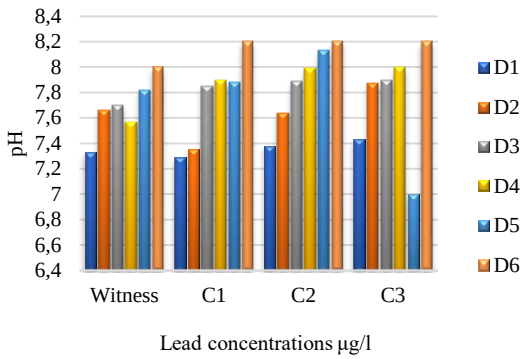

Figure 3. Flucturation of the pH solutions in time dor *Hylocomium splendens.*

For both species whatever the lead concentration, the pH of the solutions has increased, due to the absorption of some elements resulting from the dissolution of lead nitrates which implies the dissociation of its constituent ions.

Variation of Proline content

Date of mesurement	D1 (day of First Collection) S (collected from its natural habitat)	D2 (day of second collection		
		T	C1	C2
Proline content in *lichens*	0,0004	0,0001	0,0005	0,0007

Figure 4. Variation in Proline content in *Xanthoria parietina* and *Hylocomium splendens.*

The proline content of the sample (S) was very high in both species, tended to stabilize for the concentration C1, and increased respectively With C2 and C3 indicating acute stress during the sudden disturbance of plan.

Variation of chlorophyll a, b and a+b content

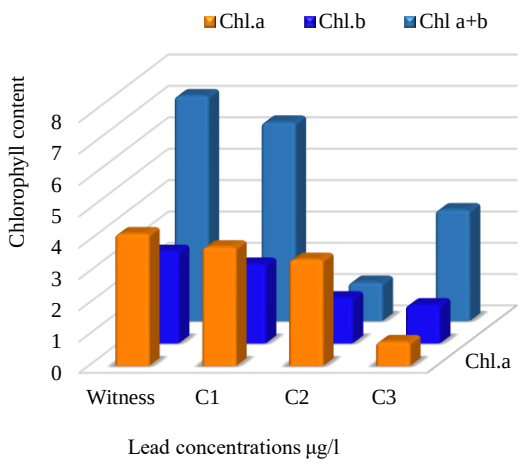

Figure 5. Variation in chlorophyll content in *Xanthoria parientina*

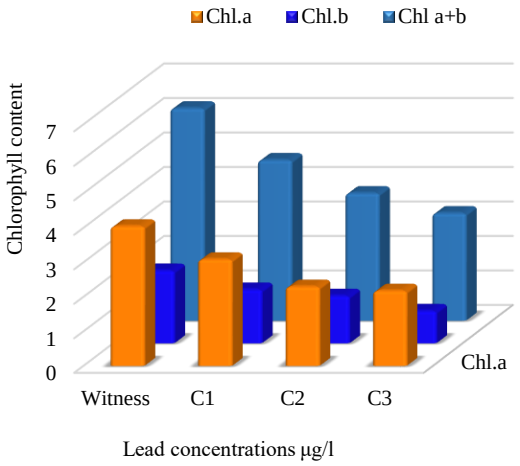

Figure 6. Variation in chlorophyll content in *Hylocomium splendens*

In a chlorophylien plant as we can see in the witness, the content of Chl a is superior than Chl b, but chlorophyll a, b, and a+b is conversely proportional to accumulated lead doses both in lichens and mosses, and decreases as a result of the reduction in photosynthetic intensity.

The content of lead related to the contamination test

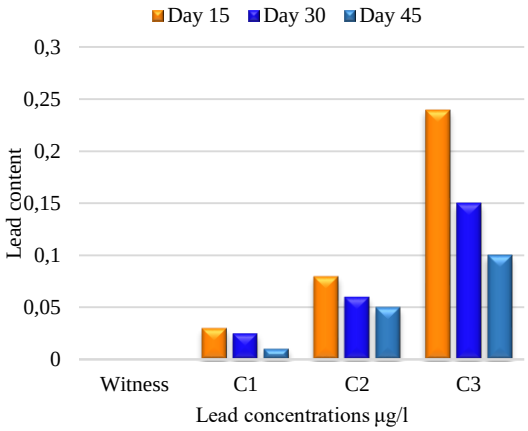

Figure 7. Temporal variation of the lead content of sollution where *Xanthoria parietina* has stayed.

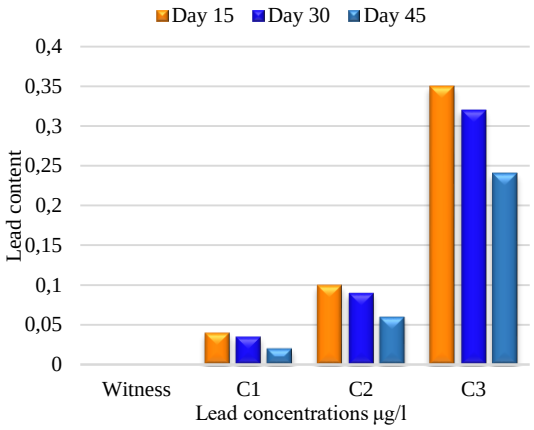

Figure 8. Temporal variation of the lead content of sollution where *Hylocomium splendens* has stayed.

For both species, the lead content in the three solutions is reduced depending on the exposure time with lower content in solutions where lichen has stayed.

Solutions at different concentrations	Accumulated lead Mass in lichens	Accumulated lead Mass in mosses
Witness	**0,03**	**0,01**
C1	**1,6**	**1,2**
C2	**2**	**1,6**
C3	**10**	**3,6**

Figure 9. Content of accumulated lead μg/g by the thallus of the two species.

The lead levels increased in the thallus of the two plants regardless of the concentrations of lead in the solutions but with a higher content in the lichen thallus. It appears globally for both species that their ability to accumulate lead in time is important.

DISCUSSION

The deterioration of the air quality and the evolution of the technologies have aroused an awareness of the public authorities and the citizen. The development of sensors capable of quantifying certain types of pollutants has contributed positively to the improvement of the quality of our environment, however, the use of indirect methods such as bio-indication proved to be very useful quickly. By analyzing the results the set of values clearly indicates that both lichens and mosses accumulate and concentrate lead in their tissues.

Both species have proven to be very good bio hyperaccumulator of lead which makes them excellent bio accumulators for the estimation of pollution especially by metals (case of lead). Nevertheless the lichen species *Xanthoria parietina* has a higher accumulator power than that of *Hylocomium splendens*. The variation in concentrations remains effectively depending on the nature of the species and the time of exposure to pollution.

As regards to variations in measured physiological parameters, chlorophyll content, proline, they can testify to the effect of lead on the two species studied, the chlorophyll content tends to decrease due to the disturbance of the photosynthetic process as well as the degradation of the fresh material, while proline content tends to increase with exposure time due to undergoes stress.

CONCLUSIONS

The deterioration of air quality and the evolution of technology have aroused an awareness of the public authorities and of the citizen. However, the use of indirect methods such as bio-indication were quickly very useful in analyzing the results, the set of values clearly indicates that both lichens and mosses accumulate and concentrate the lead in the tissues, which in its turn is responsible of many physiological disturbances.

It is obvious to mention that the two studied species are proven good bio accumulators of lead which makes them excellent bio-indicators for the estimation of air pollution especially by metals, nevertheless the lichen species *Xanthoria parietna* presents a higher accumulator power than *Hylocomium splendens*, that returns to the symbiosis that makes lichens a complex

and fantastic biological material to study, whose operating mechanisms, still little known compared to the superior plants and offer the possibility of numerous research.

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INHERITANCE OF THE DURATION OF VEGETATIVE GROWTH (DURATION BETWEEN THE INDIVIDUAL PHENOPHASES) IN SUNFLOWER HYBRID COMBINATIONS UNDER THE CONDITIONS OF NORTH-EAST BULGARIA

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ABSTRACT

The investigation was carried out during 2012 – 2016 at Dobrudzha Agricultural Institute – General Toshevo (DAI). Fifteen hybrid combinations were investigated. The heritability of the vegetative growth duration in hybrid combinations was studied in comparison to the parental lines. The periods between the different phenophases in the hybrids and the parental lines were investigated. The investigation showed that hybrids 217A x 87R (107 days) and 217A x 85R (110 days) were early. The best parent and mid-parent heterosis of the vegetative growth duration was studied in the above hybrid combinations. The duration of the vegetative growth in sterile lines was 115 days, while this duration in the fertility restorer lines was 110 days.

Keywords: *Sunflower, Heterosis, Hybrid*

INTRODUCTION

Heterosis breeding is at the basis of the modern economic production of maize grain in Bulgaria. The study on the expressions of heterosis and the genetic control over the inheritance of the traits related to yield from maize are important in the scientific approach to practical heterosis breeding. Each instance of breeding work begins with investigation on the behavior of the plants from different agricultural crops under field conditions.

The phenological observations are carried out during the entire vegetative growth of the plants (from planting to maturity and harvesting of the crop). The separate stages of the development of the plants and the periods (number of days) between these stages are observed. All changes occurring in the plants in the process of their growth and development are noted.

Simultaneously, their reaction to the changes in the environment is taken into consideration (high and low temperatures, high amounts of rainfalls, drought, attacks of pests and diseases), as well as their effect on the plants' development by phenophase. Heterosis is the main genetic factor determining the high productivity of hybrid sunflower (Genchev, 1973; Spreg, 1987).

A peculiarity of the quantitative parameters is their permanent variation due to two factors: the large number of genes, which determine this variation, and the influence of the environment on them (Genchev et al., 1975). The intensive competition in the domestic and the world markets imposes the necessity of constantly developing new sunflower hybrids with higher genetic potential for seed yield from unit area, wider range of disease resistance and higher adaptability than the one currently existing in the hybrids (Skoric et al., 2004). The heterosis effect in the first generation (F_1) is different for the individual parameters (Hladni et al., 2007; Valkova, 2013).

After testing 120 hybrid combinations, Goncharov (2008) came to the conclusion that the greater duration of the vegetative growth does not increase the seed yield. In the different groups formed according to their duration of vegetative growth, the seed yield was determined

by the time from emergence to flowering. Oil content was determined by the duration of the period from flowering to technical maturity.

Andrei et al. (1998) reported that the genotypes with greater duration of flowering were characterized by greater plant height, head diameter and seed set. During the flowering stage, the greater part of the structural elements of yield were formed (Leon et al., 2000).

MATERIAL AND METHODS

The field experiment was carried out in the trial field of DAI during 2013 – 2016 according to a conventional technology for growing of sunflower (Georgiev et al., 1997). Fifteen hybrid combinations were tested, which were obtained by crossing three lines with cytoplasmic male sterility to five fertility restorer lines. The experiment for testing of the hybrid combinations was designed according to the Latin square method in three replications. The plot size was 7.35 m^2. The standards used were the Bulgarian hybrids San Luka and Veleka, as well as one of the most productive and well established on the market foreign hybrid PR64F50. The data from the investigated parameter (duration of vegetative growth) were subjected to genetic analysis to determine the mid-parent and best parent heterosis of the studied hybrid combinations.

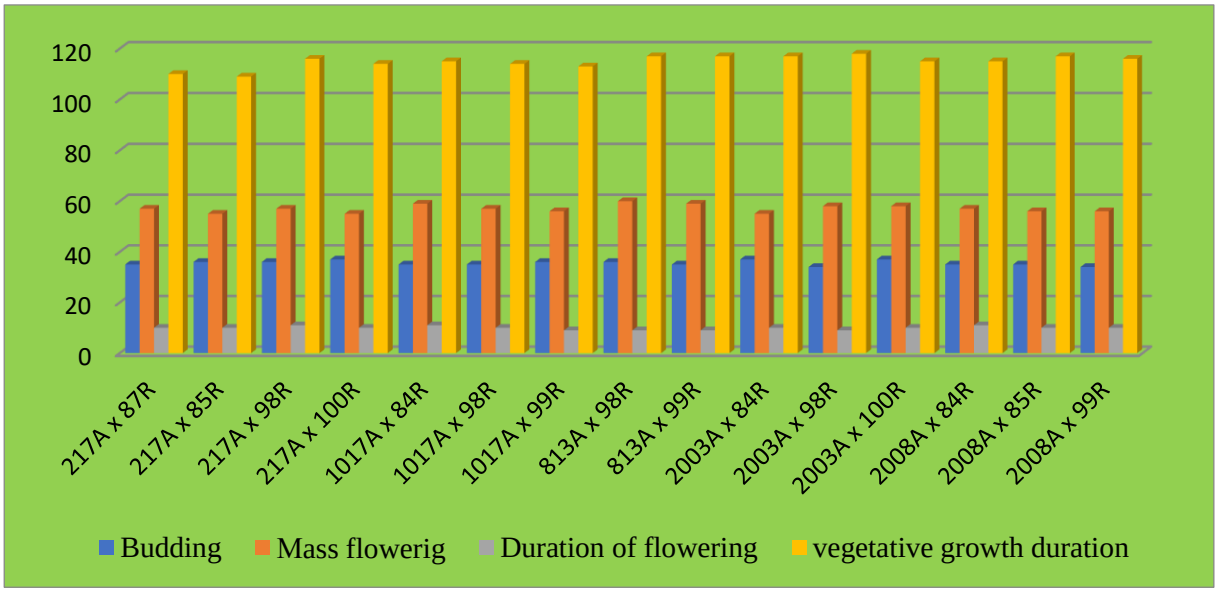

Figure 1. Duration (number of days) of the separate phenophases in hybrid combinations (budding, mass flowering, duration of flowering, vegetative growth duration)

Figure 1 presents the separate phenophases: budding (number of days), mass flowering (number of days), duration of flowering (number of days) and duration of the vegetative growth (number of days) of the hybrid combinations included in the study. The duration of budding of the investigated hybrids was longer (37 days) in comparison to the parental lines (33 days). The time from emergence to flowering (number of days) was shorter (averagely 55 days) in contrast to the parental forms (averagely 61 days). The period from the beginning till the end of the flowering in all hybrid combinations varied from 8 to 12 days, and this variation was not within a wide range over the years of study. Regarding the duration of the vegetative growth, some hybrid combinations, such as 217A x 84R (107 days) and 217A x 85R (109 days), were earlier than the parental lines (217A - 112 days, 84R – 113 days, 85R – 114 days). In other hybrid combinations, 2003A x 84R and 2003A x 98R (117 days), the duration of the vegetative growth over the years of investigation was longer than in the parental lines (2003A - 109 days, 84R - 113 days, 98R - 116 days).

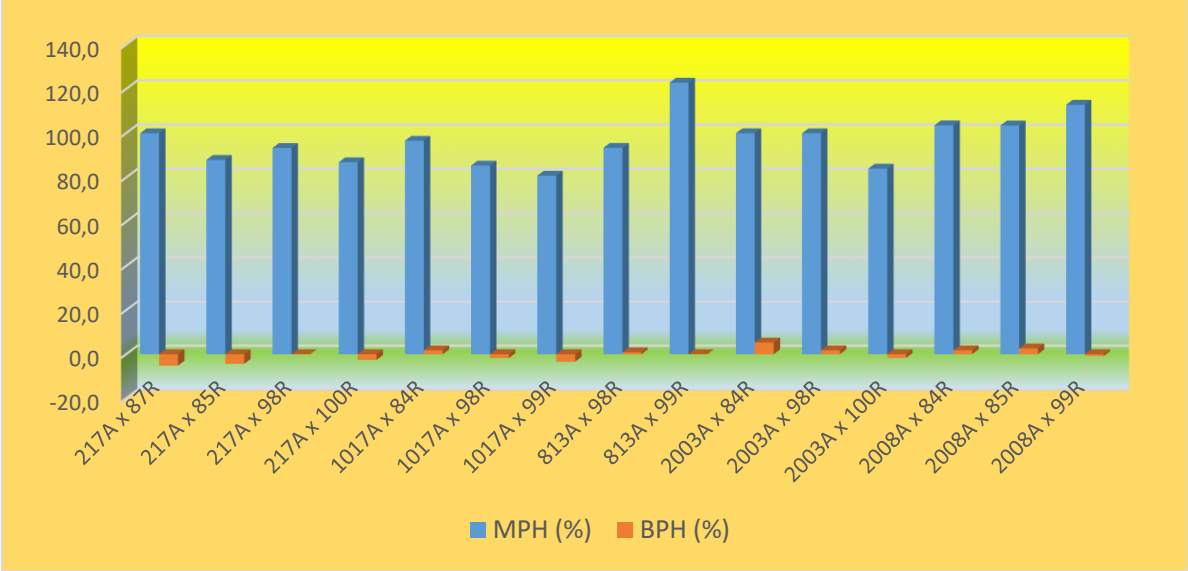

Figure 2. Mid-parent (according to both parents), MPH (%) and best parent (according to the better parent) heterosis, BPH (%), of the parameter duration of vegetative growth in the studied hybrid combinations

Figure 2 shows the data on the investigated hybrid combinations for mid-parent (according to both parents) and best parent (according to the better parent) heterosis of the duration of the vegetative growth. Negative values for best parent heterosis were established in seven hybrid combinations for this parameter. Highest values of the parameter for mid-parent and best parent heterosis were determined in the following combinations: 2003A x 84R, (MPH- 100%), (BPH-5,7%); 2008A x 85R, (MPH- 103.5 %), (BPH-2.6 %); 1017A x 84R, (MPH- 96.6 %), (BPH-1.8 %). The same father line 84R was involved as a component in two of the hybrid combinations.

CONCLUSIONS

Two of the hybrid combinations, 217A x 84R (107 days) and 217A x 85R (109 days) were earlier than the parental lines (217A - 112 days, 84R – 113 days, 85R – 114 days).

The budding stage of the studied hybrids was longer (37 days) in comparison to the parental lines (33 days).

Highest values of this parameter for mid-parent and best parent heterosis were determined in 2003A x 84R, (MPH-100%), (BPH-5.7%); 2008A x 85R, (MPH-103.5 %), (BPH-2.6 %); 1017A x 84R, (MPH- 96.6 %), (BPH-1.8 %).

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INVESTIGATION OF STRUCTURAL AND ELECTRICAL PROPERTIES OF MANGANESE DOPED ZNO VARISTORS PREPARED FROM NANOPOWDERS

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ABSTRACT

Non-ohmic properties of pure and doped zinc oxide varistors are widely used to manufacture good devices. These varistors are generally used in electrical or electronic circuits to protect them from voltage surges. In this study, pure and Mn doped ZnO nanopowders have been synthesized by a soft chemistry method, the sol-gel route. The obtained powders after calcination at 500°C are consolidated and sintered using conventional furnace at 1075°C for 15 min. The obtained pellets are characterized by: X-ray diffraction, MET. The XRD spectra indicate that pure and Mn doped ZnO powders are solid solution, with an average grain size varying between 36.15 nm to 65.92 nm. The grain size decreases with the increase of Mn concentration except for 5 mol % Mn, where there is an unexpected increase. This is confirmed by MET images. The current-voltage J(E) characteristics show that the breakdown electric field increases with increasing Mn concentration ranging from 1595V/cm to 1901.50V/cm except for the Zn-5%Mn-O varistor, where the non linear coefficient α and breakdown electric field are lower. In general the more the grain size decreases the more the varistor effect and the threshold voltage increase.

Keywords: *Particle size, Sol-Gel, Varistors, Zinc oxide nanopowder*

INTRODUCTION

Nanomaterials have received a great interest in last decades due to their many technological applications, very promising in various fields such as sensors, field-emission transistors, ultraviolet photo-detectors, photovoltaic and biomedical system such as DNA sequence detectors Wang et al. (2007). Among these materials, zinc oxide (ZnO) is considered as one of the most important in varistor behavior because of its excellent non-ohmic properties and a high non-linearity coefficient Matsuoka et al. (1969), Subasri et al. (2009). Varistors based on ZnO have been most extensively studied Levinson et al. (1975), Clarke et al. (1999). At the same time researchers have made considerable efforts to develop and investigate new properties in this material for protection against overvoltages in electrical network or a power supply. Thus, in the literature, there are varistors based on tin dioxide (SnO_2) Pianaro et al. (2009), Santos et al. (2006), titanium dioxide (TiO_2), Wu et al. (1991), De Sousa et al. (2006), Kim et al. (1999), strontium titanate ($SrTiO_3$) Rossinelli et al. (1989), barium titanate ($BaTiO_3$) Kutty et al. (1993), Yang (2004), tungsten trioxide (WO_3), Makarov et al. (1994), Dey et al.

(1992) and dioxide of cerium (CeO_2) Nahm et al. (2003). In comparison, varistors based on zinc oxide are more interesting since they present a coefficient of non-linearity α ten times more high. For this reason ZnO became the best choice. To improve the coefficient α and to favor the states of interface, several oxides such as Bi_2O_3, Pr_6O_{11}, SbO_3, CoO or MnO Hashimov et al. (2006) are added to ZnO matrix. ZnO nanopowders are synthesized by various techniques, the most widely used at this time is the sol-gel route, because of its low cost one hand, and the good quality of the varistors made with. The origin of the varistor effect is essentially due to the microstructure in one hand, and to the potential barriers of grain boundaries between two ZnO, in another hand Blatter et al. (1989), Sharma et al. (2008). The grain boundaries are therefore responsible for the non-linearity because each one of them constitutes a barrier to the passage of the current. Therefore, the size of grains in a varistor determines the number of grain boundaries that the current will encounter on its way and therefore the threshold voltage of the varistor.

The purpose of this study was therefore the investigation of the effect of manganese on the structural, optical and electrical properties of ZnO varistors.

Experimental Procedure

Pure and Mn-doped ZnO nanopowders have been synthesized by the soft chemistry sol-gel technique. Zinc acetate dehydrate ($C_4H_6O_4Zn.2H_2O$) (purity>99%, biochem-Chempharma) is used as starting material and an alcoxide: citric acid ($C_6H_8O_7.H_2O$) monohydrate is used to keep particles in suspension, monoethanolamine MEA (C_2H_7NO) and ethylene glycol are used as a stabilizer and solvent, respectively. The dopant source of manganese used is manganese chloride ($MnCl_3$). Then, the obtained solution is stirred at 130°C for 2h to obtain a homogeneous and transparent solution. Finally and after 24h the solution was calcined at 500°C for 4h in a furnace. A schematic representation of the synthesis is shown by the following sequences (Fig.1).

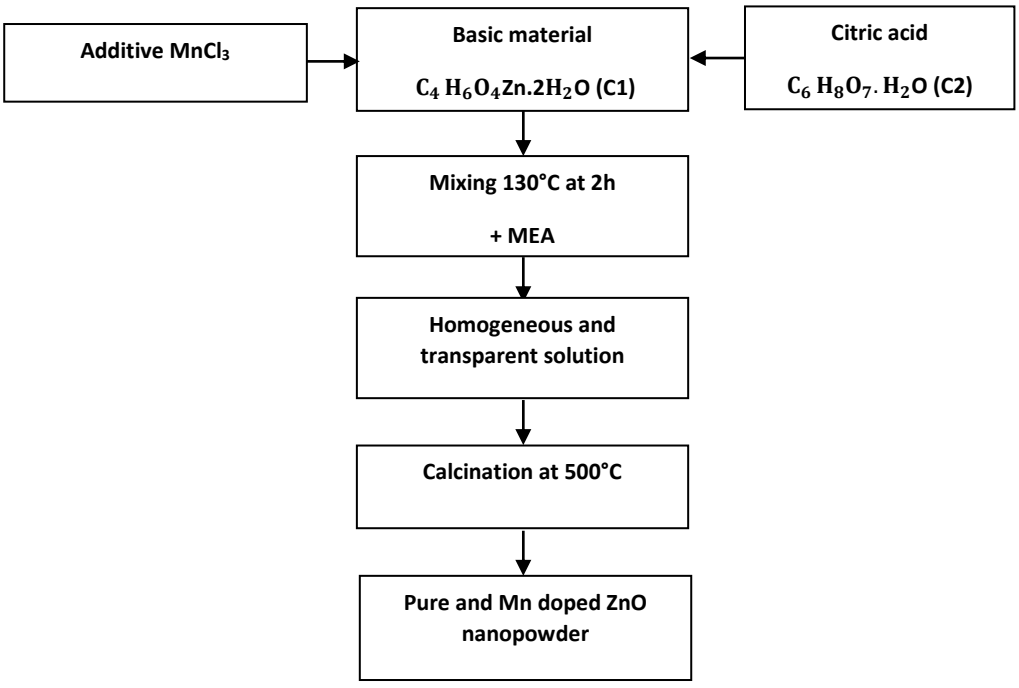

Figure 1. Synthesis of ZnO doped Mn by sol-gel route

The obtained powders are characterized by means of several techniques: XRD, using the Cukα (kα = 0.154056 nm) radiation of a BRRUKER AXS, D8 advence X-ray diffractometer, to identify the structure and calculate the grain size, TEM, using an X-Max model transmission electron microscope to identify the morphology and estimate the crystallite size, electrical characteristics J(E), using a high voltage measure unit (KEITHLEY model 237) to calculate the breakdown electric field and non linear coefficient α. In order to obtain dense varistors with high performance, conventional sintering study was performed. To do that, the obtained powders were pressed into discs of 11mm in diameter and 1.8mm in thickness at a pressure of 2 MPa. Then the pellets were sintered at 1750°C for 15 min. The size of the final samples was about 10 mm in diameter and 1.6 mm in thickness. Gold contact was deposited on both faces of the pellets of Zn%Mn-O to have ohmic contacts, which are realized by heating at 500 ◦C for 10 min to remove the organic functions.

RESULTS AND DISCUSSION

The XRD patterns of Zn1-xMnxO (0≤x≤7) powders synthesized by sol-gel method are shown in Figure 2. The spectra exhibit peaks of the würtzite structure. It is also shown that two peaks were detected at 29.39° and 33.05° only in Zn5%Mn-O corresponding to Mn_3O_4 phase as identified by Sharma et al. (2008), which reported the presence of secondary phases: $ZnMn_2O_3$ or Mn3O4 in the Mn doped ZnO.

The average grain sizes were calculated from X-ray line broadening using Scherrer formula Liu et al. (2007).

$$D = \frac{0.9\lambda}{\Delta\theta\cos\theta}$$

Where D is the grain size, λ the X-ray wave length (λ=1.5418A°), Δθ the full width at half-maximum (FWHM) and θ the Bragg angle.

Lattice parameters a and c of the samples were calculated using the following equation:

$$\frac{1}{d} = \frac{4}{3}\left[\frac{h^2 + hk + k^2}{a^2}\right] + \frac{l^2}{c^2}$$

Where a and c are the lattice parameters, d is the interplanar distance and (hkl) are the miller indices.

As it is shown in Figure 3, the grain size of Mn doped ZnO nanopowders decreases by increasing Mn concentration and vary from 65.92 nm to 36.15 nm, except for 5% mol Mn, where there is an unexpected increase, the grain size become higher (D ≈ 66 nm) because of the appearance of the new phase Mn_3O_4. However the powders keep the same structure. The values of lattice parameters are listed in Table 1.

The diffraction peaks of the ZnO matrix are slightly shifted towards the small angles after introduction of Mn atoms. It indicates that Mn^{2+} ions go to Zn^{2+} sites. The ionic radius of Zn^{2+} being 0.60 A°, and the one of Mn^{2+} is 0.66 A°.

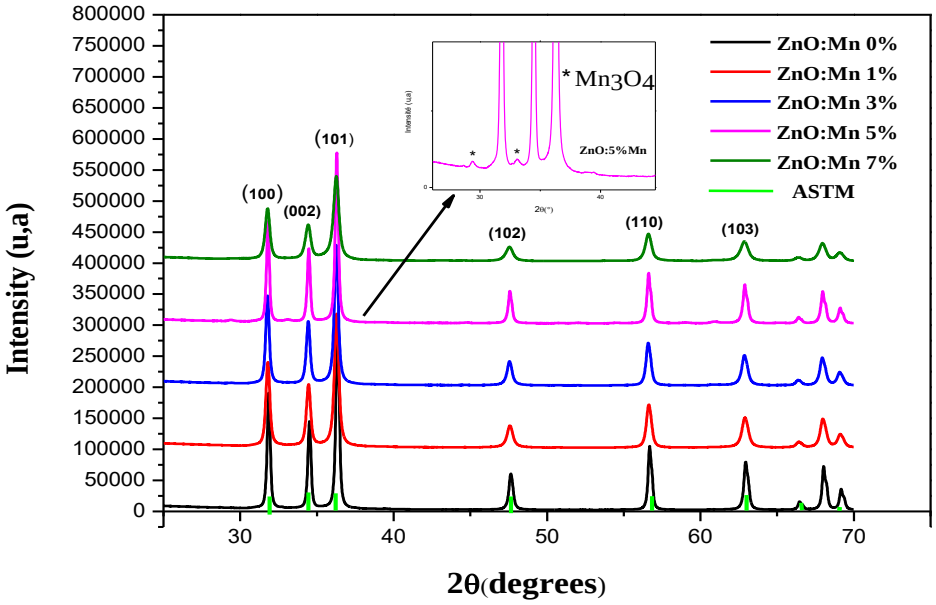

Figure 2. XRD patterns of pure and Mn doped ZnO nanopowders synthesized by sol-gel route

Table 1. The lattice parameters, crystalline size and particle size of Mn doped ZnO samples synthesized by sol-gel route

Zn% MnO	a=b(A°)	c(A°)	c/a	D (XRD) (nm)	D (TEM) (nm)
0% mol Mn	3.2425	5.2012	1,6037	65.92	59.86
1% mol Mn	3.2432	5.2064	1.6053	52.51	55.39
3% mol Mn	3.2565	5.2025	1.6000	47.47	42.13
5% mol Mn	3.2458	5.1953	1.5975	66.97	65.12
7% mol Mn	3.246	5.2021	1.6022	36.15	39.40

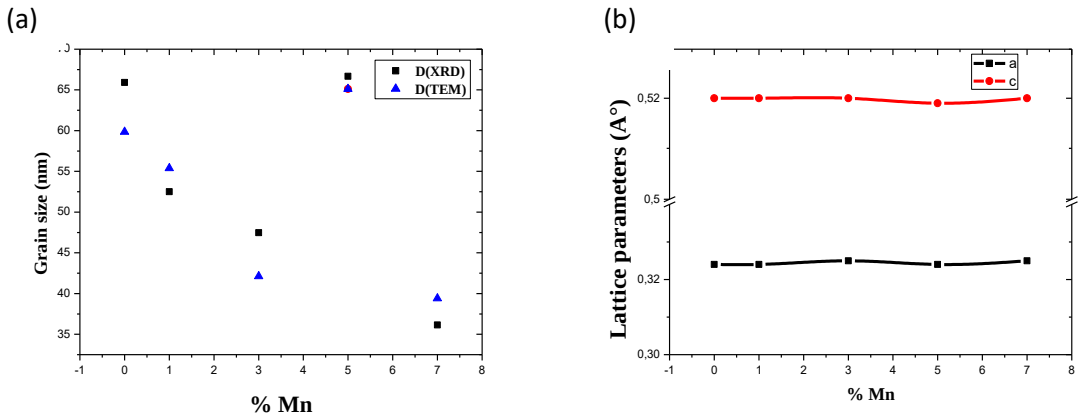

Figure 3. Variation of the (a) crystalline size (XRD, MET) and (b) hexagonal lattice parameters (a and c) with Mn concentration for Zn1−xMnxO powder samples.

In Figure 5, we present TEM images of the pure ZnO and manganese-doped ZnO nanopowders. The images show that the crystallites tend to agglomerate and form aggregating sphere. Basically, nanoparticles have a natural trend to agglomerate for two main reasons. First, the agglomeration is a more stable configuration from an energetic point of view. Then, nanoparticles tend to agglomerate to allow crystallite growth. The results presented in figure 3(a) indicate that the mean crystallite sizes measured from the TEM images is varying in the same direction than those obtained by XRD. The crystallite size histograms of pure ZnO, 1 mol%, 3 mol%, l.5 mol% and 7 mol% manganese-doped nanoparticles are shown in figure 4. For the doped samples, the crystallite size is between 10 and 80 nm. The manganese doping causes a reduction in the number of crystallites belonging to the size range from 39.4 to 79.87 nm.However, the sample doped with 5 mol% Mn contains the Mn_3O_4 phase, whose crystallite size is on average higher than those obtained with the other concentrations. Since it is not possible to distinguish the mechanism of agglomeration of ZnO crystallites from Mn_3O_4 crystallites on a TEM image, we suggest that our measurements involve crystallites of the Mn_3O_4 phase because of the larger size of these crystallites.

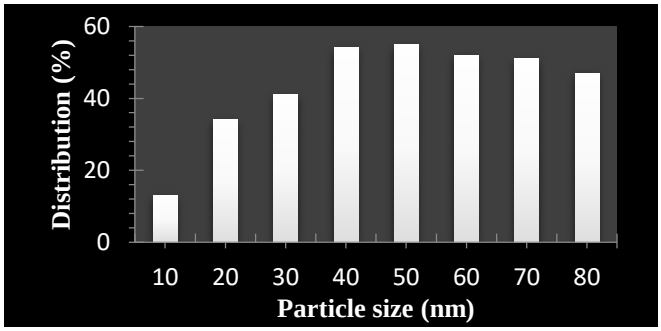

Figure 4. Average particle size histograms of pure ZnO and Mn-doped nanopowders

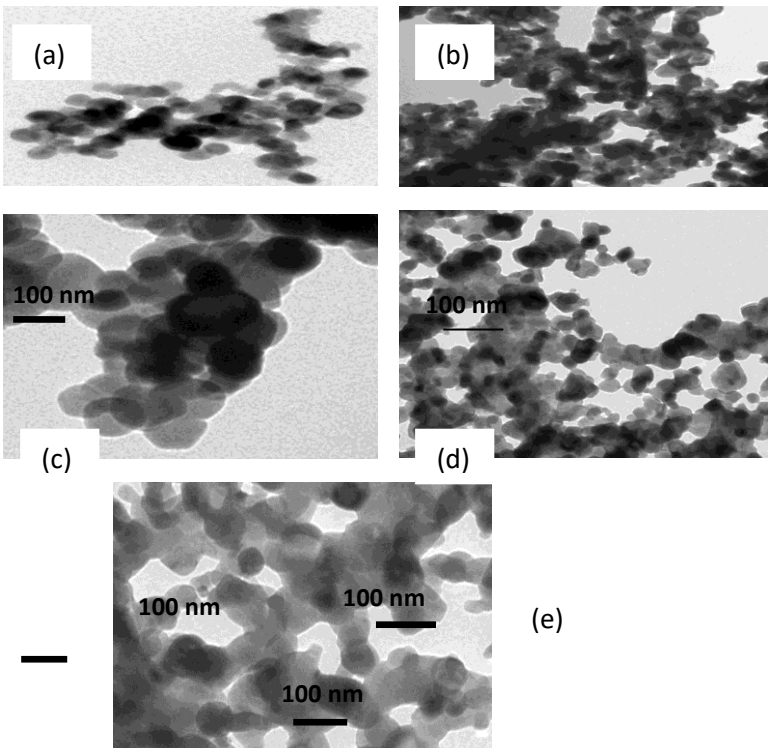

Figure 5. TEM images of pure and Mn doped ZnO nanopowders from two different regions

(a) 0%, (b) 1%, (c) 3%, (d) 5% and (e) 7%

The plotted electric field as a function of current density for different concentrations is given in figure 6. The nonlinear coefficient α was obtained by: α=log (I2/I1)/ (V2/V1) where V1 and I1 as well as V2 and I2 are corresponding values of voltage and current for two points that can be chosen arbitrarily in the non-ohmic region Duran et al. (2003).

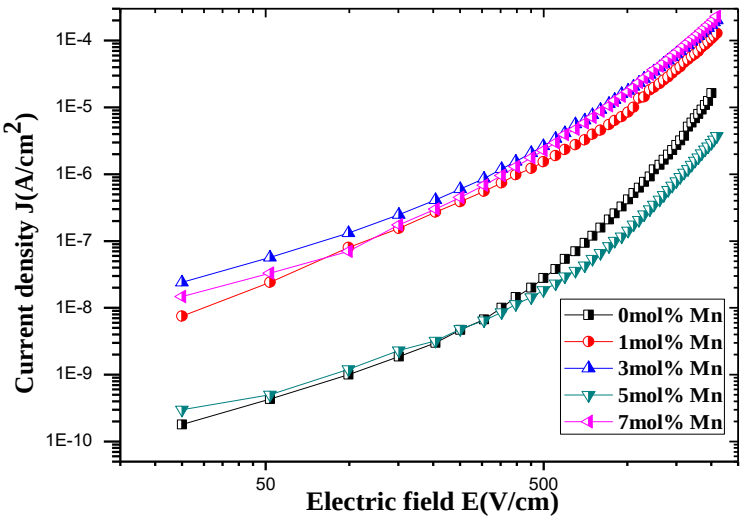

Figure 6. Characteristics of varistors as a function of Mn concentration

Figure 6 shows E(J) curves at room temperature for all samples. We can see clearly two regions: the ohmic region, known as a high resistance region and the non-ohmic one, known as a very low resistance region.

The curves show that the electric field E in the non-linear region increases by increasing Mn concentration, thus E increases with decreasing of grain and particle sizes except for 5 mol% Mn, where E is lower and the grain size higher. However the current density is reduced, particularly for the 5 mol% Mn, because the current is limited to the ohmic resistance. This is in good agreement with XRD and TEM results, and can be explained by the increase of the number of grain boundaries due to the decrease of the grain size average.

Table 2 and figure 7 show that the non-linear coefficient and the breakdown voltage increase by increasing Mn concentration up to 3mol % Mn and remain nearly constant after that. The Zn-5%Mn-O is still out the expect variation. For the coefficient α the results can be explained by the fact that this coefficient is due to solid state reactions and the formation of potential barriers between grains Mirzayi et al. (2013).

The increase of EB with increasing Mn concentration (from 1595V/cm to 1901.50V/cm except for the Zn-5%Mn-O varistor) and by the fact its increase with decreasing of the grain size of starting powders can be explained by the increase of the number of grain boundaries due to the decrease of the grain size average.

Table 2. The varistor effect parameters of pure and Mn doped ZnO samples synthesized by sol-gel route

Sample	Breakdown voltage (V/cm)	Non-linearity coefficient α
0% mol Mn	1595	2.8450
1% mol Mn	1659.88	3.7973
3% mol Mn	1851.32	3.63
5% mol Mn	1773.33	3.40
7% mol Mn	1901.50	13.63

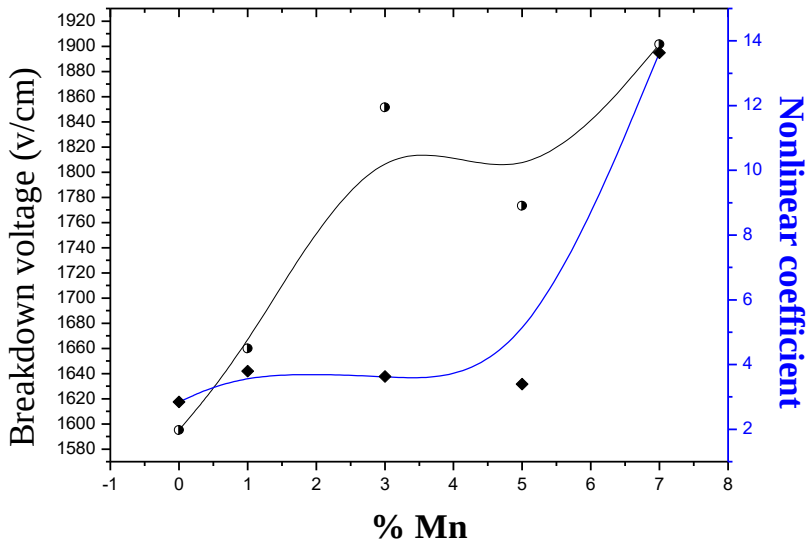

Figure 7. Variation of the nonlinear coefficient and breakdown voltage as a function of Mn concentration.

CONCLUSIONS

Nanopowders of pure and Mn (1–7%) doped ZnO have been synthesized by Sol–gel technique. The obtained nanopowders have been characterized by means of XRD, TEM and I-V to determine, respectively, their structural characteristics, morphological and electrical properties. The all obtained powders exhibit würtzite structure, where the lattice parameters a and c vary in the same direction. These powders are constituted by very small grains, which size decreases as a function of Mn concentration. On the other hand the manganese doping causes a decrease in the particles size and an increase in varistor effect for the 1, 3 and 7 mol% Mn-doped samples; the breakdown electric field ranges between 1595/cm and 1901.50V/cm except and the coefficient of non-linearity (α) between 2.8450 and 13.63.

All samples have a morphology consisting of small nanoparticles, uniformly distributed. From 5 mol% Mn and more, the powders contain a Mn3O4 secondary phase formed at grain boundaries, which is not suitable for a use as varistor. This study shows that 7 mol% Mn doped ZnO varistor has the best electrical properties.

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DEGRADATION OF PENCONAZOLE IN APPLE AND ESTIMATION OF RESIDUE LEVELS USING LC-MS/MS

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Abstract

Penconazole belongs to the class of Triazole, which is one of the largest and the most important pesticide group of fungicides to control powdery mildew, pome fruit scab and other fungal pathogens on fruit and vegetables. The aim of the study was to estimate the trend of residue levels of penconazole in apple fruit after its application. The residues of penconazole were investigated in two apple cultivars Starking and Golden Delicious, which were treated with maximum (0.05%) and minimum (0.025%) levels of recommended doses of penconazole by application of commercial formulation PEN10. The apple fruit samples were collected randomly in the interval of 1, 7 and 19 days after application and were prepared for further analysis. The samples were extracted using QuEChERS method and the clean-up was achieved using the primary secondary amine (PSA) and magnesium sulphate. The qualitative and quantitative analyses of penconazole residues in apple fruit were performed using liquid chromatography coupled with mass spectrometry (LC-MS/MS) technique.

The obtained results showed that the level of penconazole residues in all analyzed samples decreased with the time after application. Thus, the level of penconazole residues vary from 0.173mg/kg to 0.037mg/kg and from 0.306mg/kg to 0.045mg/kg in Golden Delicious with the minimum and maximum applied doses at 1st and 19th day after application, respectively. Also, in the Starking cultivar the level of penconazole residues vary from 0.239mg/kg to 0.01mg/kg and from 0.493mg/kg to 0.045 with the minimum and maximum applied doses at 1st and 19th day after application, respectively. Therefore, the level of penconazole residues on first day after the application were in general, above the Maximum Residues Limit (MRL) of 0.2mg/kg for apple fruit, except the treated samples with the minimum recommended dose in Gold Delicious cultivar. Whereas, the penconazole residues in all analyzed apple fruit samples were below MRL on the 19th day after application, showing that the application of penconazole as fungicide to control fungal pathogens in apple fruit is suitable and guarantees the food quality and safety for the consumers.

Keywords: *Penconazole, Apple, QuEChERS, Pesticide residue, LC-MS/MS technique.*

INTRODUCTION

Most fruits and vegetables are treated with pesticides which are chemicals deliberately used in households and modern agriculture, playing a major role in maintaining high agricultural productivity (Tilman et al., 2002; Iyer and Makris, 2010). Without pesticides commercial fruits production would not be economically viable in many regions of the world (Kostik et al., 2014). Their use may involve risks and hazards for humans, animals and the environment, especially, when placed on the market without having been officially tested and used incorrectly.

In order to ensure food safety for consumers and protect human health, many organizations and countries around the world have established maximum residue limits (MRLs) for pesticides in food commodities. Maximum residue levels of a pesticide residue represent highest concentrations of pesticide residues (expressed in mg/kg), which are legally permitted in or on food or animal feed when the pesticide is used according to authorized agriculture practices (EFSA, 2010; Munawar and Hamid, 2013; Jallow et al., 2017).

Among pesticides, fungicides and insecticides are likely to remain the major class used for crops protection. Because the fungicides are used increasingly in many countries around the world they can lead on high levels of pesticide residues. Thus, their analyses are important to ensure food safety and human health protection. The main objective of current study was to investigate the residue levels of penconazole in apple fruit after its application.

MATERIALS AND METHODS

Experimental field and sampling

The experiment was conducted to Korça region, which is the main market supplier with apple fruits in Albania. In the experiment two types of cultivars were studied Golden Delicious and Starking, as the most affected species by *Venturia Inequalis*. The orchard was divided in four plots as it is shown in Figure 1.

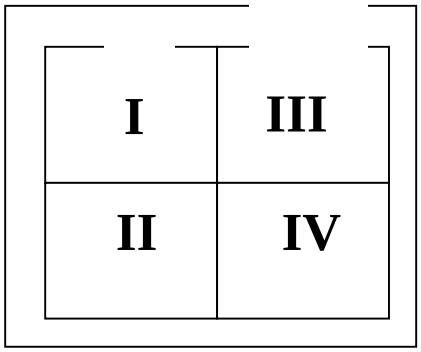

Figure 1. *Schematic drawing of experimental field*

The first and second plot represent Golden Delicious trees which were treated respectively with minimum and maximum recommended doses of pesticides, while the third and forth plot represent Starking treated with minimum and maximum recommended doses as well. The application of pesticides was done through a tractor in which two sprays were mounted.

Sampling

The apple fruit samples were collected randomly in the interval of 1, 7 and 19 days after application in accordance with European Commission Directive 2002/63/EC. The transportation of apples to the laboratory was done through cooling boxes. The temperature of boxes did not exceed 5 to 6^0C.

Extraction and clean-up

The extraction was based on QuEChERS method developed by Anastassiades et al. (2003). Thus, after homogenization of 1 kg apples with a domestic blender, 10 g of homogenized samples were weighted into a 50mL polypropylene centrifuge tube followed by addition of 10mL acetonitile and 100µL internal standard (carbofurane d-3). After they were mixed 4g anhydrous sulfate, 1g sodium chloride, 1g Na3-citrate dihydrate, 0.5 g Na_2H-citrat sesquihydrate was added and the tube was shaken again 1 min and then was centrifuged at 3000g for 5min. Then 6mL was transferred in a 15 mL PP tube and was cleaned-up with PSA sorbent and anhydrous sulfate. After it was shaken and centrifuged again, the upper layer was filtered and transferred in vial and kept in refrigerate being ready for LC-MS/MS analysis.

RESULTS AND DISCUSSION

The experiment was conducted during the years 2015 and 2016 in Korça region and results of this study were interpreted in accordance to MRLs requirements set in legal EU regulations. The data presented in Table 1 and Figure 2 showed that the level of penconazole residues in Golden Delicious cultivar in all analyzed samples decreased over time (from 0.306 mg/kg to 0.045mg/kg for cultivars treated with maximum recommended dose and from 0.173mg/kg to 0.037mg/kg for cultivars treated with mimimum recommended dose.

Table 1. Residue levels of penconazole (mg/kg) in Golden Delicious cultivar

Days	**Golden max.**			**Golden min.**		
	Year		**Mean**	Year		**Mean**
	2015	2016		2015	2016	
1	0.289	0.324	**0.306**	0.167	0.180	**0.173**
7	0.065	0.094	**0.080**	0.057	0.060	**0.059**
19	0.050	0.040	**0.045**	0.055	0.019	**0.037**

Thus, the level of penconazole residues on the first day after application which were treated with the maximum recommended doses was above the Maximum Residues Limit (MRL) of 0.2 mg/kg apple fruit, while the samples treated with the minimum recommended doses event on the first day after application showed low levels of penconazole residues, below the MRL (see Figure 2).

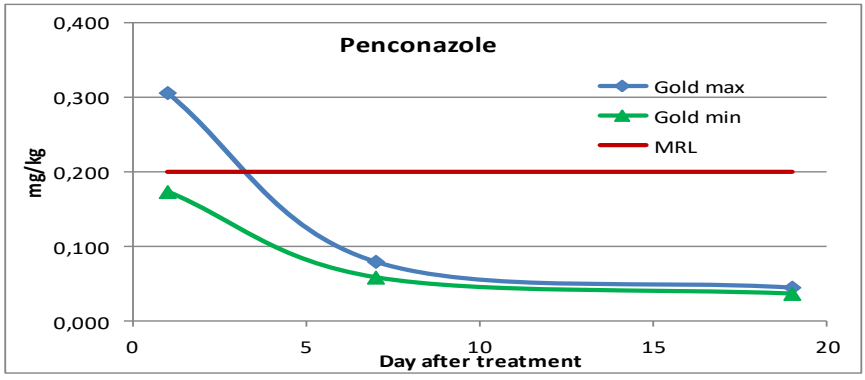

Figure 2. Decrease of penconazole residues level over time in Golden Delicious cultivar

Furthermore, presented data in Table 2 and Figure 3, show that the level of penconazole residues in the Starking cultivar vary from 0.239mg/kg to 0.01mg/kg and from 0.493mg/kg to 0.045 with the minimum and maximum applied doses at 1st and 19th day after application, respectively.

Table 2 Residue levels of penconazole (mg/kg) in Starking cultivar

Days	Starking max.			Starking min.		
	Year		Mean	Year		Mean
	2015	2016		2015	2016	
1	0.480	0.506	**0.493**	0.230	0.249	**0.239**
7	0.210	0.220	**0.215**	0.080	0.072	**0.076**
19	0.050	0.040	**0.045**	0.010	0.012	**0.011**

In contrast to the Golden Delicious cultivars, the treated samples with the minimum recommended doses which showed level of penconazole residues below 0.2 mg/kg (MRL) on first day after application, the samples of Starking cultivars on their first day after application in both cases showed level of penconazole residues above MRL.

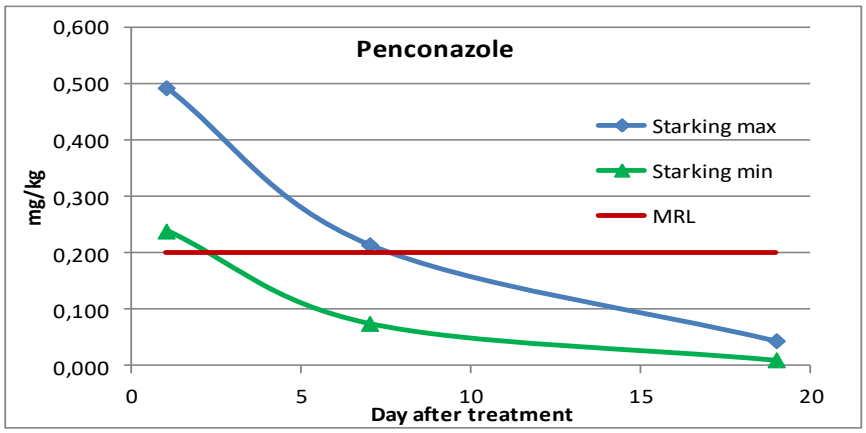

Figure 3. Decrease of Penconazole residues level over time in Starking cultivar

The Figure 2 and 3 showed that the penconazole residues in all analyzed apple fruit samples were below MRL on the 19th day after application.

CONCLUSIONS

The obtained results showed that the level of penconazole residues in all analyzed samples decreased with the time after application. The level of penconazole residues on first day after the application were in general, above the Maximum Residues Limit (MRL) of 0.2mg/kg for apple fruit, except the treated samples with the minimum recommended dose in Gold Delicious cultivar. Whereas, the penconazole residues in all analyzed apple fruit samples were below MRL on the 19th day after application, showing that the application of penconazole as fungicide to control fungal pathogens in apple fruit is suitable and guarantees the food quality and safety for the consumers.

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NEW ACCESSIONS IN THE COMMON WINTER WHEAT WORKING COLLECTION OF DOBRUDZHA AGRICULTURAL INSTITUTE, BULGARIA

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ABSTRACT

The storage and adequate management of the plant genetic resources is a main focus of the breeding programs. The priorities of the activities are determined by many factors, among which are the successful guarantee of sustainable agriculture, the climatic changes and the increasing consummation worldwide. The decrease of genetic variability imposes the necessity to apply new approaches with the aim to enrich it and to develop genotypes with improved morphological characteristics and adaptability. DAI is a main breeding center in Bulgaria and has a large plant gene pool of field crop species. The chamber for long-term storage contains a collection of 3435 accessions of genus *Triticum*. A working collection of Bulgarian and foreign breeding, which consists of 1745 varieties, is grown under filed conditions, as well as lines with specific traits. Sixty six of them were evaluated for main economic indices during three harvest seasons (2015 – 2018). The aim was: 1) characterization of the yield structure and the resistance to abiotic and biotic stress; 2) determining possibilities for hybrid combinations. The experiment was designed in plots of 10 m^2, in two replications. Phenological observations and biometric analysis were done on 25 plants per plot. The methodologies of UPOV and IPGRI were used. The accessions were well differentiated according to: date to heading, plant height, winter resistance, resistance to powdery mildew and leaf rust, yield structure specificity and productivity. Within the period of investigation, the Bulgarian wheat varieties, which realized highest yield, were Rada, Kalina, Dragana and Kristi, and the highest yielding cultivars of European breeding were Basmati, Foxil, Avenue, NS 407 and Andalou.

Keywords: *Wheat, Genetic resources, Stress factors, Yield structure*

INTRODUCTION

During the recent years, the intensive grain production has limited the set of cultivars grown on large areas. There is a permanent tendency toward narrower genetic variability, which presents a major risk under occurrence of stress factors of abiotic and biotic nature. The storage and adequate management of the plant genetic resources is a main focus of the breeding programs. The priorities of the activities are determined by many factors, among which are the successful guarantee of sustainable agriculture, the climatic changes and the increasing consummation worldwide. The decrease of genetic variability imposes the necessity to apply new approaches with the aim to enrich it and to develop genotypes with improved morphological characteristics and adaptability. A total of 102 common wheat varieties and 7 durum wheat cultivars, 13 triticale forms and 6 cultivars of winter barley have been developed at Dobrudzha Agricultural Institute. Over 35 % of the wheat areas in Bulgaria are sown with cultivars, which are the scientific product of our research institute. The rich and diverse varietal list of winter wheat allows the producers to choose the cultivars, which are best for them and to develop their appropriate varietal structure. The cultivars are with confirmed adaptability and possess good balance between productivity potential and stability of its realization under unfavorable growing conditions. Under the increasing market competitiveness, a new vision for the breeding program of the cereal crops is outlined. The strategy for improvement of a

complex of traits has been reconsidered, including the structure of yield. In compliance with the traditions, however, the accent is still on the high efficiency in production and on introduction of products for quality foods.

The aim of the research was to characterize the structure of the yield and the resistance to biotic and abiotic stress of new common winter wheat accessions included in the gene pool of Dobrudzha Agricultural Institute

MATERIAL AND METHODS

Within three growing seasons (2015-2018), 66 new accessions of common winter wheat were evaluated (Table 1). They are from different breeding centers and possess specific phenological and biological traits. They were compared to the cultivars developed at DAI, which were recently introduced in practice. The experiment was designed in two replications, the harvest plot area being 10 m^2. The sowing norm was 550 germinating seeds per m^2. The previous crop was grain peas. At the end of February, fertilization was done with 0.06 $t.ha^{-1}$ active matter of nitrogen. The biometrical measurements were in accordance with the methodology of UPOV (2008). The following indices were analyzed: days to heading (DH); number of days (from 1st January); plant height (PH), cm; productive tillers of 1 m^2 (NPT), number; grains per spike (NGS), number; thousand kernel weight (W_{1000}), g; test weight (TW), kg; lodging resistance (L), score (1 – susceptible, 3 – intermediate, 9 – resistant); grain yield (YG) $t.ha^{-1}$. The laboratory frost resistance was evaluated by the method of Tsenov and Petrova (1984). The resistance to the cause agents of powdery mildew *Blumeria graminis* f. sp. *hordei*) and brown rust (*Puccinia hordei* Otth.) were studied in an artificial infection field. The attacking rate of powdery mildew was read according to the scale of на Saari & Prescott (1975), and the type of infection – according to Mains et Diets (1930). The scale of Cobb, modified by Peterson et al., (1948) was used for leaf rust. The experimental data were processes with the help of Microsoft Excel^{xp} и STATISTICA, release 7.0 (StatSoft Inc., 2004).

Table 1. Core collection of new common winter wheat accessions

Origin	Accessions
Austria (AT)	1. Albertus; 2. Antonius; 3. Balitus; 4. Fabius; 5. Fidelius; 6. Papageno; 7. Plinius; 8. Rainer; 9. Ubicus and 10. Urbanus.
Bulgaria (BG)	11. **Aglika (St)**; 12. Bozhana; 13. Dragana; 14. Fani; 15. Kalina; 16. Korona; 17. Kristi; 18. Kristalina; 19. Lazarka; 20. Nikodim; 21. Pchelina; 22. **Pryaspa (St)**; 23. Rada; 24. **Todora (St)** and 25. Zhana.
Croatia (HR)	26. Alka; 27. Andelka; 28. Katarina; 29. Kraljica; 30. Lucija; 31. Renata and 32. Srpanjka.
France (F)	33. Anapurna; 34. Andalou; 35. Andino; 36. Apache; 37. Avenue; 38. Basmati; 39. Exotic; 40. Foxil; 41. Solehio and 42. Toskani.
Germany (DE)	43. Athlon; 44. Attraktion; 45. Balaton; 46. Bitop; 47. Eduard; 48. Etana; 49. Felix; 50. Genius; 51. Joker; 52. Joseff; 53. Katarina; 54. Laurenzio; 55. Lukullus; 56. Midas; 57. Mulan; 58. Peppino; 59. Philipp; 60. Tobias and 61. Vulkanus.
Serbia (RS)	62. Ilina; 63. NS 40S; 64. Pannonia; 65. Renesansa and 66. Simonida.

North-east Bulgaria, where Dobrudzha Agricultural Institute is situated, is characterized with soil and climatic conditions favorable for the development of cereals. The low temperatures without snow cover during the winter months are critical. The absolute minimum temperature for this region is – 29.4° C, and the absolute maximum +41.1° C. Due to the frequent flows of cooling ground-level air currents coming from the sea, the spring here is late with 10-15 days. The summer is cool, and the autumn is long, with gradual cooling of the weather. There are two distinct periods of drought, in March – April and July – August. The mean annual sum of

rainfalls is 510 mm. The leached chernozem soils are predominant in the region. Due to the heavy composition of soil, the values of the hydrological indices are comparatively high.

RESULTS

The years of study allowed very good differentiation of the investigated accessions with regard to their development and peculiarities of yield formation (Figure 1). The moisture reserves and the high mean daily air temperatures during the autumn of 2015 – 2016 were favorable for the normal emergence of the crops and their thick stand. The combination of the meteorological conditions ensured good hardening at a suitable stage of the plants development. The tillering was intensive and in some genotypes the crops became too dense. Due to the high temperatures during the spring months, the plants entered the booting stage comparatively early, and the photoperiod was not a significant limiting factor. The plants accumulated sufficient biomass, a prerequisite for formation of high productivity. Favorable conditions were registered for mass occurrence of powdery mildew, brown and yellow rust. The intensive rainfalls at the end of the vegetative growth of the plants were the reason for lodging to various degrees and for the deterioration of the physical properties of grain. The late sowing date (November) and the low mean daily temperatures in December and January were determining for the phenological development during the next vegetative growth period (2016 – 2017). The emergence started during the last decade of February. Subsequently, there were favorable conditions for tillering and the crops reached normal density.

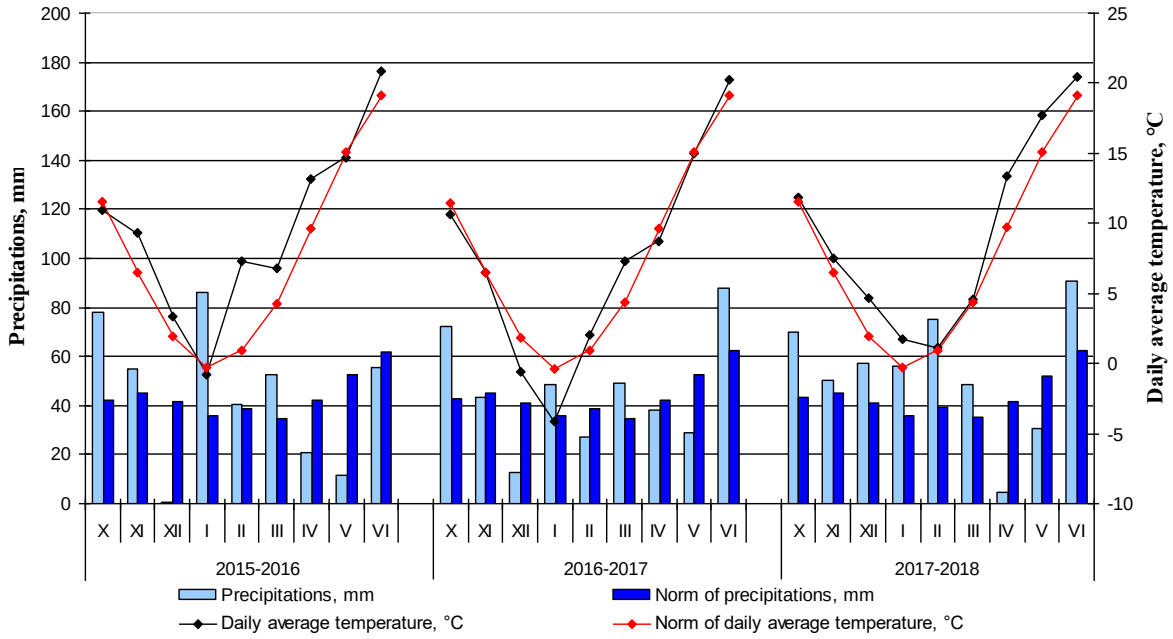

Figure 1. Agro-meteorological characteristics, 2015-2018

In spite of the elongated photoperiod, the booting stage was late. The main reason were the low mean daily and radiation temperatures. Heading started later than usual. During the next stages of vegetative growth, the amount of rainfalls and their even distribution favored the formation of good physical properties of grain. A strong drought during the spring months characterized harvest season 2017 – 2018. The entire booting period, when the reproductive organs were formed, occurred under comparatively unfavorable conditions. There were favorable conditions for the occurrence of brown rust. The high infection rate in the susceptible genotypes caused fast decay of leaf mass and early entering in the economic maturity stage.

The analysis of the variances revealed different percents of the genotype and the conditions of the environment for formation of the studied traits (Table 2). Greatest differences between the accessions were found with regard to plant height, number of productive tillers, and duration of the time to heading. Within the investigated period, the effect of the conditions on the test weight and the rate of lodging was significant.

The correct interpretation of the results requires noting that only in one of the years there were conditions for lodging and the differentiation was not good. The portion of the genotype and the conditions was similar for the formation of the number of grains and their weight. This indicates that the studied accessions had specific response to the conditions during the vegetative growth of the plants and to the combination of stress factors.

Table 2. Relative portion of genotype and environment and interaction in the total variation of investigated traits

Traits Sum of squares, %	DH	PH	NPT	NGS	W_{1000}	TW	LR	YG
Genotype (A)	52.4	69.3	56.6	68.7	39.5	27.8	24.3	38.2
Year (B)	26.4	19.4	22.8	15.9	41.4	56.4	58.2	30.5
A x B	7.3	5.5	10.7	10.2	8.7	6.3	9.8	24.7
Residual	13.9	5.8	9.9	5.2	10.4	9.5	7.7	6.6

The results present the main tendencies in the realization of the genetic potential of the accessions from the working collection. At the contemporary stage of development of the breeding programs, within the open European market and under intensive exchange of materials, it is difficult to make a categorical differentiation by center of origin. The established differences are the result primarily from the pressure in the process of breeding. It is determined by the priorities of the respective programs and by the limiting factors of the concerned ecological and geographic regions. The cultivars from Austria and Germany were with the longest time to heading, followed by the ones from Croatia and France (Table 3). The variation of the latter group was within a wide range. Cultivar Avenue headed earliest, and Foxil, Toskani and Basmati – latest. Considerable variability according to this trait was found between the accessions from Bulgaria and Serbia. The field observations showed they had similar phenological development. Among the new Bulgarian common wheat cultivars included in the study, cultivar Kalina was with the earliest date to heading, while cultivar Dragana was with the latest. The variability with regard to plant height was considerable. The accessions from Croatia were with the shortest stem. Even their maximum value was much below the mean height of the rest of the groups. The accessions involved in the investigation were from the same, and from the biggest breeding centers in that country. Probably, the shortening of the stem and the use of a concrete *Rht* gene is a specificity of the program. The accessions from France had low values of this trait. The cultivars from Austria were with the highest stem. Nevertheless, during 2015 – 2016, the registered percent of lodging did not exceed 40 %. The variation was highest in the group of the Bulgarian cultivars. The predominant part was within the range 86 – 95 cm. Cultivars Korona, Kalina, Zhana, Fani and Dragana were with the shortest stem, while Bozhana was with the highest.

In comparing the structural components of the yield, considerable variability was observed; it was highest with regard to the number of productive tillers and the number of grains in spike. The Bulgarian breeding was with the lowest potential for tillering. It was preceded by the cultivars of Serbia and Austria. Higher variation was established in them. The accessions from

Croatia and France were with highest mean number of productive tillers. Cultivars Basmati, Toskani, Andino, Lucija and Renata are worth mentioning. A peculiarity of these two groups was the lower number of grains in the spike and the lower weight of grain. Exceptions were cultivars Basmati, Exotic, Foxil, Andalou and Solehio.

The Bulgarian cultivars were with highest mean values for number of grains in spike and absolute weight, which compensated for their lower number of productive tillers. Similar was the tendency in the group of the Serbian accessions. The accessions from Germany were with a balanced combination of the traits, and to a lesser degree – the ones of Austria, which were characterized by lower absolute grain weight.

Test weight was the trait with the lowest variation within the working collection. Lower values were registered during harvest seasons 2015 – 2016 and 2017 – 2018. The reasons for this are various. During the first season, lodging of the crops deteriorated grain filling, and during the second – the high rate of leaf rust occurrence caused fast defoliation of the susceptible accessions.

The determined mean productivity between the groups of different origin was 6.45 and 7.60 $t.ha^{-1}$. In comparing the range of variation, it was observed that in each of them there were accessions with very high potential realized under the conditions of this region. Lowest mean yield was obtained from the Austrian and Croation accessions. This was not incidental since the predominant part of them were from the group of the quality wheats and a comparison is inappropriate. Similar was the reason for the variation in the German accessions. Among the French cultivars, there was the highest number of medium wheat types and medium wheat types with increased strength. In this group, the highest productivity of 9.24 $t.ha^{-1}$ was determined. Within the period of investigation, the Bulgarian wheat varieties, which realized highest yield, were Rada, Kalina, Dragana and Kristi, and the highest yielding cultivars of European breeding were Basmati, Foxil, Avenue, NS 407 and Andalou.

Table 3. Mean, minimum and maximum values for characters in wheat accessions

Origin	Traits	DH	PH	NPT	NGS	W_{1000}	TW	YG
AT	mean	132	101.5	638.8	42.7	38.2	77.8	6.47
	min-max	130-134	90-120	580-788	37-44	32-44	76-80	5.49-7.82
BG	mean	127	94.0	563.8	45.5	46.6	80.4	7.74
	min-max	123-130	82-108	552-676	38-48	43-50	78-83	6.33-8.68
HR	mean	125	69.0	775.4	35.6	35.6	79.4	6.45
	min-max	124-127	60-76	668-888	32-40	32-38	77-81	5.31-8.05
F	mean	128	80.7	724.4	39.3	40.2	78	6.90
	min-max	123-132	68-90	620-908	35-43	34-44	76-79	5.75-9.24
DE	mean	131	98.4	688.2	40.1	40.4	80.3	7.09
	min-max	125-136	70-128	560-840	35-42	32-46	76-84	4.72-8.51
RS	mean	127	89.4	630.4	44.6	44.1	79.7	7.60
	min-max	125-129	80-100	584-804	37-48	38-47	76-81	6.15-8.22

The study on the laboratory frost resistance according to the methodology adopted at DAI showed that the Bulgarian cultivars were with the highest level of cold resistance, followed by the cultivars of Serbian origin. The predominant part of the rest of the groups was at the level of the standards Rusalka and San Pastore, which is rather insufficient. Such results were to be expected. They are related to the specificity of the region where the genotypes were developed and the frequency of occurrence of critical abiotic factors during the winter and the vegetative growth of the crop.

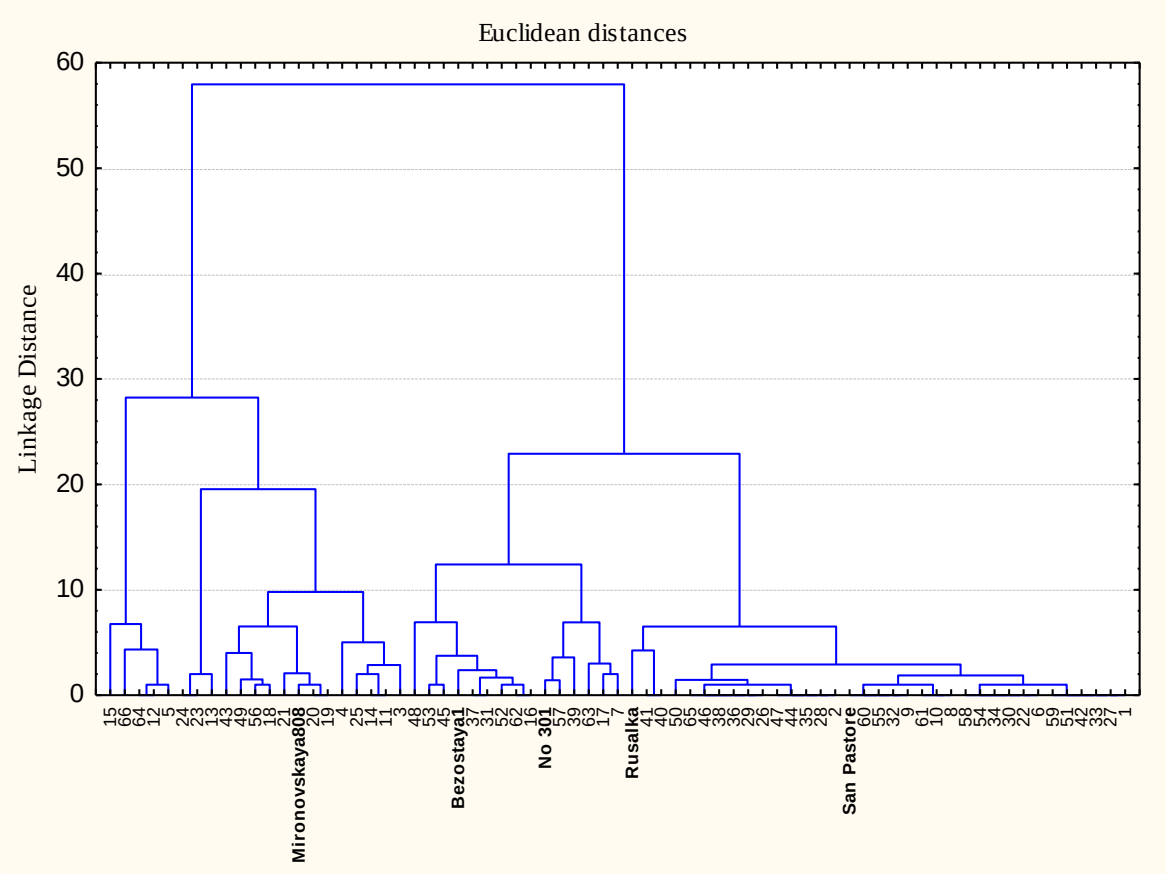

Figure 2. Level of frost resistance of common winter wheat accessions

The phytosanitary situation in Bulgaria is currently complicated due to various reasons. High attacking rates of infection caused by diseases with previous sporadic occurrence are becoming more frequent. The initial studies showed high tolerance of the tested accessions to the main diseases on wheat in this region – powdery mildew and leaf rust (Table 4). During harvest season 2017 – 2018, the situation was rather different. Most of the accessions with high level of resistance suffered a severe attack by leaf rust. The main reason for this was probably the change in the population of the pathogen resulting from improper varietal structure and dominance of a small number of genotypes.

Discussion

The updating of the breeding program of wheat in DAI imposes the necessity to search for new sources with the aim to develop genetic variability in a number of directions. These are the specific aspects: 1) High level of winter resistance; 2) Faster resumption of vegetative growth in the spring months allowing faster rate of biomass accumulation by utilization of the autumn and winter moisture reserves; 3) Earlier date to heading allowing flowering, pollination and fertilization to occur under more favorable conditions, as well; 4) Expressed dynamic relationship between duration and rate of grain filling; 5) Tolerance to drought.

Table 4. Common winter wheat accessions with tolerance to diseases (results from artificial infection field)

Origin of accessions	Powdery mildew	Leaf rust
AT	Fabius; Fidelius.	-
BG	Bozhana; Dragana; Kalina; Kristalina; Rada.	Aglika; Fani; Rada; Pchelina; Zhana.
HR	-	Andelka; Lucija.
F	Avenue; Basmati; Exotic; Foxil; Solehio; Toskani.	Anapurna; Basmati; Exotic; Foxil; Toskani.
DE	Athlon; Bitop; Joseff; Katarina; Lukullus; Midas; Mulan; Philipp.	Athlon; Attraktion; Bitop; Eduard; Genius; Katarina; Midas; Philipp; Tobias.
RS	Pannonia.	Pannonia; Simonida.

The cultivars released during the last decade represent a step forward in the breeding of this crop. An indication for their adaptability potential is the high yields realized, which are stable over years under changeable soil and climatic conditions (Mihova et al., 2018; Tsenov et al., 2012a; Tsenov et al., 2012b).

When investigating the physiological specialization of powdery mildew on wheat, genes *Pm 3c*, *Pm 7* and *Pm 3b* demonstrated highest efficiency to the studied populations (Stanoeva and Iliev 2014). Genes *Pm 4в*, *Pm 5* and *Pm 3d* had low efficiency. Completely inefficient were genes *Pm 6*, *Pm 8* and *Pm 2+6*. With regard to brown rust, genes *Lr* 9, *Lr* 19, *Lr* 40, *Lr* 41, *Lr* 42, *Lr* 43 and *Lr* 51 were with absolute efficiency. Genes *Lr* 24, *Lr 25*, *Lr* 29, *Lr* 35, *Lr 36*, *Lr* 47, *Lr* 50 and *Lr* 52 were highly efficient, while *Lr* 3ка, *Lr 11*, *Lr 15*, *Lr 18*, *Lr 26* and *Lr* 30 were absolutely inefficient (Ivanova 2014).

The yield is a resultant trait and the efforts for its enhancement relate to a number of theoretical and applied researches on certain qualities and properties. Morphological traits have been improved, which are related to the more efficient utilization of the environmental factors, including also the nutrition regime. Until recently, quite different genes were involved in Bulgarian breeding for reduction of stem height (Tsenov et al., 2009) in comparison to the West European breeding. The reasons for this are numerous, mainly the higher susceptibility to abiotic stress (primarily drought), the later date to heading, and a significant negative pleiotropic effect on the yield. The higher spike productivity was at the basis of the breeding strategy in wheat, primarily through higher number of florets and grains formed per spikelet at the expense of lower number of productive tillers. The contemporary high level of breeding and the demands of the market impose the necessity to search for new approaches to increase productivity. One of the options to do this is to simultaneously increase the number of productive tillers (Panayotov, 2013). Some of the *Rht* genes used in the breeding centers abroad are especially interesting. They ensure a favorable stem/spike ratio allowing the use of intensive production technologies.

In this respect the conservation and management of the genetic resources is especially important for the breeding program of DAI. The initial results from the evaluation of the biological traits of the new wheat accessions and their combining ability showed that they can be successfully used as parental components in the breeding and improvement work.

CONCLUSIONS

The working collection of common winter wheat includes 65 accessions with origin from Austria, Bulgaria, Croatia, France, Germany and Serbia. Best differentiation was found according to the traits plant height, number of productive tillers and date to heading. Higher

level of similarity was established between the Bulgarian and Serbian breeding. The cultivars from Austria and Germany were with the longest time to heading, followed by the ones from Croatia and France. Significant was the variability with regard to plant height. The Croatian accessions were with the shortest stem. The accessions from Croatia and France had high mean number of productive tillers. They were characterized by a lower number of grains in spike and lower grain weight.

Within the period of investigation, the Bulgarian wheat varieties, which realized highest yield, were Rada, Kalina, Dragana and Kristi, and the highest yielding cultivars of European breeding were Basmati, Foxil, Avenue, NS 407 and Andalou.

The new accessions Athlon (DE), Felix (DE) Balitus (AT), Fabius (AT), Fidelius (AT), Midas (DE), Pannonia (RS) and Simonida (RS) were with a level of laboratory frost resistance acceptable for the conditions of Bulgaria.

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INVESTIGATION OF OLEANOLIC ACID FROM FLOWERS OF EUGENIA CARYOPHYLLUS, USING OF MODERN SPECTROSCOPY

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Abstract

We obtained 0.03 gm from Oleanolic acid by using 1 gm of acetonic and ethanolic crude extracts from the flowers of *Eugenia caryophyllus*, using multiple column chromatography and using of chloroform-methanol, (1:10) as solvent system. Oleanolic acid was identified by modern spectroscope methods like (^{1}H, ^{13}C NMR and MS spectroscopy) and chromatographic methods, that available in Bangor university (UK) and the bands was related to 48 protons and 30 carbon atoms belong to Oleanolic acid. Mass spectroscopy was showed the extract molecular weight 456.3621. Also the test of inhibitory effect of isolated Oleanolic acid against three types of MO (*Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*) by using spectrophotometer that provided with LT-4000 plate reader that contend 96 cells for to concentration from (512-1) µg/ml and using MIC method. Moreover the Oleanolic acid gave different inhibitory effect against three mentioned MO.

Keywords: *Eugenia caryophyllus, Oleanolic acid, ^{1}H, ^{13}C NMR and Mass spectroscopy, Antibacterial activities.*

INTRODUCTION

Eugenia is a genus of flowering plants, the myrtle family Myrtaceae and the *Eugenia caryophyllus* is evergreen shrubs tree with a conical shape, flowering with a four-part flower, it has a strong aromatic odour, the height average of the clove tree is 10-12 meters and sometimes reaches 20 meters, it is one of the oldest and most famous spices and its seeds are like nails, they are also refered to as light bulbs and their colour is red and turns brown when it becomes dry and for medical uses, clove is described as a fever repellent, antiseptic, and sterile stomach heals from sores and headaches protects from epidermis and helps digestion and alert of the heart (Pharmazie, 1999). Moreover the medically part used is the flower buds were that they are harvested and dried for use in the extraction process to produce the oil, as well as they used leaves and stems to get the crude extract as oil (Shovalie Indero, 2010).

The most important active ingredients are their contained of the volatile oil, where it reaches 14-21% which consist of phenols, (alpha & beta) caryophyllene, terpenes, esters, ketones, alcoholics and sesqua terpenes which are anticancer compounds, it also possible to extract the eugenol compound which has the therapeutic properties of dental pain, using flower buds and clove oil contains 13% of the oleanolic acid, and gellotannins, moreover the oil is contained 97% of eugenol, also the following compounds were presented in the clove oil (methylbenzol,

furfuryl, β-pinene, α-methyl furfural, methyl-n-heptyl carbinol, vanillin, benzyl alcohol, furfuryl -n-benzyl alcohol, furfuryl-n-heptyl ketone, methyl-n-aryl carbinol (Kamatou et al., 2012).

Oleanolic acid is one of the important of triterpenes with molecular weight of 456.70 g/mol with five six rings with formula of $C_{30}H_{48}O_3$ and presented in a wide range in the kingdom of plants and in nature is presented either free pole or a free acid and it is aglyconic intiator for cytonic terpenes that is attached with simple or multiple of sugars, and the oleanolic acid synthesized in a number of plants by conversion of squalene and commercially used in addition to be as anticancer as well as in the activation of heart muscle cells, which have given great importance to the medical aspect (Jacob and Goossens, 2012) and it is also used in the treatment of some chronic diseases such as diabetes (Xiao et al., 2012, Madlala, 2012).

MATERIAL AND METHODS

Preparation of the crude plant extracts by using continuous soxhlet apparatus

The crude extracts were carried out by method that mentioned of Al-Daody (1998), and this method was depended on the nature of active constituents that isolated from the plants and also on the solvent that we used in the separation process and using the sequence solvents systems in the extraction, moreover we used five solvent systems with different polarity of following pet.ether (60-80ºC), chloroform acetone, ethonol 95% and methylated spirit industrial (IMS).

The extraction process was carried out in the different temperature degrees according to the boiling point of each solvent provide that temperature was not increase above 80ºC, so we put 100 gm of the plant powder of the flowers in the patch to soxhlet extractor and the extraction process was reached to the end of each solvent by appearance of no colour in the soxhlet. The process was repeated for each solvent using the same batch.

Also, each solvent was concentrated using rotary vacuum evaporator (RVE) at the degree of temperature 40ºC and put all crude extracts in the dark bottoms and kept in the freeze till we are used latter.

Separation and purification of oleanolic acid from acetonic and ethanolic extracts of bud flowers of Eugenia caryophyllus

Dissolve of 1 gm of each acetonic and etholic crude extract at ratio of (1:1) and we gave the symbol of E_3 and E_4 of bud flowers of *Eugenia caryophyllus* that was prepared with solvent solution and separation of components of extract E_3 and E_4 by using column chromatography with silica gel 60A for flash chromatography and saturated with mixture eluent of pet.ether-Ethyl acetate (5:1, V/V) that was chosen by the best separation of the run of TLC technique and the system was called as (P:E) and the E_3 and E_4 extracts were put in saturated of P.E. on the silica gel and the separation was carried out step by step, and we collected every 5ml from the part of solvent system (mobile phase) in glass bottomed.

The detection of the resulted fractions from the column chromatography as according to the similarity of spots at position and shapes on TLC-plates on the same solvent system that used in the column, after that we get four fractions which gave as (E_3 and $_4$ and F_3A, E_3 and $_4$ F_4A, E_3 and $_4$ F_1A, E_3 and $_4$ F_2A) and changed the polarity of mobile phase of column for the same extracts, so we chose (Chloroform-Meoh) at ration of (10:1) as (Ch:M) as solvent system.

Only two fractions were obtained from this polarity and gave as E_3&$_4$ F_1B and E_3&$_4$ F_2B and also we changed the polarity another one that we used (Ch:M) with ration of (5:1) and two fractions were appeared too, and gave as (E_3&$_4$ F_1C, E_3&$_4$ F_2C) and then evaporate the solvent

by (RVE) and dissolve some of each fraction in (Methanol-d_4) to measure it by NMR instrument (Harbourne, 1998).

The inhibitory activity test of oleanolic acid by turbidity method

The method of microbial sensitivity test of isolated oleanolic acid from *Eugenia caryophulls* so that, the test was carried out by spectrophotometer instrument from FFFF JASCO-550 UVNIS that presented in Bangor university (UK) also the turbidity test was measured by the mentioned instrument which provided from the Fishcher scientific company and using LT-4000 plate reader that contained 96 cell as cleared in the Image 1.

The primary concentration was prepared which netroulized 1024 µg/ml that dissolve of 0.1 gm of isolated pure compound in 1 ml of DMSO after that added the mixture to the test tub that contend 1 ml N. broth in cells beginning from the second cell of plate. So 200 ml was added to the primary concentration of active compound to the primary cell of plate and the formation of diluted solutions and transfer of 100 µl from the primary cell and added to second cell an so over till we reach to the tenth cell.

Microbial suspension was prepared with concentration of 1×10^5 cell/cm^3 by addition of 0.1 ml of bacteria to 10 ml from N. broth. 100 µl from microbial suspension was added to tenth cell and we get of concentration as (512, 256, 128, 64, 32, 16, 8, 4, 2, 1) µg/ml.

The test was carried out with the average of repeated there of each concentration and each type from the using microbial types on the plat it self and the complete of diluted solution. The plate was covered with adhesive adaptor and reading of spectrophotometer and using incubator at 37ºC for the period of 14-16 Hrs. after that we measure the turbidity by the instrument it self and the reading with wave length of 492 nm. So the limitation was carried out of the effect was pure active compound for comparison with the positive stander compound (Pessin et al., 2003).

Image 1. A. Spectroscopy B. Plate 96 cell

RESULTS AND DISCUSSION

Separation and identification of oleanolic acid from the acetonic and ethonolic crude extracts from the bud flowers of *Eugenia caryophyllus*, using CC technique solvent system of (P:E) (Pet.ether-ethylacetate) as mobile phase was used with ration of (5:1, V/V) in the column chromatography that packed with silica gel 60A, in the separation of oleanolic acid, and obtained of four fractions and from them, the fourth fraction that identify as oleanolic acid by NMR spectroscopy and it was not purified and gave as E_3&$_4$ F_4A by weight of 0.14 g (Image 2) and it is clear that the fourth fraction was appeared at the bottom of TLC, so the polarity was increased for choosing solvent system (Ch-MeOH) with ration of (10:1, V/V). So, this solvent system more polarity then will compete with silica gel to put of oleanolic acid and separated

and when we saw the chemical structure of oleanolic acid that we found it as glycosides from as presented in the plant, so the oleanolic acid is a triterpene with glucose sugar and it is contained of two type of hydroxyl group (OH), first is carboxylic acid at (C_{24}) position and the other is alcoholic at position of (C_2) and Figure 1, so that, the sugar will attach, with OH of carboxylic of alcoholic and also we have solvent contained of chloroform that non polar that is dissolved the aglycone part and the glycone compound will dissolve by methanol (polar compound) and in this case, the oleanolic acid will liberate from silica gel, or acid hydrolysis was carried out inside of column chromatography as a result of silica gel as silicic acid, then the oleanolic acid liberated as free pole.

There are two fractions by using of the solvent system as (Ch:M) and after of the testing of them by NMR technique, we found that oleanolic acid is presented in the first fraction with 100% purity and gave as E_3&$_4$ F_1B as clear in the (Image 3) so, the B is presented as second choosing polarity (Ch:M) and the weight was 0.03gm from the origin 1 g extract of each acetonic and ethanolic with ration of (1:1, V/V).

Oleanolic acid is polar compound that, it is elucidated to appear after the change of polarity of mobile phase and also was separated from acetonic and ethanolic that are usually known as polar solvents and this compound is not appeared in the non polar solvents.

The Image 4 was appeared that oleanolic acid had white colour after evaporated the solvent by RVE or it had white colour with power from when it evaporated and had crystal material when it inside of the solvent. It is considered that oleanolic acid was separated as pure from the bud flowers of *Eugenia caryophyllus* and this study is the first in Iraq and it was differed from the first study in India as a result of different culture by Rangari & Banarase that get the patent (Rangari and Banarase, 2010).

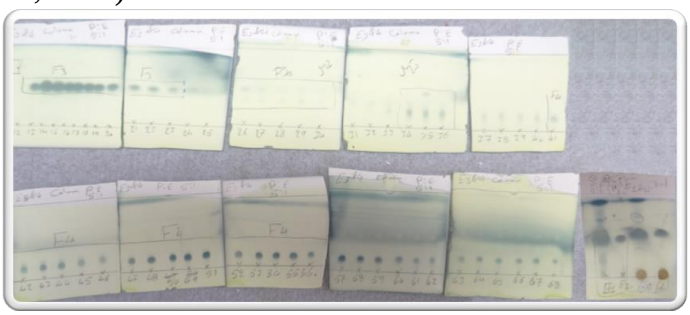


Image 2. Oleanolic acid in E_3&$_4$ F_4A by TLC technical

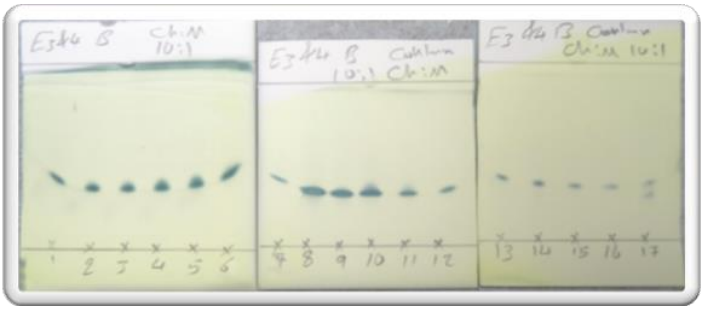


Image 3. Oleanolic acid in E_3&$_4$ F_1B by TLC technical

Image 4. Pure oleanolic acid from *Eugenia caryophllus*

Identification of oleanolic acid

The oleanolic acid was identified by using multiple of techniques as following below: **First:** by ^{1}H-NMR, that used of NMR instrument with 400 MHz for proton, and 101 MHz for C^{13}, so that the oleanolic acid which was isolated by column chromatograph dissolve in chloroform-d^{1} as (CD Cl_3) so, it gave band at 7.27 ppm in ^{1}H-NMR & gave the band at 77.00 ppm related to ^{13}C-NMR.

A: Identification of oleanolic acid by ^{1}H-NMR

The identification was carried out through limitation of the bands that appeared in $C_{30}H_{48}O_3$ as clear in the Figure 1. The broad band b as triplet band (t) for on proton at 5.29 ppm, ^{1}H, J3.4Hz, b.t and this proton was carried the no. 13 and the integration was also 1.00. The band (dd) for one proton was also appeared at 3.22 ppm, ^{1}H, 10.8 Hz, J4.32 (dd) and carried no. 2 and this proton was appeared (dd) for adjacent of two proton, one of them is cis and the other is trans as clips & couch and the integration is 1.01. Also the band of (dd) for one proton was appeared at 2.83 ppm as (dd), ^{1}H, J4.28, 14.04 Hz and carried the no. 18. And in case of previous proton it self, with no. 2 as adjacent two proton but the difference that, this proton was Endo while the proton of no. 2 was Exo and integration was 1.04.

So that, a band (tt) at 1.97 ppm was also appeared for one proton and the integration was 1.16 as (^{1}H, tt J4.04, 13.4 Hz which was carried the no. of 33 and it carried the carboxyl group. Also, the multiple bands were appeared for two proton (4, 10) at (1.88-1.92) ppm as m, 2H and integration was 1.91. Multiple bands were also appeared belong to 12 protons at (1.53-1.81) ppm as 12H, m, belong to atoms (6+7+8+14+20+22) as from CHz and integration was 11.43. The same multiple bands were appeared at (1.05-1.48) ppm belong to 12 proton (1+15+16+19) as well as of proton of OH at 27 carbon atom, and also triple multiple band belong to three protons related to CHz group no. 32 was appeared at 1.14 ppm and integration was 12.35 as 1.05-1.48.

Also, single six bands were appeared belong to three protons for CH_3 groups at 0.99 ppm related to atoms 28 (5, 3H) with integration of 3.38, and the atom 2, at position of (5, 3H, 0.93 ppm) with integration 8.74. The action 36 at 0.92 ppm, 3H, S with integration 8.74 and atom 35 at 0.91 ppm. S, 3H, with integration of 8.74, and the atoms 25 at 0.78 ppm (3H, S) and integration of 3.81 and atom 26 at position of 0.76 ppm (3H, S). The OH group of carboxylic acid was not appeared at normal position 9 ppm and over that because as exchange of part of acid with $CDCl_3$ to change to $CHCl_3$ and for that the acid was appeared at position with high shielded and appeared of $CHCl_3$ at position of deshielded as 7.28 ppm. The resent results of study was equaled to the study (Corey and Lee, 1993). So that the identified position that he obtained by ^{1}H-NMR for oleanolic acid that prepared it by chemical methods was similar to

the recent study. Also, the current study was nearly equal with results of (Marquina et al. 2001) who was identified oleanolic acid that isolated from the roots of *Viguiera decurrens* by ^{1}H-NMR. Also, identified oleanolic from *Sebania sesban* by ^{13}C-NMR and the results was nearly equal to the current study (Das et al., 2011).

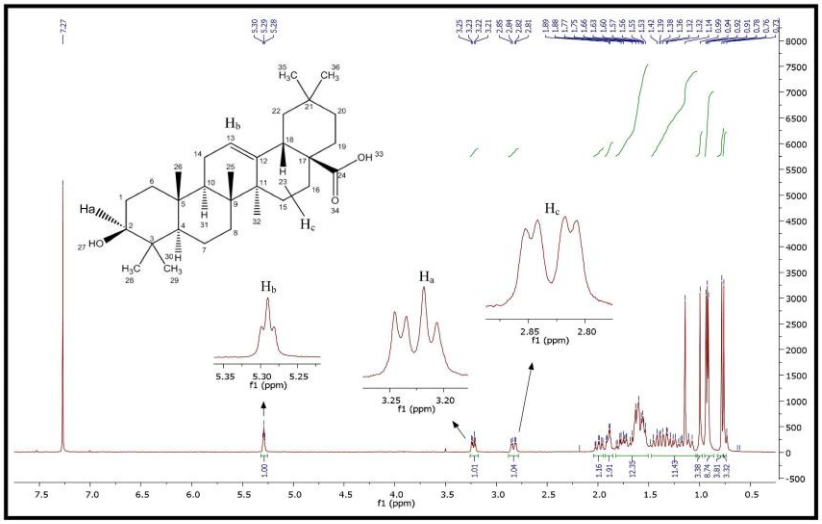


Figure 1. ^{1}H-NMR of oleanolic acid

B. Identification of oleanolic acid by ^{13}C-NMR

The Figure 2 was cleared that the various bands of oleanolic acid which contained of 30 carbon atoms $C_{30}H_{48}O_3$ and measured by ^{13}C-NMR with 101 MHz and using $CDCl_3$ as solvent that gave the band at 77.00 MHz, so the number of carbons from C1-30 as following below:

C_{12}=143.6	C_{13}=122.6	C_2=79.6	C_{22}=55.2	C_8=47.6	C_7=46.5
C_1=45.9	C_6=41.6	C_{32}=41.0	C_{36}=39.3	C_{35}=38.7	C_{28}=38.4
C_{29}=37.1	C_{26}=33.8	C_{25}=33.1	C_{19}=32.6	C_{16}=32.4	C_{14}=30.7
C_{18}=28.1	C_{11}=27.7	C_4=27.2	C_{17}=25.9	C_3=23.6	C_5=23.4
C_9=22.9	C_{20}=18.3	C_{15}=17.1	C_{10}=15.5	C_{21}=15.5	

There results were equal to the results of Bednarczyk-Cwynar et al. (2012); Martinez et al. (2013); Moreira et al. (2013), relatively.

Mass spectroscopy MS is analytical technique for limitation of components that composition of the compound or the part that used to clear the chemical formula of molecules e.g. peptides and other chemical compounds.

The Figure 3 was spectrum of mass of oleanolic acid was started from appeared the primary essential band as presented as molecular ion (M^+) and truly mean the molecular weight to the measure compound and fragmentation of this compound according to appear of fragmented bands.

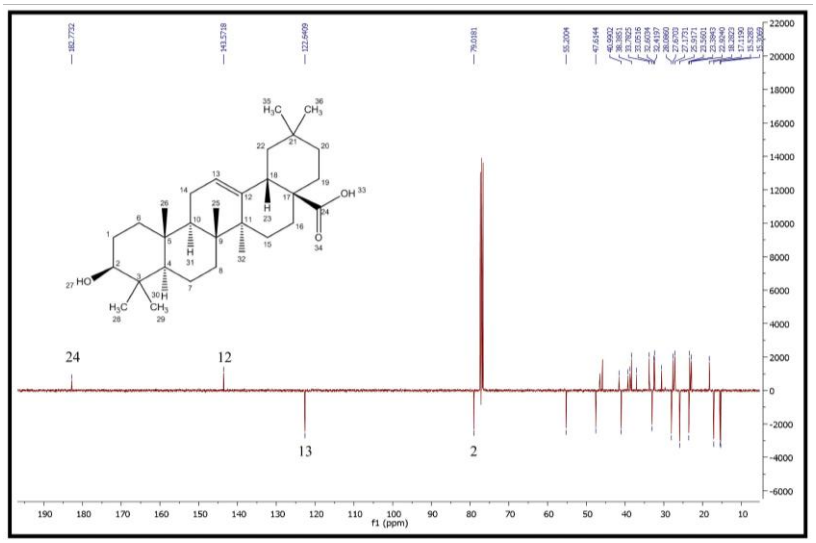


Figure 2. ^{13}C-NMR of oleanolic acid

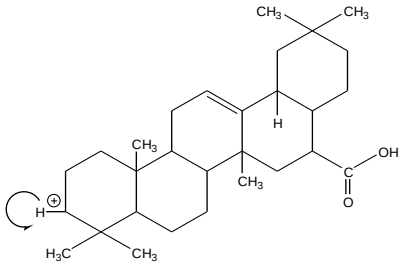
Second: Identification of oleanolic acid by using Mass spectroscopy (MS)

(1) $M^+ = 456.3621$

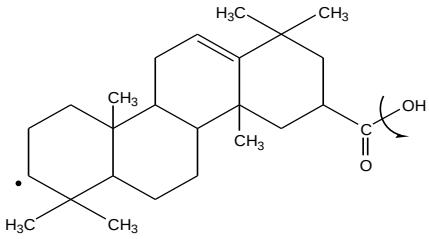
(2) $lC^{13}+ = 457.4449$

(3) 17(OH alc.) – = 439.4449

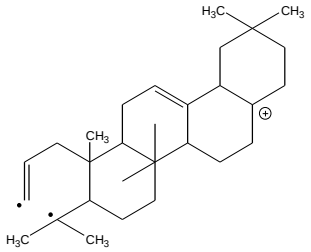
(4) 18(H_2O) – = 438.3390



(5) 438.446-17 (OH) = 421.44



(6) 438.3390-28 (CO) = 410.3488

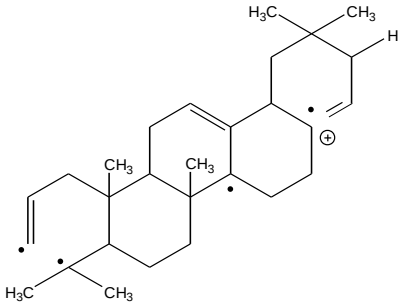


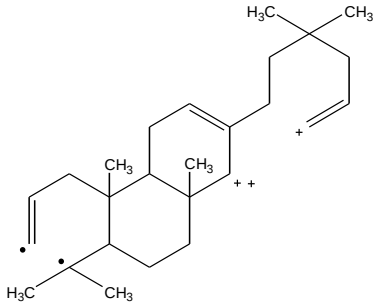
(7) 392.368 + ($1C^{13}$) = 393.3940

(8) 438.3390-46() = 392.3268

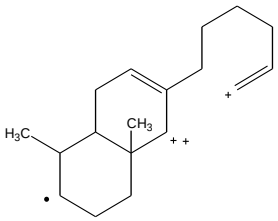
(9) 392.3268-15 (CH_3) =377.3151

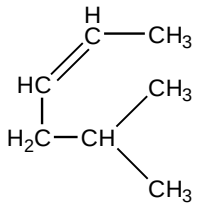
(10) 377.3151-28 (CH_2=CH_2) = 349.2776





(11) 349.2774 - 100 =249.2331



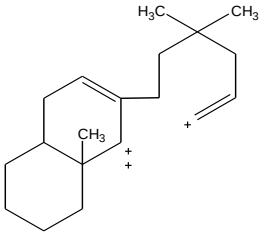


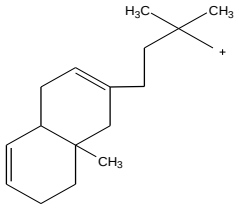
(12) 249.2331 + ($1C^{13}$) = 250.1889

(13) 249.2331 - (H) = 248.1057

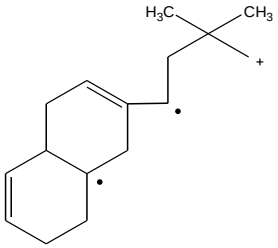
(14) 248.1057 - 15 (CH_3) = 233.1265

(15) 233.1265-26 ($H-C\equiv C-H$)=207.1523





(16) 207.1523 - 18 (CH_4) = 191.1314



(17) 191.1314 - (1H) = 190.1314

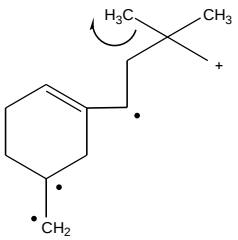
(18) 190.1314 - (1H) = 189.1226

(19) 189.1226 - (1H) = 188.1304

(20) 188.1304 - (1H) = 187.1304

(21) 187.1301-26 ($H-C\equiv C-H$) = 161.2345

(22) 161 - (1H) = 160



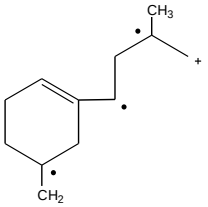
(23) 160 - (1H) = 159

(24) 159 - (1H) = 158

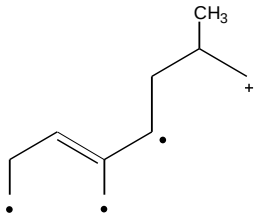
(25) 158 - (1H) = 157

(26) 161.2345 - 16 (CH_4) = 145.0947

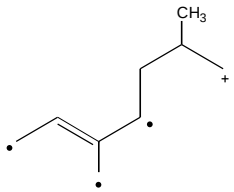
(27) 145.0947 - 16 (CH_4) = 129.1122



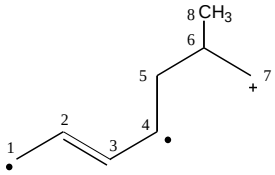
(28) 145.0947 - 26 ($H-C\equiv C-H$) = 119.0715



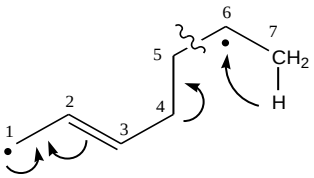
(29) 119.0715 - 12 ($1C^{12}$) = 107.0697



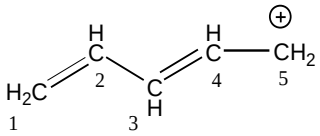
(30) 107.0697 - 12 ($1C^{12}$) = 95.0705



(31) 95.0705 - 14 (CH_2) = 81.0507



(32) 95.0705 - 28 (CH_2CH_2) = 67.0990



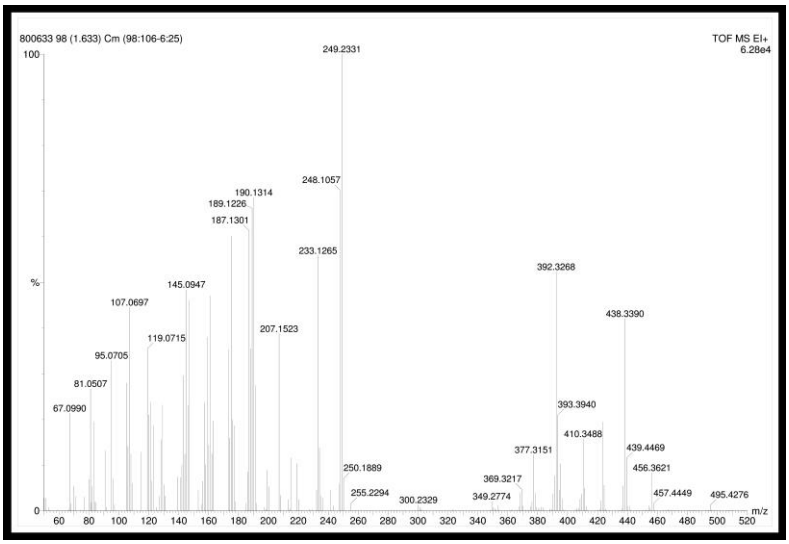


Figure 3. Mass spectra of oleanolic acid

Antimicrobial activity

We take the isolation microbial of (*Staph. aureus, E. coli and C. albicans*) it was done in one of the laboratories of Dept of Biology/ University of Bangor/ British and the isolation bacteria was identified worldly and raping with lyfolyzer and so that the summation of isolation bacteria was reached to 48 isolation and there were 30 isolation as Gram positive and 18 isolation with Gram negative.

The experiment was carried out with test turbidity method by using JASCO V-550 UV/VIS spectrophotometer and it provided from Fishcher scientific company with using plate of type LT-4000 plate reader and content with 96 cell.

The method was carried out in the biological was in Bangor University (UK) and preparation of diluted solution as clear from the material and methods. So, it was prepared the concentration of oleanolic acid compound (512, 256, 128, 64, 32, 16, 8, 4, 2, 1) µg/cm^3 and after incubator at 37°C so, the results were appeared as following.

The sensitivity test of oleanolic acid

The experiment was appeared clear sensitivity the turbidity test of microbial (*Staph. aureus, E. coli and C. albicans*) against isolated oleanolic acid with pure from the put flower of *Eugenia caryophllus* with comparison with positive stander control that it content from N. broth media and microbial using under study for each one that it is noticed in the Table 1 using of microbial was a highly sensitivity against oleanolic acid. Oleanolic acid was inhibited the growth of *staph. aureus* in a mount 0.09 at the concentration 512 µg/cm^3 and the same case in the rest of used of concentrations as clear in the figure (4). Moreover the oleanolic acid was effected at *E. coli* by 0.04 with compared with stander control as clear in the Table 1 and the Figure 4. And the same case for *C. albicans* so the oleanolic acid was a good affected against *C. albicans* so the growth of these microbial was few with turbidity of 0.20 with concentration 512 µg/cm^3 against oleanolic acid and the effect was inserted directly with were compared with stander control. As in the Table 1 and the Figure 4.

Table (1): Sensitive of some microbial to pure oleanolic acid from *Eugenia caryophllus*

Microbial / Treatment		Concentration µg/cm^3										
		512	256	128	64	32	16	8	4	2	1	Control
Oleanolic acid	*Staph. aureus*	0.09	0.19	0.30	0.57	0.69	0.87	0.98	1.99	1.00	1.07	1.52
	E. coli	0.04	0.09	0.22	0.53	0.77	0.95	1.09	1.20	1.33	1.41	1.55
	C. albicans	0.20	0.44	0.62	0.79	0.86	0.99	1.04	1.09	1.23	1.40	1.53

Control (+ve) 100 µl from broth and 100 µl from microbial suspension.

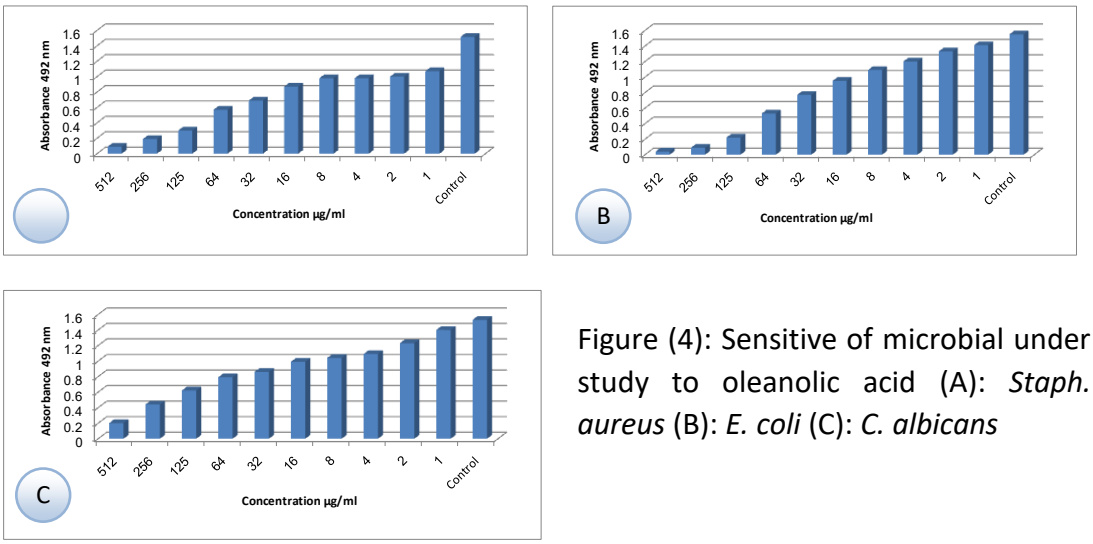


Figure (4): Sensitive of microbial under study to oleanolic acid (A): *Staph. aureus* (B): *E. coli* (C): *C. albicans*

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ULTRASOUND DIAGNOSIS OF HEPATIC LIPIDOSIS IN COWS FOR MILK PRODUCTION AS INDICATOR OF SARA (SUB ACUTE RUMEN ACIDOSIS)

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ABSTRACT

Metabolic diseases in the cow have been and remain the challenge of veterinarians around the world. The main problem is related not only to nutrition and the environment but also to the individual characteristics of animals and the physiological period. Immediately after calving is the most favorable terrain for the development of many metabolic diseases. Very common pathologies are Sara, ketosis, puerperal paresis, etc. Studies show that there is a strong relation between ruminal environment disorder by subclinic acidosis and hepatic lipidosis. Since sub clinic acidosis or SARA presents many diagnostic difficulties, hepatic lipidosis as frequent episode of this pathology, may serve as an important indicator for Sara's diagnosis. The simplest, fast, non-invasive and low cost method for diagnosis is ultrasonography of liver. Through this method, it is possible to see the hepatic changes caused by the increased deposition of the adipose tissue. The main purpose of this article is to inform the reader that hepatic lipidosis serves to raise doubts that dairy cows are suffering from SARA condition.

Keywords: *Sara, Hepatic lipidosis, Ultrasonography, Cows, Diagnosis*

INTRODUCTION

Subacute rumen acidosis (below SARA condition) is the most important economic problems on farms and cows for milk production and is characterized by daily episodes of pH reduction in the rumen content between values of 5.5 - 5.0, (Enemark et al., 2002). Poverty or lack of clinical signs do not easily identifiable pathology. Clinical signs may appear to reduce the amount of dry food consumption, laminitis, rumenitis, liver abscesses, pulmonary bacterial embolism, loss of body weight and % reduction of fat in milk, require weeks and months to follow the negative impact of SARA condition (Underwood, 1992; McDonald, 1995; Mortensen, 1999; Kleen et al., 2003). The decline in pH of ruminal content and rumen movement disorders in cows with SARA condition, caused by the local accumulation of volatile fatty acids only as a result of feeding the animals with food ration easily fermentable and not by accumulation of lactic acid (Nocek, 2003; Kleen et al., 2003; Gianesella, 2012).

Subacute rumen acidosis (SARA), is recognized as a disorder of the processes of fermentation and digestion of food in the rumen (Oetzel, 2003; 2005). This syndrome is found in particular in well-managed farms of cows for milk production (Nordlund et al., 1995; Hughes, 2001). SARA condition is the most common disorders in ruminants and deviation from the norm underlying processes in the environment of ruminal fermentation. SARA condition is characterized by episodes of lowering daily pH of rumen content in values between 5.5 and 5.0, (Underwood, 1992). This disorder is a result of feeding the animals with high levels of concentrate (Radostis et al., 2003). SARA condition can also be defined as a decrease of pH in the content of rumen in nophysiological levels after receiving concentrated foods and not to favor environmental adaptation in terms of ruminal microflora and mucous membranes. This condition is not associated with obvious clinical signs.

Cows are always regarded as an essential source of animal products (meat, milk, leather, bones) and as natural land fertilizers. One of the permanent tasks of mankind has been and remains the improvement of the productive and reproductive performance of dairy cows. This exaggerated obsession is related to increased demand for products and by-products originating from cows. Such levels requirements tend to exceed all genetic capabilities of animals and constitute the major ubiquitous cause of metabolic problems (Nordlund, 1995). The liver is an organ of great importance in the body. It actively participates in the synthesis of glucose, in plasma protein formation, in formation and excretion of bile salts, in pigment excretion, in the formation of prothrombin, in detoxification and excretion of many substances including photodynamic agents. Ruminants liver has a remarkable functional reserve. Clinical manifestations of liver diseases becomes apparent only if 70% of parenchyma is totally in functional inactivity. Using imaging methods especially ultrasound for diagnosing of hepatic diseases in cattle is indicated because other diagnostic methods including the determination of hepatospecific enzymes, in most cases are insufficient (Nordlund, 1995; Shabani et al., 2013). Hepatic ultrasound examination is rewarding because it defines the hepar size, position, shape, condition, dimension of vessels and different types of hepatic disorders (Shabani and Robaj, 2016). Hepatic lipidosis is the most important metabolic disorder of dairy cows during early lactation and is responsible for ill-health and poor reproductive performance of the animals. Efficient application of diagnostic and preventive strategies for this syndrome has great economic importance. Transitional period between late pregnancy and early lactation is a situation where creates an excessive drainage of different nutrients. Hepatic lipidosis is a condition that usually develops in the period near of calving and in early lactation (Kojouri, 2003). Hepatic lipidosis develops when hepatic lipid intake exceeds the level of oxidation and exretion of lipds from liver. This condition is characterized by high concentration of free fatty acids metabolized by adipose tissue. Lipid excess is deposited as triglycerol who is primarily responsible for reducing the liver's metabolic functions. Liver can be categorized as normal or average, moderate or severe hepatic lipidosis, encephalopatic lipidosis, and hepatic incefalopatia (Braun, 1990; Grummer, 1990; Ametaj, 2005; Brown, 2000). Syndrome of hepatic lipidosis affects almost half of herd immediately after calving. The amount of fat accumulated in liver in the first 10 days after calving ranges from 60 to 120 grams per day. Lipids accumulated in liver occupy 12 to 25% of liver wet weight. In some cases, hepatic lipidosis followed by severe inflammation resulting in patient's death. If patients who suffer from this syndrome are not treated, mortality captures the values 25% (Ametaj, 2005). SARA causes negative energy balance resulting in hepatic lipidosis. Hepatic lipidosis can be identified by an ultrasound study and can serve as a SARA condition indicator (Shabani and Robaj, 2016).

Results of recent studies show an interesting correlation among the unfavorable breeding conditions (bad hygiene, inadequate ventilation, inappropriate layer, density of animals above the permitted levels, poor lighting, poor approach to water and food) with the frequency of metabolic diseases.

Cows with high milk production experience a period of energy scarcity in early lactation. In this period the cows mobilize body reserves to cope milk production. These facts constituting the real causes of moderate hepatic lipidosis which affects the best individuals in the herd. Fat is deposited in all tissues of the body, especially in sceletal muscles. Statistical processing of data from different studies shows that hepatic lipidosis syndrome is part of the general fats mobilization and not just a specific tissue or organ (Kida, 2003). Margolles et al. (1988) demonstrated that hepatic lipidosis happens in such circumstances when the energy distribution in animals at late pregnancy is insufficient. Animals with double pregnancy are extremely vulnerable and sensitive to energy deficiencies in resent moths of pregnancy. Recent studies shows very clearly the importance of nutrition of animals before and after calving on

the etiology of ketosis/hepatic lipidosis syndrome. Braun (2003), states that transitional period (3 weeks before and 3 weeks after calving is the most critical moment in the biological life of dairy cows.

Always it has been reported a correlation between mastitis and metabolic diseases. Hyperglycemia followed by hypoglycemia it has been reported as part of the inflammatory phase. It is believed that the hypoglycemia phase is associated with the reduction of the level of glucose released from the liver (Katoh, 2002) reported a transitional growth of plasma concentration in NEFA after intravenous injection of *E.coli* lipopolysaccharide in heifers. Inflamatory responses are part of the etiology of hepatic lipidosis. Hepatic lipid accumulation is stimulated by the growing of concentration of proinflamative cytokines and tumor necrosis factor alfa (Braun and Gerber, 1994; Mammdouh, 2004). Inflammatory conditions and the response of the acute phase activate the macrophages that release a wide variety of products known as cytokines. The most important cytokines are TNF alpha, interleukin 1 and interleukin 6 that promote the production of a large range of proteins in the liver. These proteins are known as SAA and haptoglobina which reach high concentrations in the plasma of dairy cows immediately after calving. The levels are similar with the situations when we inject endotoxin intravenously (Ametaj, 2005**;** Radostitis et al., 2005). Endotoxins are components of cell membranes in all gram negative bacteria and play a very important role in the development of many matabolic diseases among them and hepatic lipidosis (Mundron et al., 1999; Ametaj, 2005; Bobe et al., 2008).

Goff and Horst (1997) concluded that phosphorus deficiency after calving plays an important role in the pathogenesis of hepatic lipidosis. The same author recomended phosphorus supplements as a useful prophylactic and therapeutic measure for postpartum hepatic lipidosis. This scientific facts were emphasized even more by (Multu et al., 1998; Radostitis et al., 2003) who describet post- mortem examination in cattles with haemoglobin deficiency after calving. The liver of this animals appears enlarged and with great fatty infiltration. Changes of degenerative fatty infiltration were found also in histopathological examiantions. Sayed (1991) studied 180 cattles of Holshtein breed 40 days after calving. From this study, the author reported that hypophosphatemia in 10.7% of cases was associated with hyperacetonemia. Morrow et al. (1979) and Oetzel (2001) sugested that hypophosphatemia comes as a secondary metabolic event, as a result of subclinical condition of ketoacidosis in the period before calving.

Hepatic lipidosis it is a pathological change which is not yet very clear on the causality, but many authors suggest and give as the main problem disorder of the intrahepatic blood circulation (Acorda et al., 1994). Brown (1990) showed that degenerative local changes of hepatic lipidosis can be identified with special diagnostic techniques (ultrasound). Hepatic accumulation of triglycerides due to increased level of hepatic intake of NEFA stimulate an increased activity of diacylglycerol acyltransferases (Acorda et al., 1994; Manal et al. 2005). Recent studies have managed to explain why the liver has a limited capacity for oxidation of fatty acids. Lack of ocsalacetate which is needed to keep in view the tricarboxylic acid cycle, lack of carnitine which is necessary for mitochondrial transport and oxidation of acetyl coenzyme A, lack of niacin, disturbed endocrine factors of patient, are the correct answers of the question WHY (Goff and Horst, 1997). Hepatic lipidosis occurs when the unesterified fatty acid concentrations reach levels 1000 uEq per L (Multu et al., 1998). Hepatic triglyceride level is negatively correlated with plasma level of alfatocopherol (Van Den Top et al., 2005). Microzomal hepatic triglicerides transfer the protein activity and the body mass index it is not affected by nutritional status of cows that are not in lactation (Acorda et al., 1994).

Morrow et al. (1979) and Grummer et al. (1990) described hepatic lipidisis as a specific clinical condition that occurs in obese dairy cows. This illness situation causes serious health problems at the time of calving. The same author noted that the level of morbidity was 82%

and mortality 25%. Cows with a very good condition at the calving period are more likely to be affected from hepatic lipidosis and cows with hepatic lipidosis are very likely to develop the ketosis condition (Salem et al., 2003). Hepatic lipidosis syndrome may be developed within 24 hours and lasts for a long time. By histological, cows classified into 3 groups based on the level of fat content in the hepatic level at individuals one week after calving

1.Less than 20% lipids corresponds to less than 50 mg per gram of liver weight.
2. 50 to 100 mg lipids per gram of liver.
3. More than 40% is more than 100 mg lipids per gram of liver.

These concentrations correspond to the average rates, moderate and severe of hepatic lipidosis. Cows with less than 20% lipids in liver, in a week after calving are considered normal, individuals with over 20% are considered with hepatic lipidosis syndrome. Clinical evidences of liver disease do not pull the attention of a doctor or a farmer until such time as concentrations of lipids in liver do not reach the values 35%, 45% or more (Radostitis et al., 2003). The total of hepatic lipids, mainly triglycerides, come to a climax on the day of calving or between 1 and 5 week after calving. This situation persists until the 12th week of lactation (Radostitis et al., 2003; Ametaj, 2005) in the total of affected animals of a herd, generally 25%, fatality level in affected individuals is 90% (Radostitis et al., 2003; Beitz et al., 2004; Shabani and Robaj, 2016). The vital indicators (body temperature, cardiac and respiratory frequency) are within values of the norm. Rumen peristaltic is weak or absent and faeces are generally scarce. Periods of lying for a long time are common in patients who suffer from this illness condition. Heavy ketosis condition which does not answers in ordinary treatment is frequent. Patients demonstrate full anorexia. This fact makes the animals physically weaker and forces them to stay lying. Animals with lying syndrome die within 7 or 10 days (Clain et al., 1984; Braun, 2003). Clinical findings in patients with serious hepatic lipidosis are yellowing of the mucous membranes, anorexia, ketosis, frequent movement and unintentional of the head, the left shift of abomasum, lying and coma (Nordlund, 1995; Braun et al., 1996). However, it should be noted that this illness condition it is not characterized by specific clinical signs. A clinical moment which should never be forgotten is that the hepatic lipidosis is a physiological deviation that occurs at the beginning of lactation. Special attention should be shown to animals in early lactation because other diseases as ketoza, paresis, mastitis, metritis, displacement of abomasum and the rest of the placenta are more frequent in animals with hepatic lipidosis (Braun, 1996; 2004). Almost in all cases hepatic lipidosis is coexistent with the above diseases (Radostitis et al. 2003; Sayed, 1991; Nordlund, 1995; Van Den Top, 2005).

Ultrasound examination of liver

The complete ultrasound examination of the liver must provide detailed information on shape, size, position, ultrasound model of hepatic parenchyma, size of the gall bladder, size of the internal and external liver duct and topography of the large blood vessels. Ultrasound allows doctors to accurately trial hepatic pathological processes and helps setting the correct diagnosis (Braun, 1990; Bremmer et al., 2000; Shabani and Robaj, 2016). Ultrasound diagnosis methods are safe and do not cause damage to the liver cells (Braun , 1990; Shabani and Robaj, 2016). The best region for ultrasound examination of the liver in cows is the area between the ribs 7 and 12 on the right side. Care should be taken in patients with right abomasal displacement or with diaphragmatic hernia or different congenital malformations which relocate liver and make it invisible on ultrasound examination (Bobe et al., 2005; Shabani and Robaj, 2016). Normal pattern of normal cow's liver during the ultrasound examination consists of a poor echoich homogeneity distributed throughout the liver area. The lumen of the portal and hepatic vein is anechoic. The normal liver ultrasonograme consists in a number of weak echo distributed

homogeneously throughout the liver area, with a thin longitudinal anechoic line, veins and wall of the arteries are thick and hiperechoic (Salem et al. 2003). Hepatic ultrasonogram is rewarding in assessing the degree of hepatic fatty infiltration in dairy cows (Bobe et al., 2005). Ultrasound diagnosis of liver using liver/kidney contrast has only limited use in dairy cows (Acorda et al., 1994). Braun (1990) and Bobe et al. (2005) indicated that the breed and the cow's age does not influence the liver ulttrasound appearance. Different echo models of bright patterns, light stains in deep hepatic vessels and different angles can be used to distinguish varying or diffuse hepatocellular disorders in dairy cows. Ultrasound methods can also be used as a screening test before using other invasive techniques (Acorda et al., 1994). Ultrasound findings of hepatic lipidosis is associated with hepatomegaly appearance, round borders of liver, hiperechoic parenchyma close to the abdominal wall, the weak echoic view with increasing distance from the abdominal wall and poor appearance of the liver blood vessels (Braun, 1990). Local hepatic lipidosis not cause displacement of adjacent blood vessels (Grummer at al., 1990; Shabani and Robaj, 2016). By increasing the fat content in the liver observed reduction in the diameter of the portal vein and increase the size of the gallbladder (Kida, 2003). Digital ultrasound tests have the potential to classify the level of triglycerides in the liver infiltration and evaluate the hepatic triglyceride content. These analyzes are appropriate and safe to be realized in a large group of animals on the farm. The diagnosis of hepatic lipidosis makes successful treatment and significantly lowers the level of mortality in patients suffering from this pathological condition and SARA (Bobe et al., 2004).

CONCLUSIONS

From this study of literature were some important moments which should be always in consideration during the management of herds of dairy cows, in order to successfully avoid the productive and reproductive health problems. The application of biochemical examining methods for animals during negative energy balance is rewarding for the diagnosis and prevention of hepatic lipidosis but imaging test are the most accurate especially to associate Sara with lipidosis. Ultrasound diagnostic techniques are safe, noninvasive and rewarding in early diagnosis of hepatic lipidosis and Sara condition. The combined application of biochemical examining and ultrasound leads in early and correct diagnosis of hepatic lipidosis and Sara and helps prevent and treatment of this syndromes.

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TREATMENT OF POLLUTION FROM TEXTILE DYES WITH OZONE METHOD

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Abstract

The textile industry uses a large number of different colors and pigments. More than 50% of the dyes used in the textile industry are azo dyestuffs. These dyes usually contain at least one and at most four azo groups bound to two radicals, at least one or both of which are aromatic groups. The increased use of cotton also causes significant increases in the use of reactive dyes. However, since reactive azo dyes are separated by carcinogenic aromatic amines, they are potentially harmful to the environment and are of high importance. In this study, COD and color removal were investigated by ozonation (2 g O_3/hour) method of selected dyestuff species (Reactive Red 195 and Acid Blue 284) used in the textile industry. COD and color removal were found to be very low during the two hour reaction period. The total reaction time was 4.5 hours. As a result, ozonation method has not provided a significant elimination of COD removal from these dyestuffs. The color removal efficiency for both paints was found to be high.

Key words: Ozone, Advanced treatment, Dyestuff, Chemical oxygen demand, Color removal

INTRODUCTION

Waste water from the textile industry is one of the most important pollutants. Particularly reactive dyestuffs are widely used in the textile industry due to its easy dyeing process and stability. In addition to these, dyestuff groups which are different according to fiber type are also widely used. Waste water containing these paints causes serious environmental problems when drained. In addition to color removal from textile waste water, it is very difficult to treat chemical substances that are harmful to environment and health contained in the contents of paints. Therefore, advanced oxidation processes have been used for wastewater treatment in recent years.

Textile wastewater is an important source of water pollution. In general, textile wastewater contains high organic compound, heavy metal, high temperature, high COD, high pH, and concentrated color (Wijannarong et al., 2013). In general, the dye molecules are organic compounds that contain chromoform groups linked in a single bond. Ozonation oxidizes the double bonds of the chromoform groups in the dye molecules (Wijannarong et al., 2013). In many studies, color removal from synthetic dyes is provided by ozonation (Wu et al., 2007; Glaze et al., 2007). Ozone is one of the best methods for breaking paint and eliminates formation in other problems (Iranifam et al., 2011).

Pigments are small molecules consisting of two main components: chromophore, which usually gives color and functional group, which binds dye to yarn. The adsorption of the paint on the yarn varies depending on the textile yarn and the type of paint. There are hundreds of

types of paint classified in the literature, according to the chemical structure or the fiber type applied. Therefore, the chemical variety that affects purification systems is very high.

Oxidation with ozone is one of the most effective methods for removing reactive pigments. There are probably two ways of oxidizing with ozone: Molecular ozone mechanism and free radical mechanism (Shu et al., 1985). Color removal by ozonation method:
Sludge formation is low, (2) Minimum risk, (3) Color removal and disintegration occur in one stage, (4) Application is easy, (5) Space requirement is low, (6) All residual ozone easily decomposes oxygen. Ozone-based oxidation is the most appropriate method for removing azo dyes (Sharma et al., 2013). In this work, it is aimed to purify by the ozonation method of various dyestuff groups(Reactive Red 195 and Acid Blue 284) used in the textile industry.

MATERIALS AND METHODS

Materials

Ozone production was carried out using Degremont Technologies brand Triogen model ozone generator with ambient air. The homogeneous distribution of ozone production in the water and diffusion formation in the wastewater were supported by KNF (D-79112) brand air compressor. Experiments were carried out by placing 400 milliliters of sample in a glass reactor called a 500 milliliter gas wash bottle. There are three gas washing bottles beside the reactor in the experimental setup. The number 1 bottle shown in Figure 1 is the reactor in which the dye solution is placed as the waste water sample. Bottles 2, 3 and 4 are gas-flush bottles connected in series with the reagent with a 2% KI (Potassium iodide) solution in order to determine the amount of ozone that escapes without reaction.

Potassium iodide (KI) in the experiments: Cas no. 7681-11-0 Sigma-Aldrich 99.0-100.5% purity, Sodium thiosulfate pentahydrate (Na2O3S2 * 5H2O): Cas no. 10102-17-7 Merck ≥ 98.0% purity, Sulfuric Acid (H_2SO_4): Cas no. 7664-93-9 Merck 95-97% purity. Acid Blue 284(0.5 g/l) and Reactive Red 195(0.5 g/l) paints were used for color and COD removal with ozone.

Figure 1. Ozone Experiment System

METHODS

In the ozonation experiments, 0.5 g /lt dyestuffs solutions were used as waste water samples. The dyestuffs used in the experiments were obtained from Setaş Kimya San. A.Ş. During the experiments, the following charts were followed according to the numbering given in the Fig. 1 apparatus: The number one bottle was filled with 400 ml of dye solution. Two, three and four wash bottles were filled with 2% KI (Potassium iodide) solution. The flow meter for the ozone generator was set to 10 l/min. Air pump activated. The ozone generator was activated after some air passage was achieved. The sample was taken from the reactor with a 30 minute interval. Sampling continued throughout the planned ozonation period.

RESULTS AND DISCUSSION

Acid Blue 284 (Acid Paint) and Reactive Red 195 (Reactive Paint) type paints were used in the experiments. The measurement results of the sample taken during the 4.5 hour ozonation of 0.5 g/l Acid Blue 284 solution are given in Figure 2. The influent COD concentration is 537 mg/l. At the end of the reaction for four and a half hours, the effluent COD value is 511 mg/l. The pH change range during the reaction is 1.6-5.4.
COD removal as a result of the ozonation experiment with Acid Blue 284 (Acid Paint) is given in Figure 2. Depending on the duration of the ozone, the COD removal was approximately 12% at about 3 hours. The COD increase at 180 and 270 minutes is thought to be due to the formation of organic acids and intermediates, which are completely non-cleavable (acetic acid, aldehydes, ketones) in the reaction of the dyes with ozone. The color removal (Figure 3) as a result of the 4.5 hour ozonation process is shown in Figure 3. No change was observed in the color removal during the first 1.5-2 hour period with the ozonation process. As a result, COD elimination seems to be low. From these results it was seen that during the ozonation the paint structure turned into intermediate products, no final products. The change of color from very dark to slightly darker shows that the paint structure is deteriorated.

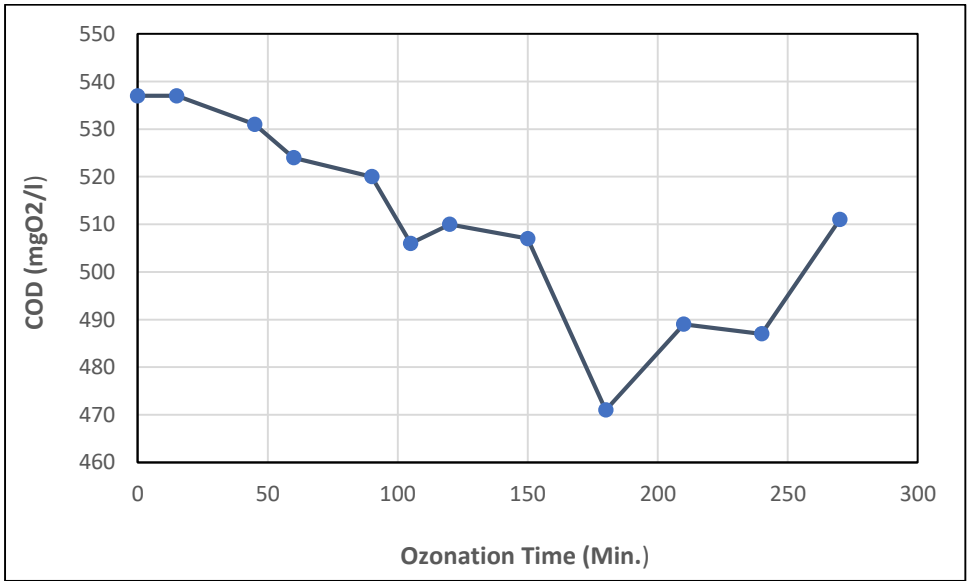

Figure 2. COD change with time as a result of ozonation (Acid Blue 284)

Figure 3. Ozonation and change of color with time (Acid Blue 284)

COD removal as a result of ozonation of Reactive Red 195 (Reactive Dye) is given in Figure 4. The results of the sample measurements taken during the 4.5 hour ozonation of the 0.5 g / lt Reactive Red 195 solution are shown in figure 3.3. The influent COD value is 287 mg/l and the effluent COD value is 186 mg / l after 4.5 hours of reaction. Depending on the duration of the ozone, the COD removal was approximately% 29 at about 3 hours. The color removal (Figure 5) as a result of the 4.5 hour ozonation process is shown Figure 5. No change was observed in the color removal during the first 1.5-2 hour period with the ozonation process. As a result, COD elimination seems to be low. From these results it was seen that during the ozonation the paint structure turned into intermediate products, no final products. The order of color change from very dark to slightly dark indicates that the structure of the reactive dye is deteriorated.

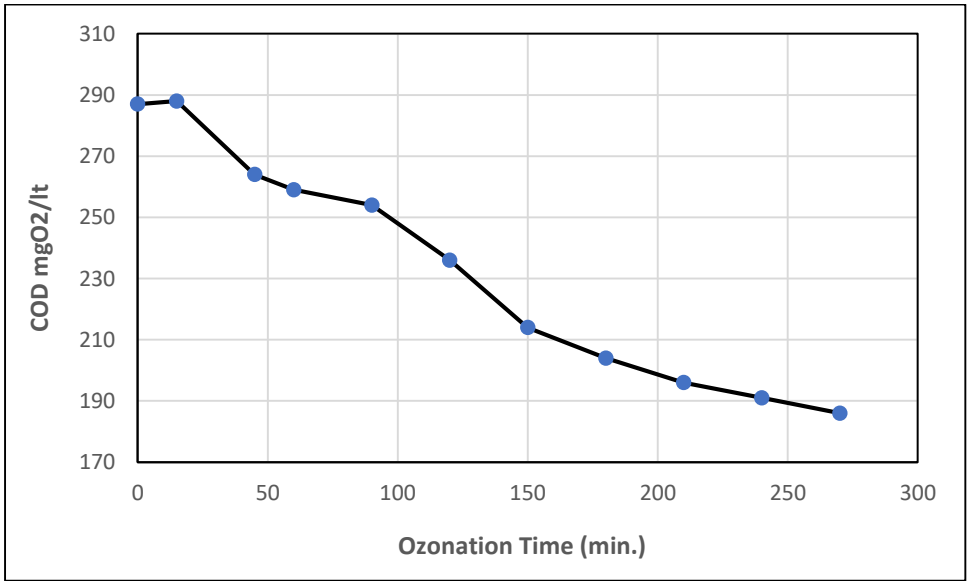

Figure 4. Change in COD Removal with Ozonation Time(Reactive Red195)

Figure 5. Ozonization and change of color with time (Reactive Red 195).

CONCLUSIONS

In this study using acid and reactive dye, it was found that the removal of COD is very low for both paint samples as a result of 4.5 hour ozone reaction. The decrease in pH value is thought to be due to the formation of organic acids. The color removal for both dyes did not change for the first few hours. In the 4.5 hour ozone reaction, a change in color from a very dark to an open color was observed. This indicates that both dye molecules are deteriorated at the end of the reaction.

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THE FINDING OF SUITABLE BIOCOMFORT AREA MAPPING FOR KARABUK CITY CENTER

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ABSTRACT

People experience nominal temperature, precipitation, and humidity, and in certain ranges of environmental conditions, such as wind, they feel healthy and dynamic. In the appropriate range for the people of these values, it is called biocomfort. When biocomfort will be in the range of fair value, people in the area would become bothered and want to get away from the area. Hence, biocomfort areas used for tourism are important.

In this study, biocomfort is examined by mapping the Karabuk, and thus, this study aims to build pad similar studies in urban or forest areas with similar structures. To this end, the climatic data of Karabuk are obtained; based on the equivalent temperature from the physiological index, biocomfort maps are prepared. To determine the structure of the biocomfort field, climatic data are collected from meteorological stations. The obtained data are evaluated using the RayMan 1.2 program, and geographic information system is used to produce a thermal perception map with the help of a software. As a result, the most appropriate time and area for outdoor recreation activities are identified by thermal perception maps.

Keywords: Landscape plan, Forest, Karabuk, Biocomft

INTRODUCTION

Urban development in developing countries in recent years has mostly been rapid and unplanned. As a result, the city is made the center of the crowd, and a dreary point has occurred. People who live in these areas that provide them opportunities transform themselves to become comfortable with their conditions. The air temperature and moisture content in this area significantly affect human health and productivity. People experience nominal temperature, precipitation, and humidity, and in certain ranges of environmental conditions, such as wind, they feel healthy and dynamic. In the appropriate range for the people of these values, it is called biocomfort. When biocomfort will exceeds in the range of fair value, people in the area would become bothered and want to get away from the area. Therefore, people live in areas in which biocomfort is very important. The per unit area in residential areas where biocomfort is more felt by people is of great importance (Milne, 2013; Olgyay, 2015; Cetin, 2015; Cetin, 2016).

Air and surface temperatures are higher in urban areas than in rural areas. Many cities are situated in natural land, and air temperature can be warmer than 13 °C. The main reason for this is that the current space in the crowd is the temperature that is linked to human activity. Increased temperature causes people to feel uncomfortable. Temperature and humidity are two of the environmental factors that affect people's comfort (Altunkasa, 1990; Cetin et al., 2010; Topay, 2012; Cetin, 2015; Cetin and Zeren, 2016; Cetin et al., 2016).

Biocomfort has been on the agenda in recent years and is an issue of growing importance. People have started to consider the values of biocomfort in landscape planning and design. When the value of biocomfort is appropriate, people are healthy, and when the dynamic range is not in the range of biocomfort, people refrain from staying in that area. Based on the urban areas nowadays, the biocomfort value range has not helped much in terms of landscape

planning and design. However, the most important factors that shape the climate biocomfort are the most important factors in determining the lifestyle of people. Air and the climate of the person's behavior and physiological state have significant effects. Human performance can be distinguished based on the climate change. Comfortable climatic conditions make these positive responses. Climatic conditions or conditions that are healthy for human thermal comfort of mood and dynamic weather conditions refer to the human sense of satisfaction with the thermal environment (Cetin et al., 2010; Topay, 2013; Cetin, 2015; Cetin and Zeren, 2016; Cetin et al., 2016).

In the middle latitudes where our country is located, the perceived temperature value that is considered to be suitable for bioclimatic comfort, depending on humidity and wind, ranges from 17 °C to 24.9 °C. According to Altunkasa (1990), 21–27 °C temperature if all other conditions are normal and showed that together create a comfortable environment with relative humidity of 30–65%. Below or in value on these conditions, to achieve the bioclimatic comfort temperature or radiation or energy or shadow report that they are needed in specific wind and humidity. However, considering these negatives in the planning is crucial in fulfilling the purpose of landscaping (Cetin et al., 2010; Cetin, 2015; Cetin and Zeren, 2016; Cetin et al., 2016).

This study evaluated whether the Karabuk detailed analysis of meteorological and climate data for landscape planning was transferred into a database. This database is intended for climate balanced planning and design purposes. Planning and design and other criteria, such as climate balanced planning and design, are used to show the creation of a bioclimatic assessment.

MATERIALS AND METHOD

The study material is Karabuk and the surrounding area. The Karabuk district, located in the Black Sea coast, the Western Black Sea Region. It is with coordinates of 41° 11' north latitude and 32° 37' east longitude and the values were evaluated and processed using GIS maps, and the map obtained is shown in Figure 1. The surface area of the region of Karabuk has its natural, cultural, and recreational resources have a high value. In the region, the majority of the year is dominated by the Black Sea rainy climate. The average annual temperature is 19.4 °C. The highest temperature is 22.2 °C to 23.2 °C, which usually occurs in July and August. January is the coldest month at −5.2 °C. The northern slopes of the area get more rain (Municipality, 2016; Meteorology, 2016).

In this study, the climatic data of Karabuk were identified; the equivalent temperatures based on the physiological index in biocomfort maps were prepared. To determine the structure of the field of biocomfort, climatic data were collected from meteorological stations. The obtained data were evaluated using the RayMan 1.2 program, and geographic information system (GIS) was used to produce a thermal perception map with the help of a software. The results showed that the best biocomfort fields using ArcView software has been tried to be determined.

Working primarily in Karabuk, data were obtained from the meteorological station of the research area. The annual temperature was determined using data obtained from the annual average value and converted using the RayMan 1.2 program (Matzarakis et al., 2007, Matzarakis et al., 2010). To view the map associated with it, humidity and wind speed maps were created. The theoretical basis of the study of climatic factors related to climate comfort was evaluated in terms of the Karabuk. This study provides the most accurate map of the climatic data obtained in the field. ArcView GIS mapping with linear interpolation Kriging

interpolation option ESRI software was used. As a result, the climatic factors in Karabuk and bioclimatically suitable areas were identified and evaluated in terms of comfort level.

Figure 1. The location of study

RESULTS

The temperature and relative humidity values of the Karabuk between 1968 and 2015 at 2.00 p.m. were obtained from the general directorate of the State Meteorological Service (Meteorology, 2016).

The resulting ArcView 10 software maps, climate with the Kriging interpolation method was used to form an appropriate space. To evaluate the data, the universal linear extension system was used because it provided the most accurate climate data distribution in the area. The annual average temperature of the study area was evaluated. The biocomfort value of the town of Karabuk ranged from 15 °C to 21 °C, and the temperature with the value of the study area was determined to focus on the northern and southern regions.

The optimal relative humidity range of the Karabuk district in terms biocomfort was determined to be 51%–56%. The annual average relative humidity values in the northern part of the study area examined are increasing. This condition is linked to the presence of the sea and reduces the comfort value of the northern part of this work area. The optimal values in terms of the annual average wind speed biocomfort were 1.7 and 2.1 m/s. The average annual wind speed value in the study area in the northern and central regions was 2.02 m/s, and therefore, the annual average wind speed in terms of biocomfort value of the parts was the highest. The study area was determined to be the north and midlands.

Comfort area produced maps for the study area was classified for each month. The average value for each field on the resulting bioclimatic comfort conditions, considering the 12-month map, allowed us to obtain the map that coincided with the annual value of the sensed temperature. The classified 12 month program was assessed using the ArcGIS raster data function. As a result, average values were calculated for each 12-month scan. The annual sensed temperature ranges divided in the map were subjected to grading. The obtained data were evaluated and shown all over the map based on biocomfort suitable or unsuitable areas and areas designated as a suitable comfort zone. Consequently, a bioclimatic map of the Karabuk district was analyzed. Temperature, relative humidity, and areas for optimum wind speed are shown in Figure 2.

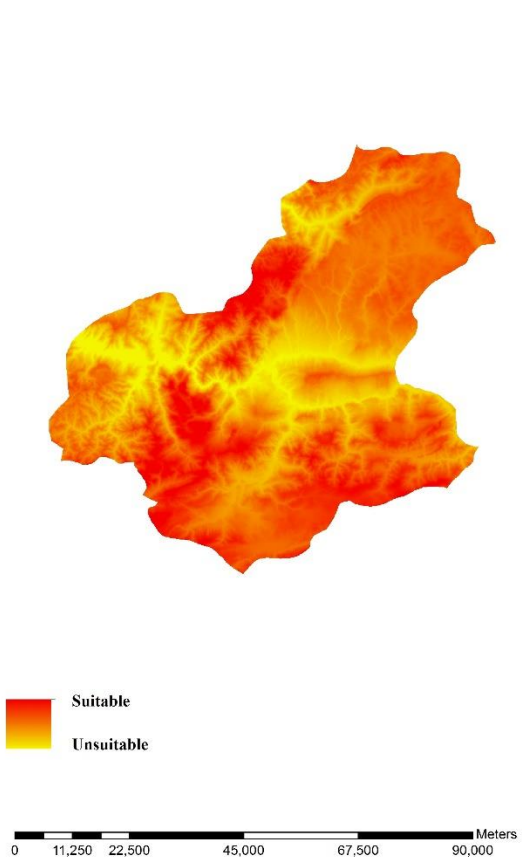

Figure 2. Karabuk biocomfort areas

DISCUSSION AND CONCLUSIONS

The values obtained in this study affect the comfort bioclimatic conditions of the residential area being studied and reduce biocomfort. The bioclimatic comfort temperature range of Karabuk is from 15°C to 21°C. However, the humidity is changing the sensed temperature value. The study results, is the temperature, when the relative humidity and evaluated in terms of wind speed, the Karabuk district of the remaining forest and coastal strip outside the city residential area, in the northwestern area shows the western and the southern part is suitable areas for bioclimatic comfort.

Based on the results obtained in evaluating the map, the total surface area of the available space for bioclimatic comfort is approximately 211 km^2. The town of Karabuk appears to cover the portion of approximately 183 km^2. The total area of the town of Karabuk. Approximately 80% of this area is considered suitable for biocomfort section. The total area of 28 km^2 is eligible for bioclimatic comfort. The ideal value of the relative humidity in the region bioclimatic comfort can be interpreted as a consequence of being present.

Not very convenient in terms biocomfort, namely, negative values with bioclimatic comfort areas, mostly located in the northeast part of the county were determined.

The absolute comfort of bioclimatic conditions of the area should be considered during landscaping. Improper conditions in the planning and design of bioclimatic comfort can create extremely unfavorable conditions because the natural vegetation is under stress and prevents the formation of hot or cold areas suitable for intensive bioclimatic comfort in areas.

In this study, the bioclimatic comfort zone for the Karabuk was identified. The resulting maps and data can be useful for future town planning. Bioclimatic comfort, so the removal of the regional planning structures Karabuk. The highest level of comfortable climatic conditions will help in determining the limits of comfortable housing population. The study results related to new residential and recreation areas can help in planning the locations of public buildings.

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THE ASSESSMENT OF ECOTOURISM POTENTIAL FOR THE CASE STUDY OF KARABUK AREAS

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ABSTRACT

Karabuk has an important historical value and great potential because of its outstanding natural and cultural heritage. It is an area that can make an important contribution to the entire region. But in order to do so, making long-term plans for the area and its surroundings, creating the necessary infrastructure, and promoting it locally and internationally are required. This study deals with the ecotourism resources of Karabuk that include its historical, cultural, and natural landscape to determine its potential classification in terms of values that can be a source of tourism activity and are intended to be mapped. For this purpose, the value of the tourism resources that constitute Karabuk's values such as maps, photos, and surveys were evaluated in light of data collected as a result of work done in the area and of existing and potential ecotourism activities. Then the appropriate fields for the specified activities creating a digital base were determined. During the evaluation and processing of data, they were used to map the ArcGIS program. In conclusion, Karabuk's ecotourism resources including its historical, cultural, and ecological values were identified and mapped.

Keywords: Forest, Karabuk, Ecotourism, ArcGIS

INTRODUCTION

Natural resources are vital to human life. However, especially in the last century, the irresponsible use of natural resources has become one of the most alarming problems threatening nature and the environment. Therefore, global warming, climate change, air pollution, the irresponsible use of water resources, and the sustainable use of natural resources in all areas have been brought to the agenda (Cengiz, 2007; Maple et al., 2013; Cetin and Sevik 2016a,b). For centuries, people have been inspired by nature, and tourism activities have been carried out to see different natural landscapes. In particular, coastal tourism is the hippest form of tourism in recent years. Visiting coastal areas, swimming, water sports, and hiking have been quite attractive for people, and millions of people have started to carry out tourism activities in this manner to forget, albeit for a while, problems in the city and to participate in natural and cultural activities, desiring to make it even more valuable. Natural, cultural, and historic sites are seen with great interest. However, the intensive use and increasing interest in the area also brings the risk of damage, so establishing the right balance between the protection and the use of these areas is of paramount importance (Nouri et al., 2008; Cetin 2015a,b,c,d; Cetin 2016).

Removing the environment and wildlife conservation from the forefront and the importance of ecotourism from tourism, this process has increased and continues to increase. Ecotourism focuses on unusual properties, promoting natural and cultural areas, and providing a positive contribution in many areas. Areas where ecotourism is applied become the center of attraction. Ecotourism activities are carried out with a general awareness in the use of the natural environment. Besides holding activities for the protection of natural and cultural values

for economic use, to adjust the balance between protection and use is the sine qua non of sustainable tourism, and it can be achieved through the natural and cultural aspects of sustainability in the field of the foreground and environmentally sensitive ecotourism, as well as aesthetic, recreational, or cultural activities that protect delicate ecosystems. Education can be brought in the forefront as awareness (Powell et al., 2008; Nouri et al., 2008; Cetin 2015a,b,c,d; Cetin 2016).

Ensuring cooperation among the local population is one of the most important requirements for planning in the tourism sector. In the long term, natural resources, tourism, alternative tourism, ecotourism, and nature tourism are becoming an important choice based on sustainable use. In this study, the natural and cultural aspects of coastal areas are intended to reveal the potential of ecotourism in Karabuk districts.

MATERIALS AND METHODS

The study material includes Karabuk districts. The geographic location of Karabuk districts with rich natural and cultural areas are found between the coordinates 41° 11' north latitude and 32° 37' east longitude and the values were evaluated and processed using GIS maps, and the map obtained is shown in Figure 1. The position of Karabuk district is shown in Figure 1.

Figure1. Karabuk geographical location

Located in coastal areas in the West Black Sea Region, Karabuk is a mountainous and rugged terrain. The average altitude is around five meters in the central district. It is 121 km from the city center through the district (Municipality, 2016; District Governorship, 2016).

Temperatures in winter fall as much as 5 degrees and in summer around 30 degrees. The beach continues to increase toward the interior upgrade. With a mild climate, during winter season on the beach, the altitude of the large amount of snow in the inner part is over 1000 meters. Winter rain is experienced in coastal areas, while inland, snow is seen. The summers are hot and arid.

While old houses are made of wood, because of the concrete forest district, new homes are now made of wood and brick. In indigenous places, slate stones are used in the roof. Due the forested and mountainous region made up of many streams are merged through the Black Sea.

Meseta and traces of the castle by the sea can be seen in the fishing ports in the district center. Glade is the oldest inhabited settlement. In Ilyasbey, there is nothing more than ruins.

Our district is full of natural beauty for tourism due to take place on the beach. It has unique landscapes that combine the sea and the forest, the green and blue intertwining.

In the study, geological, hydrological, topographical, and soil maps were obtained. Then the climate of the region and flora and fauna information were collected. The obtained data can be fed into computers belonging to the area with the help of ArcGIS maps, and the data is processed on the maps. During the fieldwork in the study area, surveys, opportunities, and constraints were determined by considering various pieces of information regarding the local population. SWOT was used in analyzing the obtained data, and these data were evaluated using maps. All the data and created maps of areas suitable for ecotourism were evaluated to develop an alternative to the current use of the area's strengths and weaknesses in terms of ecotourism, and threats and opportunities were identified.

RESULTS

The coastal areas, forest areas, forest openings, potential areas for accommodation, and recreational facilities in the Karabuk districts are rather limited. Landscape, coastal areas, nature and culture, vegetation and wildlife, geological and hydrological conditions, and soil have values in Karabuk. Given the topographical features of the Karabuk districts, the slope of the high woodlands and narrow coastal strip limit the number of suitable areas for construction.

The geological, geomorphological, and geological structures of the soil groups in the region have interesting features, such as coastal and mountain formations. The height and slope analysis of Karabuk are shown in Figure 2. Different geomorphological character movements in the valley are characterized by ridges and peaks. This geomorphological structure creates an extraordinary natural landscape with lush forests.

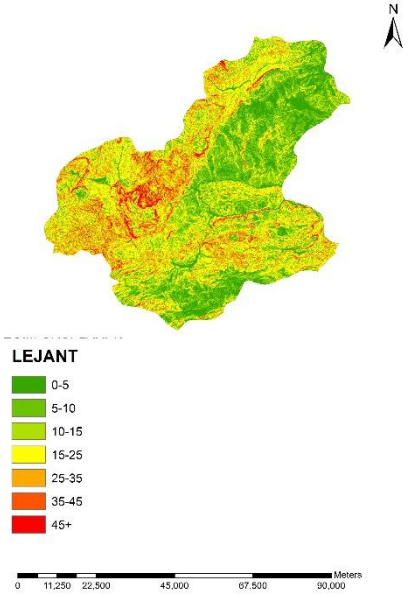

Figure 2. Karabuk slope analysis

The Black Sea climate prevails in the region. Summers are hot, and winters are usually mild. Adequate rainfall is experienced in all seasons. The number of visitors per day during the tourist season ranges from 15,000 to 20,000. During this period, the following activities can be done: hiking, camping, photo safari, trekking, cycling, and amateur fishing. Many recreational activities, such as botanical tourism and air sports, are also offered. The Karabuk survey was conducted to determine the protection of the coastal area in the protection-use balance and to determine the level of awareness. The SWOT analysis was conducted, and the opportunities

that may be encountered in the future, as well as the current strengths and weaknesses and threats identified in the area.

Areas under ecotourism activities in the study area, coastal tourism, historical sites, bird observation areas, natural monuments, springs, rivers, canyons, bike tours, hiking, photo-jeep safari, camping, rock climbing, cave, winter tourism hunting-fishing, paragliding, wind, water, surf, cultural, and thermally processed tourism maps are shown in Figure 3.

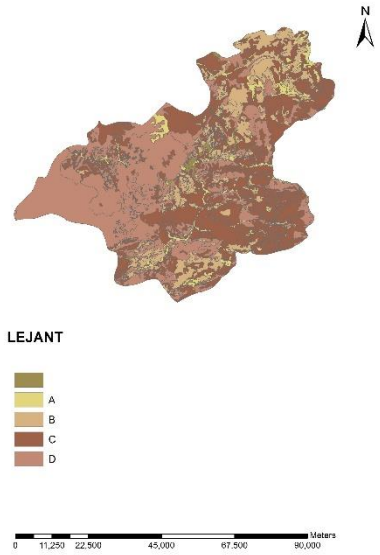

Figure 3. Potential Karabuk ecotourism map

DISCUSSION AND CONCLUSION

Once it is known enough to become one of the most important tourist areas in the Karabuk, even the Black Sea will take its rightful place in tourism. Enough accommodation is needed so there is no reason to eliminate openness to tourists. There are those rare places where natural beauty remains intact in their natural state. On weekends, families can come and go easily, although there are tea gardens and recreational areas where they can have a picnic in Karabuk and the surrounding counties that are not known.

For the development and economic growth of countries, the importance of sustainable tourism activities with an ecological approach is great. Sustainable ecotourism can be achieved only with a proper approach to the ecological characteristics of tourism development and management plan. To set up an ecotourism strategy in the area of tourism and recreational activities, there is a need to organize and have a good planning. The study area has quite a high tourism potential in terms of tourism and recreation. Karabuk's scarce resources and assets allow different tourism activities in different seasons. The Karabuk District's beach, historical sites, photography, bird watching, adventure and sports tourism, historical and cultural tourism, wildlife tours, caves, camping, picnic activities, horse riding, cycling, and rich ecotourism activities such as fishing have a potential.

Karabuk's regulations on the forest and coastal for ecotourism has a significant potential. However, the routes for many activities and lack of contacts and facilities are a major problem. The solution to these problems and the use of the potential of the region, as well as the process involved, can provide important economic contributions to the region.

Karabuk is rich in vegetation, and the mountain tourism potential is high in the region. In the area of botanical gardens, arboretum structures that promote the region's biodiversity can make an important contribution to increase the public's awareness and so on. In addition, the

area contains interesting examples of different geological and geomorphological structures. In terms of biodiversity, this area may be of interest to enthusiasts and researchers in particular.

Ecotourism in the region has a high potential. Uncontrolled development, overuse, and destruction in value depending on their source cause excessive pollution. In the planning, this issue should be taken into consideration.

Areas suitable for various activities are shown in Figure 3. This map of ecotourism infrastructure, site selection, planning, design, transportation, management of solid waste pollution, and sewage systems can be used in accommodation facilities and the natural environment and can also be utilized for taking measures to protect wetlands.

In addition, historical and cultural tourism resources have been identified and are again. A natural park, an ideal area for the observation of wild animals, offers substantial opportunities for wildlife tours. Ideal plateau areas for wildlife tours are also shown in Figure 3. The use of these fields also needs to be done with trained guides and an orientation on plants.

There are problems in the area of transport, especially in regional transport, which as to be available and seamless. In addition, the area is very safe, and people are very hospitable. Promotion should be done in this regard. Also, at the entrance to the Karabuk, information about important areas in the region must be available, and promotion should be done by putting up maps. Also, the introduction of the place should be done at the national and international levels.

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THE ASSESSMENT OF ACCESSIBILITY OF URBAN GREEN AREA FOR KARABUK

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ABSTRACT

An urban green space distribution of active and functional and aesthetic qualities of size and systematic planning will be possible with the development of an urban design concept. The adequacy of the standard value of green space is usually specified in the relevant legislation and comparing it with the amount of green space available per city are defined. Size and decreasing rates per person evaluated. Even distribution of distance and accessibility of green space throughout the city is closely related to the need to provide entertainment. Different sizes of green space, recreational activities and accessibility standards unit varies depending on the city they serve. In this research, Karabuk green field distribution and distribution of the amount of green space in the neighborhood, as well as scale are analyzed. According to the digitization of parks composed of polygons in the ArcGIS attribute table for calculation, parks in the study area consist of 30 different parcels, 12 of which are 10000 m^2 or less in area. Most small parklands were 1.296 m^2; the largest urban park is at the southern entrance of the city, with an area of 5.624 m^2. Parks in the study area cover a total area of 8.358 m^2.

Key words: *GIS, Karabuk, Green space, Urban city, Sustainable*

INTRODUCTION

Urban green areas as quality active and passive recreation activities, provides many functions, such as providing environmental benefits and is a habitat for wildlife. Urban green spaces are areas that have been established to meet the recreational needs of urban people. Green spaces, but vary according to country in terms of layout and design features from one region to the country, basically has been created to allow the meeting with human nature (Arnberger & Eder, 2012; Barthel et al., 2013; Caspersen and Olafsson, 2010; Cetin et al., 2010; Cetin, 2015a; Cetin, 2015b; Cetin, 2015c; Cetin, 2015d; Cetin, 2015e; Cetin, 2016a; Cetin, 2016b; Cetin, 2016c; Cetin and Sevik, 2016a; Cetin and Sevik, 2016b; Cetin and Sevik, 2016c; Cetin and Sevik, 2016d; Cetin et al., 2016; Panduro and Veie 2013; Schipperijn et al., 2010; Wendel et al., 2012; Wolch et al., 2014; Zhou and Parves, 2012).

Urban green space and traffic emissions, air quality, microclimate, noise, accessibility, providing there are effects on the economic impact and social benefits. This affects the neighbourhood scale should be considered in urban planning and design of green space for various green areas that serve the entire urban area. Adequacy of green areas has been evaluated by several researchers (Conway et al., 2010; Coombes et al., 2010; Dai, 2011; Cetin, 2015a; Cetin, 2015b; Cetin, 2015c; Cetin, 2015d; Cetin, 2015e; Cetin, 2016a; Cetin, 2016b; Cetin, 2016c; Cetin and Sevik, 2016a; Cetin and Sevik 2016b; Cetin and Sevik 2016c; Cetin and

Sevik 2016d; Cetin et al., 2016; Panduro and Veie 2013; Schipperijn et al., 2010; Wendel et al., 2012; Wolch et al., 2014; Zhou and Parves 2012).

Cetin (2015a) criteria for determining the potential recreation areas have been set taking into account the views of the landscape architect and urban planning expert. In this context, compliance with recreational use criteria in order of priority; the presence of plants, transportation, slope, water availability, drainage, rainfall, temperature, and altitude indicators were identified as erosion. On the other hand Cetin and Sevik (2016c) increases the potential value of the recreational green areas; proximity to the presence of water, cultural values, accessibility, vegetation, slope, visual values, climate, altitude, soil and has set bond (Cetin, 2015a; Cetin, 2015b; Cetin, 2015c; Cetin, 2015d; Cetin, 2015e; Cetin, 2016a; Cetin, 2016b; Cetin, 2016c; Cetin and Sevik, 2016a; Cetin and Sevik, 2016b; Cetin and Sevik, 2016c; Cetin and Sevik, 2016d; Cetin et al., 2016; Panduro and Veie, 2013; Schipperijn et al., 2010; Wendel et al., 2012; Wolch et al., 2014; Zhou and Parves, 2012). Over similar recreational suitability criteria are defined basically by different researchers. In this study, recreational green areas of spatial magnitude affecting important that the criteria parks include the equipment and access in the use of the Karabuk considering accessibility and slope Contiguous Zone has tried to reveal the appropriateness of green areas.

MATERIAL AND METHOD

The study material is Karabuk and the surrounding area. The Karabuk district, located in the Black Sea coast, the Western Black Sea Region. It is with coordinates of 41° 11' north latitude and 32° 37' east longitude and the values were evaluated and processed using GIS maps, and the map obtained is shown in Figure 1. The surface area of the region of Karabuk has its natural, cultural, and recreational resources have a high value. In the region, the majority of the year is dominated by the Black Sea rainy climate. The average annual temperature is 19.4 °C. The highest temperature is 22.2 °C to 23.2 °C, which usually occurs in July and August. January is the coldest month at −5.2 °C. The northern slopes of the area get more rain It has significant natural, cultural and recreational resource values at regional and national scale. In the region, the climate of the Black Sea is predominant, with most of the year rainy. The average annual temperature is 16.5°C and the highest temperature is 27.4°C in July and 27.2°C in August. The coldest month with January -7.5°C. The north region of the zone gets more rain (Municipality, 2016, Meteorology 2016). Within the scope of the study, geology, hydrology, topographic and soil maps belonging to the study area were provided. Then the climate, flora and fauna of the region were collected. The obtained data are entered into the computer and maps of the region are created with the help of Arc GIS software and processed on the maps. In this study it was obtained from the Municipality of Karabuk numerical data used in the park. In the study: 1/5000 Master Plan, 1/100000 scale topographic map data and GIS is utilized (Figure 2).

Karabuk area of green space within standard that given revealed that on the basis of the slope groups; (1) are classified according to their spatial size criteria, (2) Effective service area (radius) by accessibility analysis, (3) slope analysis was conducted in the area of effective service (Cetin, 2015a; Cetin and Sevik, 2016c). Recreational slope of the distance and the distance until transportation is one of the most important factors affecting the use of recreational areas. Accessibility within walking distance defined by the people, can be reduced depending on the distance of the slopes. In this context, the slope groups, especially so

considering the accessibility of persons with disabilities (0-4), (4-5), (5-8), (8-12), (12 <) are classified into 5 groups. The showing Figure 2 are the different slope groups in Karabuk. (Cetin, 2015a; Cetin and Sevik, 2016c). The analysis performed in this study Arc GIS software (Arc Map Version 10) was performed using.

Figure 1. Geographical location of Karabuk and its immediate surroundings

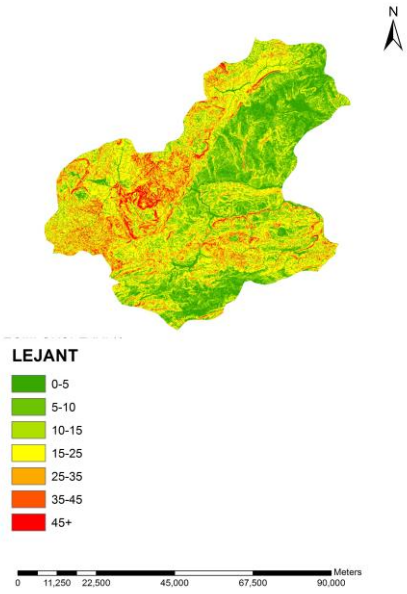

Figure 2. Karabuk slope analysis

RESULTS

In this study, Karabuk district adjacent area of the park boundary in a total of 12 were evaluated. The parks discussed under the research, based on area size and categorized in terms of accessibility, provincial district park, it was determined that the neighborhood playground and 3 different parks size can be defined as a children's playground. The classification result;

1 district park, neighborhood park, 2 and 1 has also been found that a total of 4 parking can be defined as a playground or sports field. In these studies, 1 also under evaluation to be defined as 30 parking spaces and green areas are considered substandard. Karabuk district park, neighborhood park and playground accessibility map is given in Figure 3. The city, with a total of 30 parking less than 1000 m^2 has been identified as substandard parks. The size of these parks between 8 to 1 in 12 for parking, which is made of the accessibility analysis using the effective service radius of 200 m (Figure 3). Analysis showed a total of 213 m^2 of parks in ideal conditions it was found that accessibility is possible. However, due to the creation of the position of parking availability status without considering the availability of parking space coincide. This is the availability of parking space has led to a fall from 2130 m^2 to 1800 m^2. Accessibility slope groups in the border (0-4%, 4-5%, 5-8%, 8-12% and 12% <) was evaluated in five grades. Spatial extent of these classes, respectively, 879, 97, 200, 286 and 754 m^2.

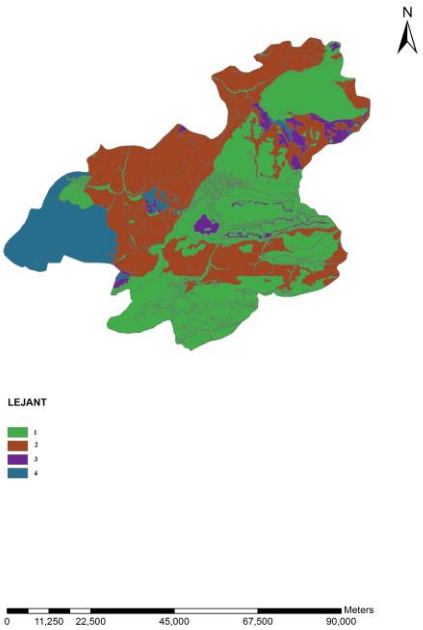

Figure 3. Karabuk district park, neighborhood park and playground accessibility map and availability of Karabuk substandard parking map

Neighborhood Park: When the neighborhood parking availability within the boundaries of the slope groups studied, 0-4% slope areas with 581 m^2, slope areas between 4-5% 62 m^2 slope area between 5-8% of 85 m^2, slope areas between 8-12% 86 m^2 and the area with the slope values above 12% were found to be 212 m^2. Looking at the present topography slope groups within the boundaries of the neighborhood parking availability, accessibility ideal slope of a significant portion of the area, especially people with disabilities, it is seen that the value will prevent access to the area. Neighborhood parks of the area of 268 m^2 as an important part of the city adjacent areas due to remain outside the boundaries of the park district has not been calculated for this section of slope group. Neighborhood Parks: The neighborhood slope groups in the availability of the boundaries of the park are analyzed, 0-4% slope areas with 63 m^2 slope area between 4-5% 16 m^2 slope area between 5-8% 91 m^2 slope area between% first 8-12 120 m^2 and the area with the slope values above 12% were found to be 473 m^2. An important part of the area of accessibility as 80% of the ideal neighborhood parks are not accessible in terms

of slope. The neighborhood is close around the intensification of the park of high slope in the availability border of the park, while the lower slope accessibility limits of the extreme accessibility in because of the concentration limit of 20% part because of the slope distribution within is not accessible. Children's Playground: The accessibility within the boundaries of the slope groups studied, the area up to the slope of 4% 103 m^2, sloping areas between 4-5% 6 m^2, sloping areas between 5-8% 5 m^2, sloping areas between 8-12% 7 m^2 and% in the area with the slope values over the 12 it was found to be 21 m^2. The standard limits the availability of underground parking inclination group in the assessment; areas with 4% slope 132 m^2, 13 areas with slopes between 4-5% m^2, 19 areas with slopes between 5-8% m^2, 10 areas with slopes between 8-12% m^2, the area has a slope of over 12% 39 was found to be m^2. Standard six parks, its effect on the accessibility of the slope is more advantageous than other parks group class.

CONCLUSIONS

Karabuk district area within the boundaries of the study for the park; 1 neighborhood park of 93 parking spaces, parking 3 different size may be 2 and 1 neighborhood park playground have been identified. 8 in the bottom of the 89 parking spaces were identified as substandard parks. Availability of these parking areas (effective service areas) respectively 1026 m^2, 763 m^2, 142 m^2 and 180 m^2. When Karabuk district area of public parks accessibility in all circumstances considered together; availability of ideal distance, and could not reach the park from 69% of the study area (Figure 3). On the other hand parks able to achieve the accessibility of the park due to conflict with each other border areas it is narrowed. Conflict with an ability to reach areas of the park are a positive feature when under normal conditions, the distribution and availability of parking space in the city has decreased recreational services when considered together. Made unplanned and the needs of more than one park to reach the city's green space system in any park shuttle when it is not possible at some point in the district indicate that at some point meet. When parking on a slope groups in the field of research evaluation in accordance with accessibility limitations; 0-4% 4-5% 5-8% slope can be achieved easily reach the park between groups. Park within the boundaries of accessibility for the comfort provided by the park district's transportation groups slope 688 m^2, 170 m^2 for neighborhood parks, a children's garden for 114 m^2, covers 164 m^2 for substandard parks. Karabuk district areas within the boundaries of the park provide comfortable areas where the transportation of the group in accordance with the inclination limit accessibility covers 1176 m^2. Within the boundaries of the park accessibility 8-12% and 12% <area between the slope groups are the areas where it is difficult to access or lack of transportation. This area of 298 m^2, respectively (neighborhood parks) 593 m^2 (neighborhood parks), 28 m^2 (playground) and 49 m^2 (substandard parks) covers. Karabuk district area according to the inclination of the accessibility limit set in the park in the adjacent area in which it is difficult to transport or transportation are not possible areas cover 1031 m^2. The availability and attractiveness of green areas have a part of urban life quality the easy availability of green spaces can help to increase the physical activity of it. Karabuk in the study of the park users to be close to the first preferred parking spaces (38%), size (20%) and easy accessible ability (18%) chose the criteria. These results demonstrate the importance of evaluating the accessibility of the park. 38% of the contiguous area within the boundaries of settlements (379.6 m^2) to enter into effective service area of the park. 62% of the residential area (630.3 m^2) is effective outside the service area of the park. In these areas, there arises a need for the establishment of the park in standard sizes. Karabuk district area Contiguous Zone will be held on considering accessibility in urban green

space planning and design studies indicating parking balanced distribution system should be established.

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THE EFFECT OF DIFFERENT DRYING METHODS ON TOTAL PHENOLIC CONTENTS OF KABAAŞI APRICOT VARIETY

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ABSTRACT

The origin of Kabaaşı apricot variety is Malatya. It is evaluated as table and dried. Amount of dry matter soluble in water varies between 24-26, the fruit meat hardness is hard texture. Malatya apricots are known to be an important source of phenolic compounds. Phenolic compounds are important in terms of food composition, their effects on the taste-odor formation, their participation in the mechanism of color formation and change, their antioxidant and antimicrobial properties. Phenolic compounds not only prevent the oxidation of foods but also protect the human body from harmful oxidative effects. This study is important to determine the specificity of identification of the phenolic compound compositions of Malatya apricots and to reveal differences among varieties. Kabaaşı apricot sample suitable for drying was applied to sun drying (SD) and sulfur drying (SuD) and oven drying (OD) methods. Changes in the amount of total phenolic contents (TPC) were determined after drying with drying methods. The amount of TPC was determined spectrophotometrically by modifying the Folin-Ciocalteu method. The TPC values of the samples were calculated as the sum of the amounts of water-soluble and methanol-soluble substances as gallic acid equivalents. The amount of TPC in the Kabaaşı apricot sample was determined as 187.22 mg GAE100 g DM^{-1}, whereas the quantities of 135.91, 229.46 and 154.06 mg GAE100 g DM^{-1} TPC were determined in SD, SuD and OD methods, respectively. It was determined that the amount of TPC was increased due to sulfur application and quantity in the SuD method.

Keywords: *Malatya apricots, Total phenolic content, Gallic acid*

INTRODUCTION

The origin of Kabaaşı apricot variety is Malatya. It is evaluated as table and dried. Amount of dry matter soluble in water varies between 24-26, the fruit meat hardness is hard texture.

MATERIAL AND METHODS

Materials

Kabaaşı fresh and dried apricot variety is presented in Figure 1.

Figure 1. Dried Kabaaşı apricot sample

METHODS

1 g of the wet sample was taken from 0.5 g of the dried sample, homogenized with 25 mL of pure methanol for 2 minutes, then allowed to stand overnight at + 4 ° C. Centrifugation was carried out for 20 minutes at 9000 rpm in a refrigerated centrifuge (Nüve NF 800 R, Türkiye). Kabaaşı wet apricot samples were taken out by dissolving in methanol and then the process was repeated by adding 25 mL of purified water to the same phase in the amount of TPC dissolved in water. The TPC amount was determined spectrophotometrically by modifying the Folin-Ciocalteu method (Thaiponga ve ark. 2006; Re et.al. 1999). The TPC amount is calculated as the sum of the water-soluble and methanol-soluble substance values. Curve was drawn by spectrophotometer with standard solution (Shimadzu UV-120-01, China) prepared at 5 different concentrations (mg / mL) of gallic acid (Sigma-Aldrich, Germany) and the absorbance results of the samples were obtained as mg GAE 100 g DM^{-1} It was calculated (Table 1.)

Table 1. Concentration of Gallic acid equivalent

0,1 mL →	100 mL	→5 ppm
0,2mL →	100mL	→10 ppm
0,5mL →	100 mL	→25 ppm
1mL →	100 mL	→50 ppm
2 mL →	100 mL	→100 ppm

KONS	ABS
5	0.043
10	0.107
25	0.302
50	0.566
100	1.091

Figure 2. Concentration and Absorbance Value of GAE

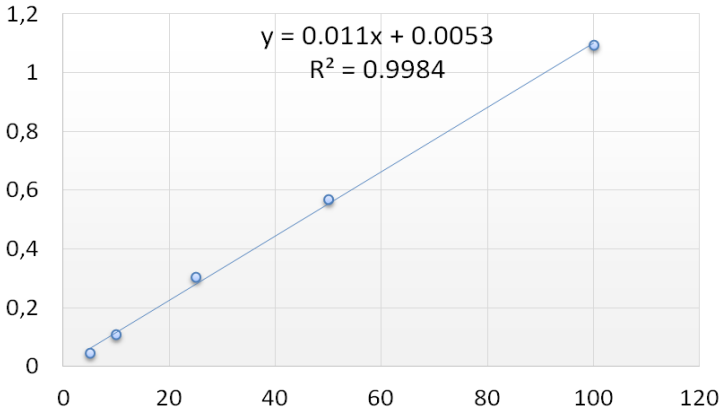

Figure 3. Calibration Curve of Gallic acid

RESULTS

The amount of TPC in the Kabaaşı fresh apricot sample was determined as 187.22 mg GAE100 g DM^{-1}, whereas the quantities of 135.91, 229.46 and 154.06 mg GAE100 g DM^{-1} TPC were determined in SD, SuD and OD methods, respectively. It was determined that the amount of TPC was increased due to sulfur application and quantity in the SuD method.

Table 1. Kabaaşı apricot samples TPC values of water + methanol extracts

Apricot sample	Water GAE100g DM^{-1}	Methanol GAE100g DM^{-1}	TFM (Water + Methanol) GAE100g DM^{-1}
Kabaaşı FF (Fresh apricot)	32.05	155.16	187.22
Kabaaşı SD (Sun Dried),	29.36	106.55	135.91
Kabaaşı SuD (Sulphur Dried),	63.37	166.09	229.46
Kabaaşı OD (Oven Dried)	19.94	134.12	154.06

CONCLUSIONS

The amount of TPC value determined 229.46 mg GAE100g DM^{-1} in SuD samples (Table 1). A reduction in the amount of TPC of the apricots with drying was generally determined. It is known that phenolic compounds are used as substrates in the enzymatic browning reactions that occur during drying in the sun.

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A NEW SIGHT IN PARTIALITY AND IN WHOLENESS ON THE ORGANISM IDIOTYPE ACTION

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ABSTRACT

In this work is *given, with a dose of conditionality, a new concept for the ingredients of the results of action of one or another combination of the genetic determinants. First is the action of the so cold ***non-Mendelian*** -, ***cytoplasmic*** -, or preferred by the author of this article ***extrachromosomal inheritance (heredity)***, valid for all the bionta, exluding the species of pre-cellular organisms. That is why, the sum of the action of these genetic determinants is *named ***ech-zigosis*** and ***ech-osis*** or ***eci-zigosis*** and ***eci-osis***, and *designated with the symbol $\pm$ d_{eci}. The second sum of actions is the *named as ***hemizygosis*** or ***hemisis***, *marked with the symbol $\pm$ d_{hem}. The third sum of actions is named ***homosis*** or - ****homozygosis,*** here *designated as $\pm$ d_{hom}. and the fourth sum of actions is - the ***heterozygosis*** or ***heterosis,*** here *symbolized as $\pm$ d_{het}. The last three single summary effects are *joined together, and *named with the terms ***ch-osis*** and ***ch-zygosis*** or ***ci-osis*** and ***ci-zygosis***, and their total sum is *marked with the symbol $\pm$ d_{ci}. *New terms are also implemented for the summary effect of the whole or holistic combination of the hereditary determinants - ***holozygosis*** or ***holosis.*** For the pre-cellular organisms are suitable only the terms ***hemisis*** and ***holosis***. But the terms ***ech-osis*** or ***eci-osis***, ***hemisis*** , ***ch-osis*** or ***ci-osis*** and ***holosis*** are suitable for all the species of prokaryotes, and during all the phases from the *so-named stadium of ***gametobiont,*** being either ****androgametobiont*** or ****gynogametobiont***, from the particular contiguous windings (generations) of the screw lines of the survivals of the birth populations of eukaryotes, either diploid, auto- or allopolyploid. The terms ***ech-zigosis (eci-zigosis)***, ***hemizygosis***, ***homozygosis***, ***heterozygosis***, ***ch-zygosis (ci-zygosis)*** and ***holozygosis*** are suitable during all the phases from the *so-named stadium of ***zygotobiont*** from the particular contiguous windings (generations) of the screw lines of the survivals of the birth populations of eukaryotes, either diploid, auto- or allopolyploid, while in these circumstances the terms ***homosis*** and ***heterosis*** have not to be used. This whole or holistic effect is *designated with the symbol $\pm$ e_{hol} , and is composed by the sum of the four summary single effects and by the addition of all possible interactions between them. Interactions are *labeled with the symbol $\pm$ b with subscripts for the respective double, triple or quadruple combination of ***ech (eci)***, ***ch (ci), hem***, ***hom*** and ***het***. Mathematical expressions of the ***holozygosis*** - or the ***holosis*** in any definite environment are given with the respective equations for the pre-cellular organisms, for the prokaryotes or during all the phases of the aforementioned stadium of ***gametobiont*** of the eukaryotes, and during all the phases of the aforementioned stadium of ***zygotobiont*** of the eukaryotes.

Key words: *Androgametation, Hemisis, Hemizygosis, Heterosis, Heterozygosis, Holosis, Holozygosis, Homosis, Homozygosis, **Ch**-Osis, **Ch**-Zygosis, **Ci**-Osis, **Ci**-Zygosis, **Ech**-Osis, **Ech**-Zygosis, **Eci**-Osis, **Eci**-Zygosis, Gametobiont, Androgametobiont, Gynogametobiont, Zygotobiont*

INTRODUCTION

In the mother Nature, or the evolution, ever since exists the *so-called ***spontaneous*** -, or ***natural (a)melioration*** of the heredity of all the organisms, incl. the human beings, whether it was ***biogenic*** - or ****biontogenic*** -, incl. ***anthropogenic influenced*** or - not.

However, perhaps from the precedent of the transition from the supply with organisms, collected directly from the mother Nature to one, mediated first by their proper (as carefully as possible) management, i. e. since the moment of the spring of the husbandry, has been being its version. This alternative practice is the *so-called ***technological*** -, or ***artificial*** -, or ***induced (a)melioration*** of the heredity of the husbanded by people bionts (beings, organisms, individuals), in principal applicable also for the mankind. But this activity has particularly benefited from the advancement of science and technology, especially during the past two centuries. The introduction of the latest achievements in these fields, such as the artificial ****androgametation*** (the artificial polenisation in the phytobionts, and in the mycobionts, the so called artificial insemination in the zoobionts, incl. the zoobionts of the species Homo sapiens) and the other branches of the reproduction biotechnologies; as cloning; as induced mutagenesis; as genomiks and as genetic engineering is becoming more evident than ever because in these circumstances we mean the use of gametes,that are produced by the male beings, not - of some seeds. This does not mean that improvements in the "classics" of it are being overlooked or that its practice would soon be stopped as well as in the natural balance activities.

On the contrary, this field as a whole, as well as in its particular elements, for instance, such as giving a general evaluation of the zoobiont (the animal) breeding value, have been nourished by the introduction of electronics and computing machinery.

Still, the extent and the direction of action of its operations' contributions do not act in synergy so that, for instance, the animal beings for final production purposes (for meat, for milk, for wool, for eggs, for work, and for etc.) would give the maximum amount, highest quality and lowest cost of production (Dimov, 2003), because the things in that direction with the other bionts are not much more different.

Historical overview

It was performed first a brief retrospective overview of the practices of the technological (a) melioration of the heredity of husbanded domestic bionts within the aforementioned period. For illustration of this explanation it will stop on the zoobionts. Initially, the emphasis was put on the recognized technology for production of pure-blood (pure-bred) animals for final utilization.

The use of this approach results in obtaining the least expensive possible animals for production of animal foodstuffs. This however does not imply that the production would also be the least expensive due to the failure to be composed the most appropriate idiotype to achieve production goals.

During the second half of the last century were implemented everywhere the technologies for creating zoobionts and other bionts for production purposes - whether single-hybrid - or double-hybrid multitudes - of single or double hybrid cultigenic varieties or varieties of cultigens - multitudes of bionts from the species, cultivated for the needs of economy (husbandry) or human life, i.e. ***cultivars of bionts.

On one side, it is aknowledged that the need for a special, very rigid and costly system for production of breeding zoobionts; seeds and nurselings of phytobionts (plants) and inoculates of mycobionts and of microbionts for multiplication, results in a very expensive production of bionts for final purposes.

The reason for this is that hybrid crossing (hybridization or metization) in both variants - primary or inverse is not always rational (Lewis, 1955). To say of production of double hybrids for final purposes, this is out of any ratio (Dimov, 2006).

As a result, we face the need to exclude from the breeding the male or female individuals of the different direct (purebred) or hybrid *cultivars in ***multipliers*** - the multiplying or breeding units in the ***nursery*** or ***pepiniere*** in Fr and Ge, ***pepiniera*** in It, from the *so-called ***(a)melioratorium*** (the system for melioration of the heredity of the majorities of one or another husbanded species of organisms, whose highest unit is the ***citadel, center, central or headquarters***, the actual part of this sacred action flows), as well as other inconveniences that are not subject of this paper.

A new concept for the ingredients of the results of action of one or another combinations of the genetic (hereditary) determinants (factors)

The biggest drawback of existing so far technologies for production of purebred or hybrid bionts for final purposes is that the most beneficial combination of genetic determinants, from an economical point of view, is achieved with lots of efforts and rather by chance.

To become this reality, we need go through, for instance in zoobionts, the "investment" in the phenotypic performance of its offspring. If we analyze this performance from the point of view of her determination, we will find four most important pools, that serve either independently or together, to achieve a certain result.

On one hand, through this approach, all the species of bionts, excl. sub-cellular -, pre-cellular - or para-cellular organisms (*regnum -,regia* - or *superdomain Acytota*), not only do not gather the beneficial versions or alternatives (alleles) of one or another genetic determinant or genetic factor (locus) from the so-called ***non-Mendelian*** -, ***cytoplasmic*** -, or ***extrachromosomal inheritance*** or - ***heredity*** (Jones, 1952, Rieger et al., 1976), situated out of the cell's nucleus in eukaryote bionts, either diploid, auto- or allopolyploid (extrachromosomal in prokaryotes or in *regnum Monera*), but a significant loss of these alleles and loci also occurs.

Thus, beings are deprived of the beneficial - and useful effects of these genetic determinants. Because the science knows the transmitted to the next generations through the chromosomes, but not according to the Mendel s laws allele convertion, genom imprinting (gamete of origin-dependent modification), either vegetative (somatic) - or germinative mosaicism, and also trinucleotide repeat disorders, the author of this paper prefers the term ***extrachromosomal inheritance (heredity)***.

That is why he *named the sum of these positive - or negative effects on the entire - and the all-round adaptability of the organisms ***ech-zigosis*** or ***ech-osis,*** but it is not wrong to be used also the analogous terms ***eci-zigosis*** or ***eci-osis*** (hereby *designated with the symbol $\pm$ d_{eci}, but the symbol $\pm$ d_{ech} is also suitable).

Here are possible three casuses. The first of them treats the prokaryotes, lately divided into the domains bacteria and archaea. In them these genetic determinants are invested in the next generation through donor ship of plasmids and / or episomes between two cells, realized, on one hand, para-sexual or asexual, on other hand, equivalent - or not equivalent reciprocal, and on the third hand, reciprocal or one way.

The other treats the cases of amphymixis of the eukaryotes. In some of them the transmission of these situated in the chloroplasts and in the mitochondria (in phytobyonts) or in mitochondria

(in zoobionts or in mykobionts) extrachromosomal hereditary factors to the contiguous generation is realized only through the ovum, i. e. by mother line.

This phenomenon is discovered after cross of the spot-leaved variety of four o clock flower or marvel of Peru *Mirabilis jalapa* by Correns (1904), one of the founders of the modern genetics. Its nature he for the first time gave the name *Status albomaculatus.*

In other eukaryotes the transmission of these extrachromosomal genetic determinants to the next generation is realized equipollent - or in some step non equipollent bought through mother -, and through father line. This phenomenon is discovered after cross by Baur E. (1909), also one of the founders of the modern genetics, of the variety with white edged leaves of pelargonium *Pelargonium zonale*. Its nature he for the first time gave the name *Status paraalbomaculatus*.

The last touches the cases of vegetative multiplication of the bionta. In it also is not transmitted the majority of absolutely all the hereditary determinants, either in their authentic matter, or in absolutely the same structure between them.

By the way the things stand so also in the cases, when during all the life or in the part of it, some lot of this heredity is due to viruses and / or rickettsia, and / or even bacteria. In result of all this it is impossible to be with fully identical hereditary determinants either parent - and filial generations or possibly the most related side relatives.

That is why in result of the different heritage of such hereditary determinants the natural (spontaneous) clonings - monozygotic (one- egged) twins (doublets), triplets, quadruplets and so on, (as well as artificial - or induced clonings) are not fully identical beings. This means that in these cases, there is always $\pm \boldsymbol{d}_{\boldsymbol{eci}}$ effect and therefore always there is some free territory for the selection, either natural or artificial.

Here is the place to give the deserved to Bateson et al. **(**1902), who for the first time introduced the terms homozygote and heterozygote. In this way they created the necessary conditions, later to be understood either partially - or in wholeness the actions and the interactions of the separate components of the idiotype.

So on the other hand, working via these approaches does not emphasize sufficiently the wanted effect resulting from one or another version (allele) of one or more genetic factors, *named ***hemizygosis*** or ***hemisis***. The sum of these positive - or negative effects on the entire - and the all-round adaptability of the organisms is *marked with the symbol $\pm \boldsymbol{d}_{\boldsymbol{hem}}$.

It presents: in pre-cellular bionts; in prokaryotes, that posses a single chromosome i. e. are one-chromosome bionts; during all the phases from the *so-named stadium of ***gametobiont***, being either ****androgametobiont*** or ****gynogametobiont***, (the so far gametophyte or the incorrectly named, why, we shall see a bit later, sexual generation in the phytobionts or in the mykobionts) from the particular contiguous windings (generations) of the screw lines of the survivels of the birth populations of eukaryota, either diploid, auto- or allopolyploid; and mostly in heterogametic eukaryote offspring.

It was put a stress on "mostly" as this phenomenon also occurs, although very rarely, in environmental (through the environment conditions), maternal (through the egg) or haplo-diploid sex determination as well as in homogametic sex with sex determination by Mendelian traits heredity, autosomo-homoallosomal (of allosome of the homogametic sex) balance and homoallosomo-heteroallosomal (of allosome of the heterogametic sex) systems of sex predetermination.

It is provoked by hemizygosity on one or another alternative of one or more genetic determinants located in homoallosomes (exhibited also in homogametic sex individuals) and / or on autosomes (exhibited in representatives from both sexes).

In addition, at least with zoobionts, the simultaneous occurrence of heterogameticity and the ability to „invest" extrachromosomal heredity only in female individuals (the carriers and the transmitters of extrachromosomal genetic determinants) is not a precedent.

What is more, the utilization of every of the aforementioned practices involves a very high risk to lose the already existing in direct cultivars maximum (by value) and positive wanted effect in the offspring resulting from inherited equal versions of one or more genetic factors during the purebreeding of bionts for final purposes, named ***homosis*** (Altshuler, et al., 1968), or - ** ***homozygosis***. The last term is used also by Shull **(**1915) and Wright **(**1933), but not in this sense.

This effect is proper only during all the phases from the *so-named stadium of ***zygotobiont*** (the so far sporophyte or the incorrectly named asexual generation in the phytobionts or in the mykobionts, because absolutely all the sporophyta are beings with sexual belongness) from the particular contiguous windings (generations) of the screw lines of the survivels of the birth populations of eukaryota, either diploid, auto- or allopolyploid.

This is a homozis - or homozygous effect in the offspring, obtained as a result of the action of the same versions of one or more hereditary determinants of pure-blooded production of bionts for final use. The sum of these positive - or negative effects on the entire - and the all-round adaptability of the organisms is *designated as $\pm$ $\boldsymbol{d}_{hom}$.

For sorry, **Anonymous,** in the world is being yet the totally wrong -, from all the sides not right practice, with the term homosis to be signed the phenomenon in which the hybrid spring is with lower - and / or less satisfying the needs of the husbandman qualities than these of the parent troops.

On the fourth place, this is attributed to the circumstance that in the process of obtaining double hybrids (offspring of sire and dam from different single hybrid cultivars), a lot of possibilities for attaining maximum (by value) and positive - and negative (according to Lewis, 1955; Turbin, 1961) outbreeding enhancement, hybrid vigor (Darwin, 1877; Gartner, 1849, Koelreuter, 1766; Mendel, 1865; Naudin, 1866; Lewis, 1955), *heterosis* (Shull, 1911, Shull, 1952, Hayes, 1952, Jones, 1952), or *heterozygosis* (East et al., 1912) are not realized.

This heterozygous effect is proper only during all the phases of the afore mentioned stadium of ***zygotobiont*** of the eukaryota. The sum of these positive - or negative effects on the entire - and the all-round adaptability of the organisms is *symbolized as $\pm$ $\boldsymbol{d}_{het}$.

It is result of inherited different alternatives of one or more hereditary determinants during the purebreeding or hybridization of zoo-, phyto- and mykobionts (fauna, flora and mykota) for final purposes.

The explanation could be found in the fact that this way, a loss of the effect formed in single hybrids (obtained from hybridization of direct cultivars) that serve as parents of double hybrids could have occurred

By the way, the ***heterosis***, sooner the ***heterozygosis***, as it will be seen later, is estimated in absolute - and in relative values. These estimations are divided also into:

- husbandrian - superiority of the arithmetical mean on a defined character or defined index of the hybrids above - or inferiority of the arithmetical mean on a defined character or defined index of the hybrids below, the together weighted mean on the same character or the same index

of the two parent cultivars, calculated according to the partnership ratio between them (Dimov, 1987);

- hypothetical - superiority of the arithmetical mean on a defined character or defined index of the hybrids above - or inferiority of the arithmetical mean on a defined character or defined index of the hybrids below, half the sum between the arithmetical means on the same character or the same index of the two parent cultivars (Omarov, 1975);

- real - superiority of the arithmetical mean on a defined character or defined index of the hybrids above - or inferiority of the arithmetical mean on a defined character or defined index of the hybrids below the arithmetical mean on the same character or the same index respectively of the higher - or of the lower parent cultivar (Omarov, 1975).

But through all these meagurements, the size of the heterozis is viewed as detached from both the magnitude of the arithmetic means of the hybrid majority and the parent cultivars, and dlso from the efficiency of the operation of the entire pyramid for production of the final hybrids.

This could lead to the choise of the hybrid cultivar with the largrst heterosis, but not with the largest productivity or with the most efficient exploitation. This can be completely avoided by applying

- ***competitive measurement of the heterosis*** - an increase - or a decrease of the arithmetic mean on a given trait - or on a given index of the hybrid majority respectively above or below the arithmetic mean on the same trait - or on the same index respectively of the expressed in highest - or in lowest degree in the competitive tests in a given region (country, continent, etc.) of a hybrid cultivar (Abramova, 1985), but after the following conditions are fulfilled.

If the profitability of the whole chain is compared - from the inicial direct cultivars to the ready-to-eat food, the test is conducted in the conditions closest to the normal mass practice. Perhaps it will be argued, that when behind each of the hybrid cultivars compared in a given region there are no grown in the same region formations of bionta from all higher levels of the mentioned pyramid, hybrid cultivars can not be evaluated in the way just described.

Provided that the data on the productive and reproductive qualities of the bionta from each pyramid level for the production of each of the hybrid cultivars are available, some economic indicators have been taken from the functioning of the pyramid to produce the basic hybrid cultivar (the standard of comparison) and it is known **** the cast of each of the hybrid cultivars, this problem can easily be solved by the apparatus of mathematical modeling.

Then, by regression analysis, it can be determined which of the above-mentioned measurements are closest to the real values of the hybrid cultivars and to the what extent the comparison indicators can be simplified in order not to substantially impair the assessment of these merits.

The terms ***ech-zigosis (eci-zigosis)***, ***hemizygosis***, ***homozygosis*** and ***heterozygosis*** are suitable for all the phases of the aforementioned stadium of ***zygotobiont*** of the eukaryota. And it is developed from the zygotes, either diploid, auto- or allopolyploid.

For the same reason and in the same circumstances the terms ***homosis*** and ***heterosis*** have not to be used. The term ***hemisis*** is suitable for all the species of organisms and in all possible their peripetia.

The last three single effects, i. e. the effects of ***chromosomal inheritance (heredity)*** - ***hemizygosis***, ***homozygosis*** and ***heterozygosis*** are *joined together with the terms ***ch-zygosis*** and ***ci-zygosis*** or ***ch-osis*** and ***ci-osis***, and the sum of these positive - or negative effects on the

entire - and the all-round adaptability of the organisms is *marked with the symbol $\pm$ ***d*** $_{ci}$, but the symbol $\pm$ ***d*** $_{ch}$ is also suitable.

For the cited yet reason, in this case the terms with ***-zygosis*** are suitable during all the phases of the aforementioned stadium of ***zygotobiont*** of the eukaryota, while the terms with ***-osis*** - for all the species of organisms and in all possible their peripetia.

Last but not least, consequently to all mentioned drawbacks, by all these technologies are produced for final purposes multitudes (massives) of bionts that are not homogeneous enough by their productivity. This circumstance considerably hinders the implementation, for instance, of contemporary zoobionts rearing technologies.

This new concept - base also for new systems for the conservation of genetic resourses, and for novel and perfected technologies for melioration of the heredity of the bionts.

The imperfections of systems existing so far could be reduced to a rather acceptable extent or could be completely eliminated by the creation on this base of advanced **novel and perfected technologies** for melioration of the heredity of husbanded cultigenes as well as for rearing of bionts for production purposes. On this stage the author of this paper has created and is able to implement such technologies only for husbanded zoobionts.

The achievement of each of these goals requires a kind of economical formation, but it is far more facilitated, flexible and faster than preexisting ones, i.e. it is more effective. Also, the wanted ***hemizygous***, ***homozygous*** and ***heterozygous*** effects are *maximally profited from*, as well as the wanted effects from the accumulated at an utmost extent the situated out of the cells nucleus (in prokaryotes out of the chromosome) ***extrachromosomal genetic determinants***.

Meanwhile, this new look upon the organism idiotype action gives an opportunity for the creation of far more sophisticated and at the same time more efficient and competent systems for genetic resource - and/or for biologic(al) diversity preservation. They are to be reviewed in other publications

A new scientific name of the effect of the whole combination of the hereditary determinants

As the novel concept implies a new label, I *suggest to implement the term **holozygosis** or **holosis**, that puts forward at first glance the whole - or holistic summary combination of the effects the genetic determinants of a new creature, i.e. a holistic or everyway zygosis.

For the exposed yet reason, in this case the first term is suitable during all the phases of the aforementioned stadium of ***zygotobiont*** of the eukaryotes, while the last - for all the species of organisms and in all possible their peripetia. In both cases the sum of these positive - or negative effects on the entire - and the all-round adaptability of the organisms will be *designated with the symbol $\pm$ ***e*** $_{hol}$.

It is composed by the sum of the four summary single effects and by the addition of all possible interactions between them. Interactions will be *labeled with the symbol $\pm$ ***b*** with subscripts for the respective double, triple or quadruple combination of ***ech (eci)***, ***ch (ci), hem***, ***hom*** and ***het***. The mathematical expression of the holozygosis - or of the holosis in any definite environment could be *given with the equation

$\pm$ ***e*** $_{hol}$ = $\pm$ ***d*** $_{hem}$ - for pre-cellular organisms,

$\pm$ ***e*** $_{hol}$ = $\pm$ ***d*** $_{eci}$ $\pm$ ***d*** $_{hem}$ $\pm$ ***b*** $_{eci\ hem}$ - for prokaryota or during all the phases of the aforementioned stadium of ***gametobiont*** of the eukaryota and

$\pm$ ***e*** $_{hol}$ = $\pm$ ***d*** $_{eci}$ $\pm$ ***d*** $_{hem}$ $\pm$ ***d*** $_{hom}$ $\pm$ ***d*** $_{het}$ $\pm$ ***b*** $_{eci\ hem}$ $\pm$ ***b*** $_{eci\ hom}$ $\pm$ ***b*** $_{eci\ het}$

$\pm$ ***b*** *hem hom* $\pm$ ***b*** *hem het* $\pm$ ***b*** *hom het* $\pm$ ***b*** *eci hem hom* $\pm$ ***b*** *eci hem het* $\pm$ ***b*** *eci hom het*

$\pm$ ***b*** *hem hom het* $\pm$ ***b*** *eci hem hom het* - during all the phases of the aforementioned stadium of ***zygotobiont*** of the eukaryota.

Among other things, the heterosis (heterozygosis) dimensions described above are also one to one applicable to the holosis (the holozygosis).

*** By the way, there are no objective reasons, either the taxonomic nomenclature of the phytobionts, of the mycobionts, of the zoobionts and the other bionts in the wildlife, or for the cultigenes, not to be unconditionally identical at absolutely all units of the taxonomic hierarchical structure, from the highest to the lowest.

Unfortunately, in terms of systematic names botanists, zoologists and mycologists not yet switched to the use of the same word for absolutely all essentially identical taxa. Here I exclude the clonings, whether phyto-, zoo-, myco- or microbo clonings.

In this case, I will not comment the Comintern mentality - and / or homemade criteria of our specialists in applied phytology (excluding their colleagues in forest culture), which, for the difference from their colleagues abroad, we must be lined up, they have not gone to the general application of the taxon cultivar for the different varieties of phytocultigenes yet.

As to the specialists in applied zoology or in applied mycology, literally from all over the world, I still can not -, and hardly ever I could figure out, why on the analogy of the universal use of taxon variety for every variety of bionta in the wild life, they do not * apply smoothly - and indiscriminate the taxon cultivar to denote the varieties of zoo cultigenes, or respectively of myco cultigenes, or of microbo cultigenes, in the latter two cases the name strain is still everywhere used.

Even Darwin himself used not the concept cultivar, but its predecessor - variety for just those varieties of zoo cultigenes. On the sensitive question how to call the so-called sortotypes and other similarities in some cultigenes, whether phyto-, zoo-, myco- and microbo, I answer this way: Although with some dose of conventionality, for example, the following hierarchical order can be considered: fraction of -, echelon of -, phalanx - or composition of -, version of -, series of -, group of -, strings from -; as well as **** the casts: ordinary - and synthetic - (compounde - or composite -) direct -; inbred - and outbred direct -; single hybrid - (twovariety -); three-, four-, and so on -variety consecutively alternately hybrid -; three-, four- and so on -variety consecutively non-alternately hybrid -; three-, four-, and so on -variety parallel hybrids; primal - and inverse -; intermediate - and terminal - (final -); monovalent -, bivalent -, trivalent -, ... and polyvalent (according to their purpose, whether productive, ie for the production of one or several products, model, ie for the biological modeling of the states -, of the processes - and of the phenomena, respectively occurring in the norm or in pathology, decorative, etc.) cultivars.

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INVESTIGATION ON SOME TRAITS OF BRANCHED SUNFLOWER FERTILITY RESTORER LINES DURING THE BREEDING PROCESS

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ABSTRACT

Five branched restorers of fertility in sunflower were investigated (27R, 154R, 185R, 240R, 242R). They possessed very good general and specific combining ability and were the father components in high-yielding hybrids. The aim was to investigate the development of the fertility restorers when left with their branches and to compare them to the development of the plants with removed branches. There were differences in the values of some main traits related to the breeding of sunflower where mainly manual labor is involved. The question arises whether and to what extent the removal of branches in this type of lines is justifiable. The traits subjected to investigation were the following: number of seeds per head, plant height, head diameter, oil content in seeds, absolute weight, germination energy and germination. The experiment was carried out for three years in the trial fields of Dobrudzha Agricultural Institute – General Toshevo. In most of the cases, the number of seeds in the branched plants was higher than in the plants with removed branches, but this was always at the expense of the absolute weight, which was higher in the lines without branches. The trait plant height was not influenced by the presence or absence of branches. The head diameter was lower in the normal plants in comparison to the plants with removed branches. In the fertility restorer lines with normal branches, the oil content in seeds was always higher in comparison to the lines without branches. The traits germination energy and germination were not affected by the presence/absence of branches in a father component.

Keywords: *Sunflower, Lines, Fertility restorers, Traits, Branches*

INTRODUCTION

Out of the four schemes of sunflower hybrid seed production developed at Dobrudzha Agricultural Institute (Velkov and Stoyanova, 1974), only the method if interlinear hybridization is now used for developing two-linear male fertile simple hybrid with full restoration of fertility. After the discovery of the cytoplasmic male sterility (CMS), the search for fertility restorer genes began exactly in CMS PET-1 (Nenov, 2002). Kinman (1970) reported the first such source of PET-1 restoration.

After the discovery of the sources of Rf genes, it was necessary to involve them in such lines that can be used for hybridization. The developing of fertility restorer lines is a main task of the Sunflower Breeding Department of DAI. Such forms are being obtained in several ways: selection and selfing of varieties, in which the presence of Rf genes has been confirmed (Vranceanu and Stoenescu, 1971, 1978); crossing of wild species to cultural forms – varieties and lines, selection, new crossing, etc. (Kinman, 1970; Nenova et al., 2014; Valkova et al., 2016); crossing of two lines, one of which is a carrier of Rf genes (Bochkarev, 1980); crossing

a line to a variety; developing synthetic populations, developing dihaploids, and selfing of hybrids followed by selection, which is the most commonly applied method in the recent years.

The main function of the fertility restorer line (R line) is to restore the male fertility of the new developed hybrid by combining its genome with the gene material of the mother line, and through the heterosis effect, to reach a higher productivity level (Singh et al. 2018). The genetic material in pollen grains of father line was transferred by honey bees and bumblebees (Basualdo et al., 2007).

The seed production of the different types of crops has high cost price and is carried out according to specific methodology and schemes (Mihova et al., 2017). The aim is to obtain authentic quality sowing material allowing the cultivar or hybrid to realize their genetic potential.

Sunflower seed production nowadays uses mainly branched fertility restorers, in which the pollen is in much greater amounts and is produced for a longer period of time in comparison to the non-branched R lines. This is very important for the process of pollination and hence – for better results in the production of hybrid sunflower seeds. Furthermore, the branched fertility restorers are very favorable for developing of hybrids, in which the two lines are with sharply different dates of flowering.

Often, in the process of initial breeding and initial seed production, when manual labor in the branched fertility restorers is predominant, sometimes branches are removed to work easier with the central head and to obtain larger seeds from a certain line, which will be further used in the next breeding stages.

The aim of this study was to follow the parallel development of the fertility restorer lines when left with all their branches and when the branches were removed. Our goal was to find out if there was any difference in the values of some main traits related to the breeding and seed production of sunflower; and also if the removal of the branches of this type of lines was justifiable and to what degree.

MATERIAL AND METHODS

The study was carried out at Dobrudzha Agricultural Institute – General Toshevo during 2009 – 2011. It involved 5 branched fertility restorer lines - 27R, 154R, 185R, 240R, 242R. These are father lines with very good general and specific combining ability. Some of them are father components in hybrids already officially registered in Bulgaria and abroad.

The trial was designed according to the block method in five replications, the size of the plot being 10.8 sq. m. The stand density was 6200 plants/da. Four-five days prior to flowering, two central heads were selected from each plot, from each line and replication; all branches of one of the two heads were removed manually, leaving only the head itself on the stem.

The study was aimed at following the development of the fertility restorer lines when left with all their branches, and how the plants developed when their branches were removed. We tried to find answers to the questions if there was any difference in the values of some main traits related to the breeding of sunflower, where manual labor is predominant, and was the removal in some instance of the branches of this type of lines justifiable and to what extent.

The following traits only of the central head were read: number of seeds in head (NSH), plant height (PH), diameter of the head (DH), oil content in seed (OC), absolute weight (W_{1000}), germination energy (GE) and germination (G).

The effects from the removal of the branches (Factor A) and the specificity of the genotype (Factor B) for the formation of the studied traits were evaluated by using two-way dispersion analysis. The experimental data was processed with the help of the software package Microsoft Excel^{xp}.

RESULTS AND DISCUSSION

Very often, in the process of sunflower breeding, manual labor is very important and is almost indispensable. Certain manipulations have to be performed, which can be done only by well trained workers. Each cross between two plants involves the so called "kissing" between two heads, so that the pollen from the first to be efficiently placed on the second. The production of seeds from parental sunflower lines under macro insulators is also primarily related to manual labor, where very often the branches of the father lines have to be removed to ensure better contact for "kissing" with the mother lines and thus provide better pollination and hence – greater quantity of seeds.

Furthermore, the seeds obtained from the father form are considerably larger and with better appearance. Such seeds are much easier to work with, and they are also preferable for the future seed production because they are much more easier to plant with the sunflower planters in which the discs of the sowing apparatus should not be with very small apertures in order not to hinder the sowing process.

The analysis of the variances (Table 1) shows that the removal of the branches in the father lines is related mainly to higher head diameter and 1000 kernel weight. The differences are with high statistical significance (P=1%).

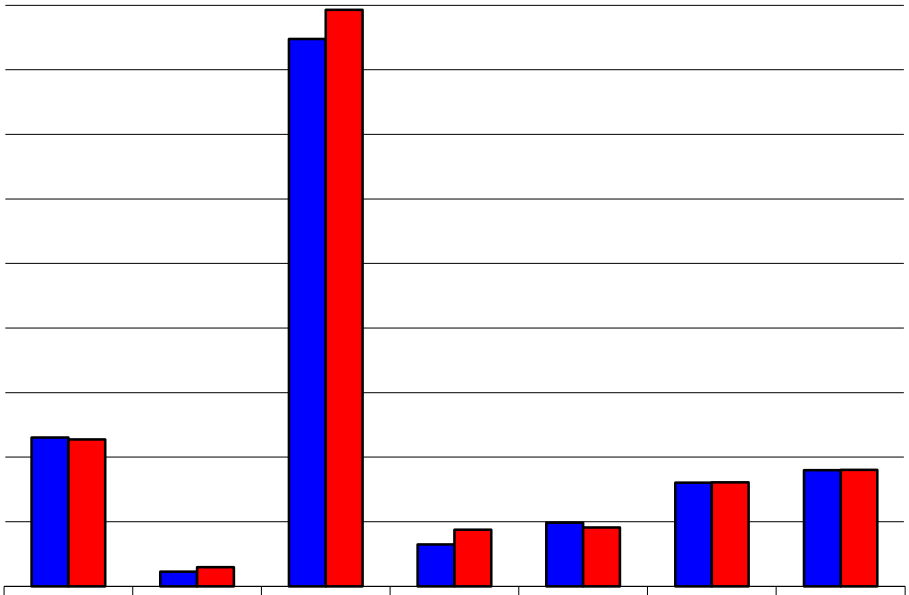
Table 1. Analysis of the variances of the studied traits in father lines of sunflower

Component	PH	DH	NSH	W_{1000}	OC	GE	G
Factor A	15.8	92.9***	3821.6	996.4***	112.5*	0.4	1.0
Factor B	523.6*	9.5	34338.8	66.3	32.7	6.0	1.5
A x B	51.7	4.7	21.805.7	6.4	2.4	0.2	0.7

Averaged for the period of investigation, the head diameter of the non-branched forms was with 3.5 cm larger, reaching 14.8 cm (Figure 1). The variations were highest in 2009 and 2011. They were within narrower limits in 2010, when considerable amounts of rainfalls were registered during June-August, especially in the flowering stage. The range of variation of 1000 kernel weight was wider, the variation being as high as 11.5 g. The mean value in the unbranched forms was 43.8 g. Under favorable conditions for the formation of this trait, the variations became greater. The removal of the branches had lower effect on oil content. This effect was significant at a lower level, P=5%. The mean value of the trait in the branched forms was 49.3%. The tendency over years was similar, although variable to different degrees in the separate lines. A probable reason for this were the phenological specificity of the genotypes, the variations in the dates to flowering and its duration, and all these are directly dependent on the combination of meteorological factors. In the rest of the traits, the differences were not significant. The deviation was greater in number of seeds per head, but the variation during the period of study and by genotypes was not unidirectional. The difference in plant height was

insignificant. In practice, there were no differences with regard to the germination energy and the germination.

The interaction of the factors was not significant, which was an indication that the removal of the branches had a unidirectional effect on the investigated traits and was not genotypically specific.



	PH, cm	DH, cm	NSH, no	W1000, g	OC, %	GE, %	E, %
with branches	115,2	11,3	423,9	32,3	49,3	80,3	89,8
without branches	113,7	14,8	446,5	43,8	45,5	80,5	90,2
GD 5%		2.9		3.8	4.0		
GD 1%	NS	4.0	NS	9.3	6.8	NS	NS
GD 0.1%		5.4		12.6	9.2		

Figure 1. Values of the traits depending on the type of development – with or without branches

The morphologically investigated father lines differed mainly by plant height (Table 2). Significant differences at the level of genotype were found only by this trait. Line 240R was with highest mean values, and line 27R – with lowest. However, the removal of the branches had low effect on this trait. The variation was within 3 cm. Higher deviation was found in line 185R – over 11 cm.

The effect from the removal of the branches on the head diameter was significant. In all lines, this was related to higher values of the trait. In 154R, the difference was almost 7 cm, but the mean deviation was within 2-5 cm. In the fertility restorer 27R, however, such a tendency was not observed and under certain climatic conditions, the removed branches did not favor a higher diameter of the central head. The higher head diameter, however, is not always related to higher number of formed seeds.

Table 2. Values of the traits by genotype

Line		PH	DH	NSH	W_{1000}	OC	GE	G
27 R	With branches	104.3	11.1	613.3	32.9	48.7	82.0	90.5
	Without branches	102.7	12.8	461.9	44.4	43.1	81.7	90.7
	Mean	103.5	11.9	537.6	38.6	45.9	81.9	90.6
154 R	With branches	120.6	10.7	454.5	25.9	50.4	80.6	89.5
	Without branches	123.1	17.1	498.0	38.7	46.2	81.4	90.4
	Mean	121.8	13.9	476.2	32.3	48.3	81.0	89.9
185 R	With branches	112.9	10.1	398.0	33.5	53.0	79.7	90.5
	Without branches	101.3	12.7	365.3	45.7	49.2	79.8	90.3
	Mean	107.1	11.4	381.7	39.6	51.1	79.7	90.4
240 R	With branches	124.9	11.9	301.5	36.9	47.6	79.5	88.7
	Without branches	126.0	15.7	391.7	44.8	43.7	80.1	90.0
	Mean	125.4	13.8	346.6	40.8	45.7	79.8	89.4
242 R	With branches	113.1	12.7	352.7	32.4	47.0	79.5	89.8
	Without branches	115.5	15.7	515.7	45.5	45.1	79.6	89.5
	Mean	114.3	14.2	434.0	38.9	46.0	79.6	89.6
	GD 5%	9.8						
	GD 1%	13.3	HP	HP	HP	HP	HP	HP
	GD 0.1 %	18.0						

In most cases, the most ostensible effect was on the increase of the absolute weight of the seeds, and the differences were with high significance. This is confirmed also by the data in the researches of Supriya et al. (2017) and Emerson et al. (2017). In lines 154R, 240R and 242R, the removal of the branches increased the number of seeds. In the other two lines, the opposite tendency was observed during most of the years of research. The differences were determined by the conditions during the vegetative growth and the presence of stress factors, such as the abundant rainfalls during flowering in the second year of the study, when the values of the trait number of seeds per head were lowest in all lines. Having in mind the necessary manual labor, the removal of the branches was justifiable only in line 242R, in which in certain years the number of seeds increased significantly and their commercial presentation became especially good. Apart from the higher number of seeds in head, this type of development is related also to a significant increase of the head diameter and the absolute weight of seeds.

With regard to the oil content in seed, the tendency was unidirectional; the seeds obtained from heads without branches had lower values of this trait. The variation of the trait in most of the lines was with 3-4 %. Further studies will check if the pollination with pollen from the two types of plants has the same effect on the obtained production of F1 seeds.

The germination energy of seeds and their germination capacity were not related to the removal of the branches. The values were similar, with very close range of variation. Tendencies and dependencies related to their weight were not observed.

CONCLUSIONS

In most cases, the number of seeds from the central head in plants with branches was higher than the seeds in plants with removed branches, but this was always at the expense of the absolute weight, which was higher in plants with removed branches. The trait plant height was not influenced by the presence or absence of branches. The head diameter was lower in the normal plants in comparison to the plants with removed branches. The oil content of the seeds in the fertility restorer lines with normal branches was always higher than in the lines with removed branches. The traits germination energy and germination were not affected by the presence or absence of the branches in a branched father component.

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ANALYSIS OF NUMERICAL METHODS FOR APPROXIMATING DDE SOLUTIONS IN WHEAT PRICE DYNAMICS IN ALBANIA

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ABSTRACT

Interactions between delay differential equations (DDEs) and economical models have been popular and developed rapidly in recent years. In mathematics, DDEs are known as differential equations in which the derivatives of some unknown functions at present time are dependent on the values of functions at previous time. Nowadays, such equations are used as a fundamental tool in describing the behavior of dynamical systems and appear frequently as mathematical models in natural sciences, economics, population dynamics, epidemiology, medicine and engineering. Many methods have been proposed for the numerical approximation of these equations. The purpose of this paper is to analyze the price dynamics of wheat in Albania, in context of mathematical modeling using linear and nonlinear DDEs. The data are obtained from the Ministry of Agriculture, Food and Consumer's Protection, Statistical Department and from Institute of Statistics for a period of 10 years (from 2007 to 2017). Fundamental methods for solving DDEs are used to study the effects of time delay on the behavior of solutions, which include steady states, periodic and oscillatory solutions, bifurcations and stability switches. In the analysis are used numerical illustrations to confirm the theoretical findings. The economical interpretations of delay effects are briefly discussed.
Keywords: *Delay differential equations, Numerical methods, Price stability.*

INTRODUCTION

Nowadays, cereals constitute the base of agricultural production. The wheat culture is one of the most cultivated crops in many countries. Wheat is a strategic crop that is found in the majority Albania's districts. This plant provides the food base of the population by being a great source of energy, proteins and fibers for human nutrition. These products today continue to be a very necessary part of modern human diet [Dukes, Toma & Wirtz, 1995]. Wheat is a plant with high energy values due to the high content of carbohydrates (amidogen, cellulose and sugars) which account for about 70% of the weight of the grains. From 1 kg of grain, an average of 3330 calories are obtained [Gooding & Davies, 1997]. In recent years, wheat consumption has increased, especially in developed countries. This is due to the significant contribution of complex carbohydrates of wheat in healthy diets. Wheat production is still a condition for human existence in poor countries [Faridi & Faubion, 1995]. Wheat production in Albania has undergone constant ups and downs over the years. The largest increase of annual growth of grain output was recorded in 2008 when about 34% more wheat than a year ago was produced. Wheat production (in tons) for the period year 2007- year 2017 is shown in the Figure 1.

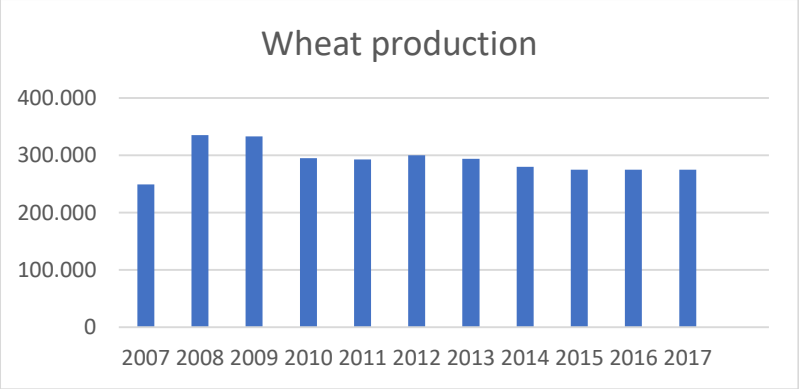

Figure 1. Production of wheat in Albania

In the study we have used Cobweb models to study the dynamics of wheat in Albania. Cobweb models describe the cyclical behavior of agricultural products' markets. These models concentrate attention on the fact that the present events depend upon the past happenings. Cobweb models use a technique to demonstrate the process of change over time and also describe a dynamic price process on a competitive market for a single non storable good with a supply response lag. Due to the production lag, suppliers must form price expectations one-time period ahead. In agricultural markets, the farmer's decision about how much should be produced is usually based on current and past experience. Within the classical cobweb model of Ezekiel [Ezekiel, 1938], producers simply form naive expectations [Dieci & Westerhoff, 2010]. Many cobweb models with different linear or nonlinear functions are used to model economic dynamics of commodity markets [Hommes, 1991; Finkestadt, 1995]. Many researchers in their findings indicate that the nonlinear cobweb model may explain various irregular fluctuations observed in real economic data. During recent years' considerable interest has been given in ignoring the potential role of delays in generating economic fluctuations using DDEs [Liz & Rost, 2013; Howroyd & Russel, 1984]. Such equations appear in various areas such as neural networks, ecology, medicine, engineering and particularly we have used them in agriculture area. DDEs describe mathematical models for systems in which the rate of change depends not only on the current study period but also on their history in the past. Techniques for solving ordinary differential equations (ODEs) and DDEs are based on numerical approaches of the solution. Nowadays, there are known a large number of methods for building numerical approximations of the initial value problem in ODEs and DDEs [Bellen & Zennaro, 2003; Al-Mutib, 1977; Butcher, 1987].

MATERIAL AND METHODS

We are based in the records of price and production of major market centers in Albania to study the price dynamics of wheat. The data are obtained from the Ministry of Agriculture, Food and Consumer's Protection, Statistical Department and from Institute of Statistics (INSTAT) for a period of 10 years (from 2007 to 2017). We have used linear and nonlinear DDEs of demand and supply, equations which are formulated from the data and then employed these equations to derive cobweb models which are used in order to study the price dynamics of wheat.

Table 1. Price and Production of wheat in Albania

Year	*Average prices of wheat (ALL/Kg)*	*Production of wheat (Metric ton)*
2007	32.00	249.5
2008	47.75	335
2009	39.25	333.1
2010	30.67	294.9
2011	42.08	292.8
2012	40.41	300.2
2013	42.75	294
2014	38.00	280
2015	37.47	275
2016	39.00	275
2017	46.16	275

Table 2. Descriptive Statistics for price and production of wheat

	Mean	*Median*	*Max*	*Min.*	*Quant.*	*Sum.*	*Std.Dev*	*Skew.*	*Kurt.*
Price	39.59455	39.25000	47.75000	30.67000	39.25000	435.5400	5.208350	-0.215270	2.409208
Production	291.3182	292.8000	335.0000	249.5000	292.8000	3204.500	25.35870	0.446621	2.655265

The model

Cobweb models describe a dynamic price process on a market for a single non storable good with a supply response lag [Dieci & Westerhof, 2010]. We describe the price by a sequence of numbers $p(1), p(2),\ p(3), \ldots, p(t), \ldots$ where $p(t)$ represents the price for the t year, and so forth. The model involves: -the price $p(t)$ of the product in year *t*; -the supply $S(t)$ for the product in year *t* – that is, how many units of the product are being made in this year; -the demand $D(t)$ for the product in year *t* – that is, how many units of the product are being bought in this year. A time lag exists between the decision to produce a particular product and actual production. Farmers determine how much they will plant in the spring based on the price they received the preceding year and they try to charge the same price in the fall. Producers are assumed to base production plans on current price. For agricultural commodities the production plans are made after the harvest. The output corresponding to these production plans appears on the market a year later. Due to the time lag, the current supply is a function of the price last year $S(t) = S\{p(t-1)\}$ (1)

It is assumed that no producer is left with unsold stocks and no consumer with an unsatisfied demand, that in other words means that the current demand is equal to the current supply $S(t) = D(t)$ (2)

Consider the linear demand function of price

$D\{p(t)\} = a - bp(t)$ (3)

and the linear supply function of price

$S\{p(t)\} = c + dp(t-\tau)$ (4)

where a, b, c, d and τ are positive constants, b represents the slope while a represents intercept for the demand function and also c and d are corresponding constants for the supply function. The slope of the demand curve is taken to be negative, that of the supply curve positive. At price 0, demand and supply are a and c, respectively [Ezekiel, 1938; Goldberg, 1961].

The market price is determined by the rate of change of the price between supply and demand as in the equation

$$p'(t) = D\{p(t)\} - S\{p(t)\} \quad (5)$$

$$p'(t) = (a - c) - bp(t) - dp(t - \tau), \tau > 0 \text{ on } [0, v], v > 0 \quad (6)$$

This is a DDE with a single delay. Analytically a large number of DDEs are solved with a well-known method called the method of steps. The DDE would have initial function (the history function) as $p(t) = \varphi(t)$ defined over the interval [-τ, 0] and then its solution is mapped onto solutions of other functions. Thus the solution of this equation is going to be a mapping from functions on the interval $[t - \tau, t]$ into functions on the interval $[t, t + \tau]$, $[t + \tau, t + 2\tau]$, etc., from time points $t = 0, \tau, 2\tau, \ldots$

In other words, the solutions of this dynamical system can be considered as sequence of functions $p_0(t), p_1(t), p_2(t), \ldots$ defined over contiguous time interval of length τ [Roussel, 2014]. In practice this problem is often solved numerically. In this paper we have used MatLab solver dde23 to solve DDE (6). Considering a simple nonlinear DDE of quadratic form for the supply function of price

$$S\{p(t)\} = c + dp(t - \tau) - ep^2(t - \tau) \quad (7)$$

and linear demand function of price (3) we have

$$p'(t) = (a - c) - bp(t) - dp(t - \tau) + ep^2(t - \tau), \tau > 0 \text{ on } [0, v], v > 0 \quad (8)$$

where a, b, c, d and τ are positive constants. The equation (8) is a nonlinear DDE and cannot be solved using an analytical method. We have used Matlab solver dde23 with history function of $p(t) = \varphi(t)$, on $[-\tau, 0]$ to solve this equation numerically.

RESULTS AND DISCUSSION

We study the price dynamics of wheat in Albania in the context of mathematical modelling using real economic data of wheat price and production. We have used E-Views program to find the parameter estimates of the model and Matlab program to find the numerical solutions of the DDEs in linear and nonlinear case. The Figure 2 below shows the time series plot of wheat price in Albanian currency (ALL) in the period year 2007- year 2017. The price and production data are checked for stationary status by applying time series techniques in E-views program. The figure indicates that the price data is stationary.

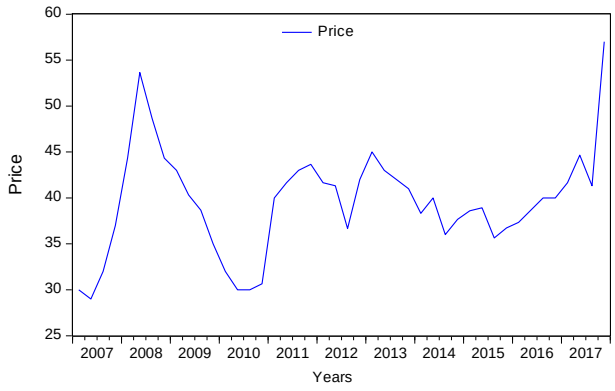

Figure 2. Time series plot of wheat price

Analysis of the model

First, we have checked the data and correct any errors and then used E-Views to verify the stationary status for price and production before formulating the demand and supply functions of price using regression analysis. In the end the DDEs (linear and nonlinear) are solved using Matlab solver dde23.

Table 3. Coefficients of the model

Model	*Coefficient*	*Std. Error*	*t-Statistic*	*Prob.*	
Price	-2.8062	0.292210	-3.113695	0.0170	Demand function
	Dependent variable: production				
Price	4.041262	1.290247	3.132161	0.0140	Supply function
Price	12.61504	1.610353	7.833714	0.0000	
Price^2	-0.130796	0.039203	-3.336394	0.0087	
	Dependent variable: production lag				

In Table 3 are given the parameter estimates of demand and supply function after the data are checked to be statistically significant.

The equation (9) for the demand function was obtained from price data of order two differencing and production data of order one differencing.

$D\big(p(t)\big) = -2.8062p(t)\,, a = 0$ (9)

Equation (10) below for supply function was obtained from price data of order one differencing and production data of order two differencing.

$S\big(p(t)\big) = 4.0412p(t-\tau),\ c = 0$ (10)

Equation (11) for supply function was obtained with no order of differencing

$S\big(p(t)\big) = 12.6150p(t-\tau) - 0.1307p^2(t-\tau),\ \ a = 0, c = 0$ (11)

In equations (10) and (11) the presence of the delay τ expresses time that is needed to realize change of supply in dependence on price. From equations (9) and (10) the rate of change of price is given by the following equation where $\tau = 1$:

$p'(t) = D\big(p(t)\big) - S\big(p(t)\big) = -2.8062p(t) - 4.0412p(t-1)$ (12)

This is a DDE which can be solved using MatLab solver dde23.

The linear model from equations (9) and (10) solved analytically provide first order linear cobweb model derived from the difference equation:

$p(t) = -1.44008\ p(t-\tau)$ (13)

The following figure is the solution of the equation (13) obtained numerically using Matlab.

The history function is set at $P(t) = 32$ (the initial price from Table 1), when $t \leq 0$, with (12) on the interval [0, 100]. The figure shows the oscillation of price around equilibrium approach. The Figure 3 above shows that the price of wheat is stabilized at equilibrium point (ALL=0.00) a long time before it started to destabilize around of equilibrium point. So, in the economic point of view, no producers would continue to supply wheat in that situation of the market price condition.

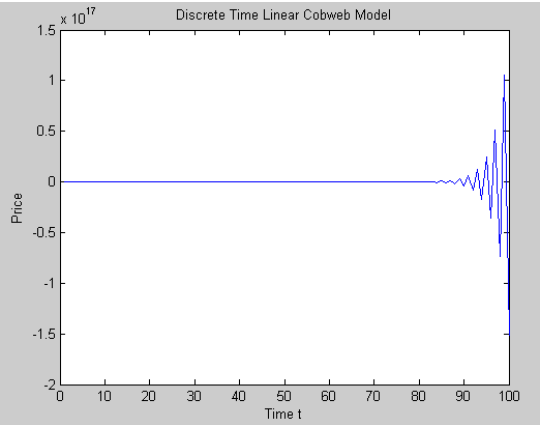

Figure 3. Discrete linear model

From equations (9) and (11), the rate of change of price is given by the following equation where $\tau = 1$:

$p'(t) = D\big(p(t)\big) - S\big(p(t)\big) = -2.8062p(t) - 12.6150p(t-1) + 0.1307p^2(t-1)$ (14)

The nonlinear model from equations (9) and (11) solved analytically provide first order nonlinear cobweb model derived from the difference equation:

$p(t) = -4.495\, p(t-1) + 0.0466\, p^2(t-1)$ (15)

The following figure is the solution of the equation (15) obtained numerically using Matlab.

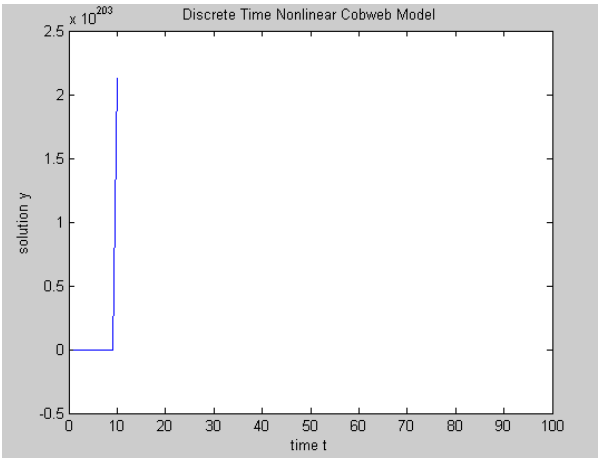

Figure 4. Discrete nonlinear model

From the Figure 4 it is clear that price stability of wheat can only be achieved at $p(t) = 0$. The reason is because of the fact that producers are sensitive to price and they would be attracted towards any other price instead of the zero equilibrium price ($p(t) = 0$) as demonstrated by the analytical solution. The price function from equation

$p(t) = -4.495\, p(t-1) + 0.0466\, p^2(t-1)$ (15)

is like quadratic equation having two price points $p_1 = 0$ and $p_2 = 117{,}93$. The price stability is dependent on the slope of supply curve or elasticity of supply. The responsiveness of demand and supply to changes in price is quantified using elasticity which is an economic measure designed for such purpose [Varian, 1992]. Equation (15) is perturbed on assumption that b remains the same, while d is varied to observe its effect on price behavior of wheat. The below figures were obtained as a result of price parameter variation using numerical analysis. In the

Figure 5 is shown that when d is reduced to 5.5572 from 12.6150 (in equation (15)), then with b still at 2.8062 so that $|d/b| > 1$, the oscillatory behavior of nonlinear cobweb model has now conformed to the condition of nonlinearity model. In the Figure 6, d is further reduced to 1.6764 from 12.6150 (in equation (15)), while b remains the same as assumed. From Figure 6, it is clear that farther the slope is reduced, more the fluctuations of wheat price are stabilized after few periods of instability.

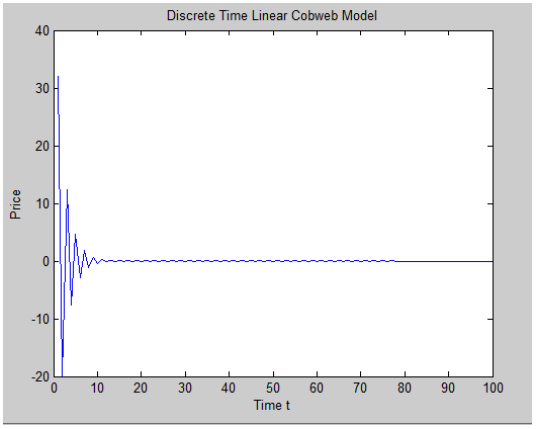

Figure 5. Slope of supply curve in nonlinear model, $|d/b| > 1$

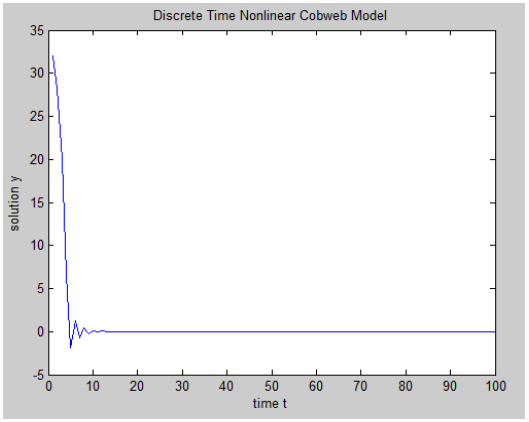

Figure 6. Slope of supply curve in nonlinear model, $|d/b| < 1$

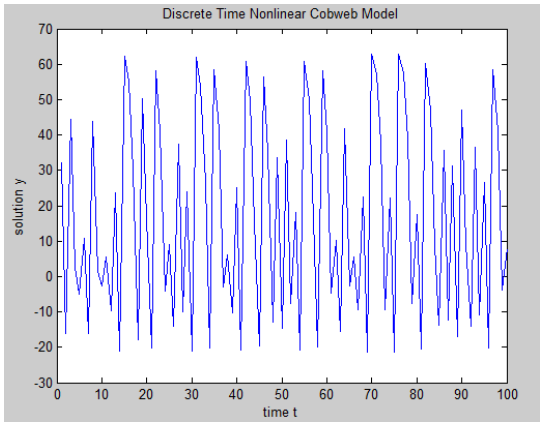

Figure 7. Slope of supply curve in nonlinear model, $|d/b| = 1$

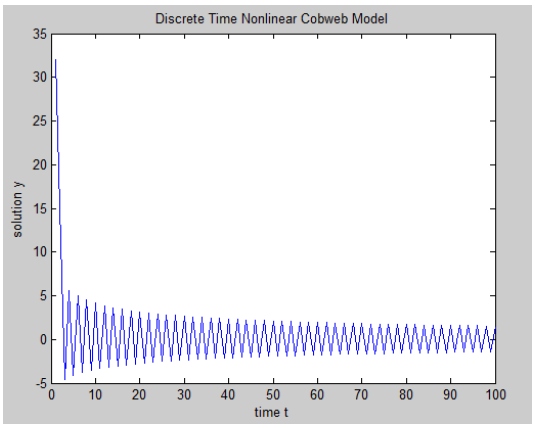

Figure 8. Slope of supply curve in linear model, $|d/b| = |0.22|$

From the Figure 7 above, the price oscillates in two cycle between two price points. It happened so because both d and b are 2.8062. From the Figure 8, when d in linear equation (10) is reduced to 0.6207 from 4.0412, the price was in stable form which is directly opposite to that displayed in Figure 3. Price of wheat would now converge towards an equilibrium price point other than the price zero (ALL=0.00). It will give few oscillations and then converge towards equilibrium.

CONCLUSIONS

In this study we analyzed the price dynamics of wheat in Albania, in context of mathematical modeling using linear and nonlinear delay differential equations. We examined the effect of changes in the wheat price in two models (linear and nonlinear) using time delay differential equations and also showed the numerical simulations of the models. The models

are based on the assumptions that wheat has no equal substitutes. The analysis of price and production data in Albania showed that the linear model provided an unstable zero equilibrium price point. This result is unrealistic because of producers' sensitivity towards price of farm produce. On the other side, the analysis of the nonlinear model showed oscillations between and around two equilibrium price points, which is realistic and a reflection of wheat price in Albania. The analysis in the study can inform the farmers about price fluctuations of wheat. The price fluctuations are reduced, if and only if, factors affected by time lag, such as buying new inputs, the time necessary for increasing supply etc. are improved. This analysis is an additional help in understanding the role of time delays in models in agriculture and should be useful for farmers in taking the right decisions and making the right plans for their products.

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COMPARATIVE VIEW OF ALBANIAN VEGETABLE PRODUCTION

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ABSTRACT

Albania has the opportunity to cultivate different kinds of vegetables based on the geographical position, climatic conditions and early tradition in their production. Nowadays, in our country are cultivated more than 30 types of vegetables and this number is always increasing. Even the total production of vegetables in the country has always been increasing. Also, more and more in Albania the vegetable production is being applied in different kinds of greenhouses. In these past years, the above mentioned factors have made the vegetable production in different areas of the country grow and extend throughout the year. The main goal is achieving the optimal levels of vegetable production in order to meet the country's needs, to reduce their import and to rise our country's export. This study, for this purpose, makes an assessment of our country's counties performance of vegetable production during 2013-2017. The performance in vegetable production is estimated using Data Envelopment Analysis (DEA). Based on this assessment, through a comparative view between the different counties, the opportunities and the appropriate ways to achieve optimal levels in vegetable production in our country are found.
Keywords: *Vegetables, Performance, DEA, Counties.*

INTRODUCTION

Vegetables are herbaceous plants of various types planted in the ground, such as tomatoes, cucumber, spinach, cabbage, etc. In Albania, over 60 different types of vegetables can be cultivated due to the favorable climate conditions, the favorable geographic position and the long experience that our country has in cultivating them. Nowadays, our country cultivates over 30 different types of vegetables and this number is growing. Considering all these positive factors, vegetable production has grown in all over the country and in some of the most important areas of the country is stretched throughout the year.

Furthermore, before the 1990s, the production of vegetables in our country occupied a special place as they served as food for the population and generated export earnings. As for the main vegetables, the main place was the field tomatoes and tomatoes and cucumbers which were produced in the existing greenhouses. For the main vegetables it is noticed that the cultivated areas have been grown in many parts of the country. This made the production of main vegetables extend. As for the second vegetables, in autumn and winter were distinguished pickles and onions, but also cucumbers, peppers, eggplant, carrot, beans, etc. Much of the production of vegetables was carried out in the coastal areas of Albania, from Shkodra to Saranda, areas which had a favorable climate for their cultivation. The main area where we cultivated vegetables in our country was Divjaka, where was carried out for the first time the production of vegetables in the greenhouses.

Nowadays, the production of vegetables in our country occupies an important place in the overall agricultural production, which is part of the country's national economic and social development strategy, and is also part of the European Integration Plan for Albania. Our

country's goal is to orient and secure a sustainable agricultural development in order to increase production to meet the country's needs, minimize imports and increase export opportunities. In these conditions, the support and management of this development gains special importance. In this sector there is an increase in production for every crop, where production growth is more evident in protected areas, where a continuous increase of protected areas is noticed and the trend of growth of production is maintained year by year.

MATERIAL AND METHODS

The data for this paper are taken from the Agricultural Statistical Yearbook 2017, INSTAT, Albania. Since the information for the vegetables in this statistical yearbook is found only for the cultivated surfaces and the production received from them, in this paper the presentation of current situation and the assessment of the performance of the counties in Albania regarding the production of vegetables is based in these indicators. Data has been collected for all of our country's counties for a period of 5 years from 2013 to 2017. Specifications include cultivated areas and total production of vegetables, cultivated area and main and second vegetable production, as well as the cultivated area and the production of main vegetables in the greenhouses.

In this study, the counties performance based on technical efficiency is evaluated using the data envelopment analysis (DEA) method, through the BCC model (Banker, Charnes, Cooper, 1984). Data Envelopment Analysis (DEA) is a relatively new "data oriented" approach for evaluating the performance of a set of peer entities called Decision Making Units (DMUs) which convert multiple inputs into multiple outputs. The definition of a DMU is generic and flexible. Recent years have seen a great variety of applications of DEA for use in evaluating the performances of many different kinds of entities engaged in many different activities in many different contexts in many different countries. These DEA applications have used DMUs of various forms to evaluate the performance of entities, such as hospitals, universities, cities, business firms, and others, including the performance of countries, regions, etc. (Cooper Seiford, Zhu, 2004). Thus, DEA is a mathematical programming method for evaluating the relative efficiency of decision making units (DMUs). Here the (pure) technical efficiency of each county (DMU) is measured in terms of the technical efficiency of all other counties in the analysis. The model will identify and differentiate efficient and inefficient ones. In BCC-I (input oriented) model, the goal is to produce the same output level with the possible minimum of input. The BCC-I model, under the VRS assumption, in its envelopment form is given below:

$$\min\ \theta - \varepsilon\left(\sum_{i=1}^{m} s_i^- + \sum_{r=1}^{s} S_r^+\right)$$

Subject to:

$$\sum_{j=1}^{n} x_{ij}\lambda_j + s_i^- = \theta x_{i0} \qquad i = 1,2,\dots,m$$

$$\sum_{j=1}^{n} y_{rj}\lambda_j - s_r^+ = y_{r0} \qquad r = 1,2,\dots,s$$

$$\sum_{j=1}^{n} \lambda_j = 1$$

$$\lambda_j, s_i^-, s_r^+ \geq 0 \qquad \forall j, i, r$$

where: n is the number of DMUs taken in the study; each DMU consumes varying amounts of m different inputs to produce s different outputs, specifically DMU_j consumes amount x_{ij} of input i and produce amount y_{rj} of output r; s_i^- and s_r^+ are additional variables (slacks); λ_j are nonnegative scalars; $\varepsilon > 0$ an infinitely small quantity; θ the relative efficiency score (scalar that determines the proportional reduction for all inputs of DMU_o).

DMU_o (DMU under evaluation) performance is fully efficient (100%) only if the conditions are met at the same time: $\theta^* = 1$ and all slacks are equal to zero. The performance of DMU_o is weakly efficient only when the conditions are met: $\theta^* = 1$ and ${s_i}^{-*} \neq 0$ and/or ${s_r}^{+*} \neq 0$ for any i and r in some alternate optima. DMU that result with the value of $\theta = 1$ are technically efficient and define the efficient frontier according to this model. Conversely, if $0 \leq \theta < 1$, the DMU_o becomes technically inefficient and extend below the efficient frontier. For inefficient DMUs, which need improvement, optimization is calculated in a two-stage process: First, the proportional reduction of the inputs used by the DMU taking the optimal value of θ as a factor and second, movement over the efficient frontier through slack variables s^+ and s^-.

Also, non-zero elements of the optimal lambdas ($\lambda_j, j = 1,2,\dots,n$) found by the model identify the reference set for each inefficient DMU. The reference set consists of efficient DMUs from the range of efficient DMUs at the efficient frontier, against which the DMU_o is evaluated. The reference set determines the point of reference for the DMU_o. This means that, the projection in the efficient frontier of an inefficient DMU will be the linear combination of the reference set of the respective efficient DMUs of this inefficient DMU with the optimal lambdas. The linear combination of the DMUs means a linear combination of inputs and outputs with the values of λ-s to obtain the input and output levels that the DMU must achieve in order to be technically efficient. This design will result the same as that gained by the improvement through θ and additional variables.

RESULTS

Based on the data of the cultivated area with vegetables and the production made there, the following graphs were made. Looking at the production graphs for each case, a clear picture of the production levels that the counties have reached during 2013-2017 will be given. This picture will also be complemented by the performance evaluated by the DEA method for each county in order to understand in which counties the production received is achieved by minimizing the cultivated area with vegetables and in which counties this is not present.

The first graphs show the general surfaces cultivated with vegetables and total vegetable production realized during the years 2013-2017 in all counties of Albania.

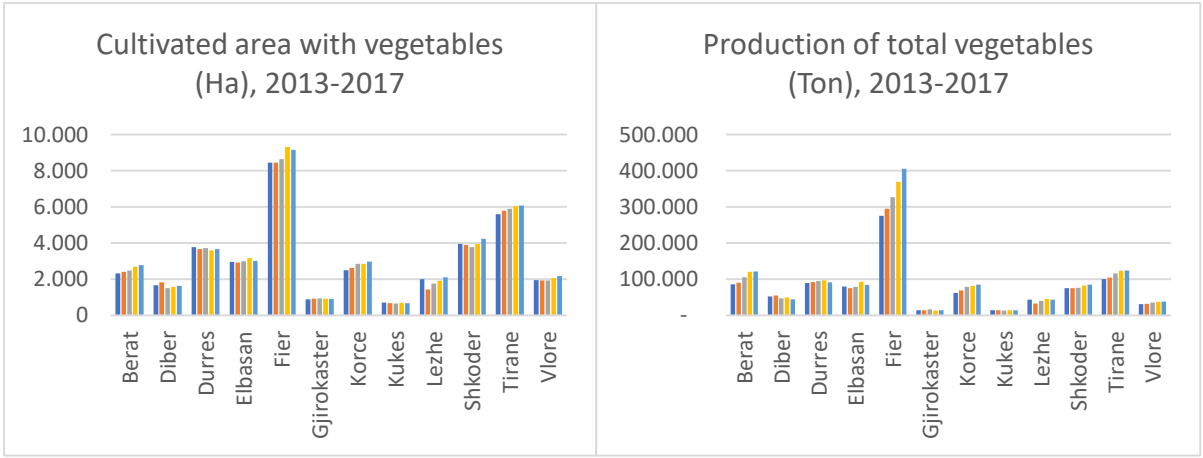


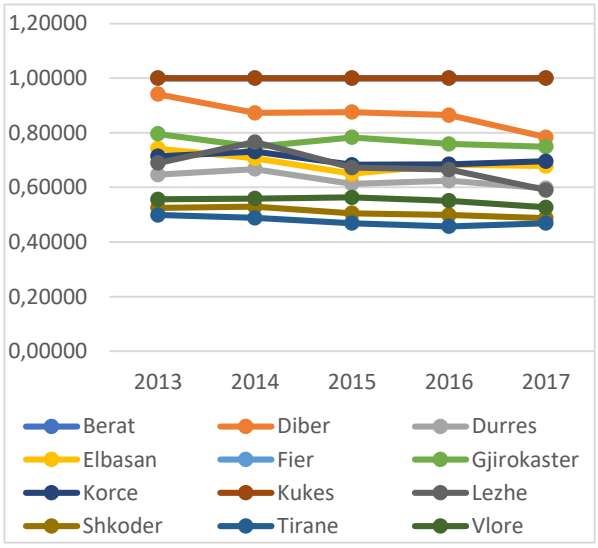
Figure 1. Cultivated area and production of total vegetables, 2013-2017

From the production chart we note that the counties, by the level of the production of total vegetables that have reached during 2013-2017, form nearly this ranking: Fier, Tirane, Berat, Durres, Korçe, Shkoder, Elbasan, Diber, Lezhe, Vlore, Kukes, Gjirokaster. Meanwhile, if we look at the production during the years in terms of output specifically for each county we distinguish: Fier county, which results in the 5 years with the highest level of production in the country, also differs from the fact that there is a significant increase in production during these years. Tirana county also with continuous production growth during 2013-2017. It follows the Berat county, which has an increase in production. Durres county has a growth in production at the beginning of the period and a decrease in production at the end. Korça county has a steady continuous growth. Then comes Shkodra county with a slight increase of production in the last two years. Elbasan county with small fluctuations around the same production level. Then Dibra and Lezha counties appear with a decrease in their level of production in these 5 years, Vlora county with very light production growth, and finally Kukes and Gjirokastra counties with slight fluctuations around the low levels of vegetable production.

The above table will be supplemented by the performance of the counties in the production of total vegetables, which is shown in Table 1.

Table 1. Counties performance in the production of total vegetables 2013-2017 (the relevant graph).

County	Technical efficiency				
	2013	2014	2015	2016	2017
Berat	1.00000	1.00000	1.00000	1.00000	1.00000
Diber	0.94116	0.87256	0.87571	0.86477	0.78345
Durres	0.64665	0.66690	0.61308	0.62448	0.59674
Elbasan	0.74215	0.70626	0.65014	0.68277	0.67809
Fier	1.00000	1.00000	1.00000	1.00000	1.00000
Gjirokaster	0.79589	0.74855	0.78292	0.75915	0.74894
Korce	0.71424	0.73117	0.68314	0.68446	0.69579
Kukes	1.00000	1.00000	1.00000	1.00000	1.00000
Lezhe	0.68904	0.76634	0.67252	0.66537	0.59012
Shkoder	0.52529	0.52903	0.50538	0.49899	0.48752
Tirane	0.49867	0.48796	0.46878	0.45686	0.46858
Vlore	0.55636	0.55908	0.56303	0.55077	0.52671
Avarage	**0.75912**	**0.75565**	**0.73456**	**0.73230**	**0.71466**



From this assessment, we find that the counties of Fier, Berat and Kukes, compared to other counties of the country, exhibit the highest level of performance, which is maintained throughout the study period. This shows that these counties have reached their level of vegetable production by optimally utilizing the area used for their cultivation, resulting in a clean technical efficiency, Thus, these counties have resulted with the best productive practice in cultivating vegetables and form the efficient production frontier. However, in a more detailed study beyond the boundaries of our work, we can differentiate between them fully efficient counties from those with weakly efficiency. Whereas, in this order: Diber, Gjirokastra, Elbasan, Korca, Lezha, Durres, Vlora, Shkoder, Tirana, result technically inefficient in the process of producing vegetables, showing during the entire study period values of relative efficiency smaller than the one, by staying below the efficient production frontier.

The following graphs show cultivated areas with main vegetables and their production during the years 2013-2017 in all regions of Albania.

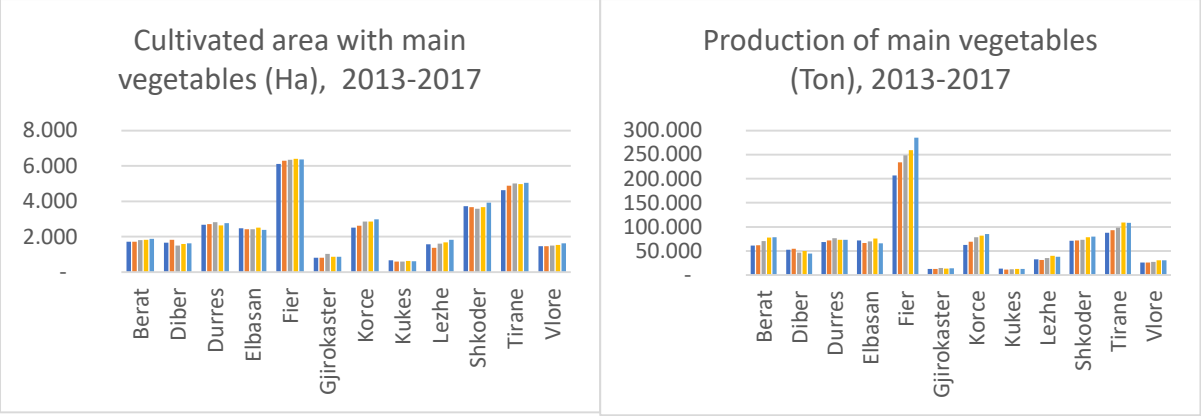


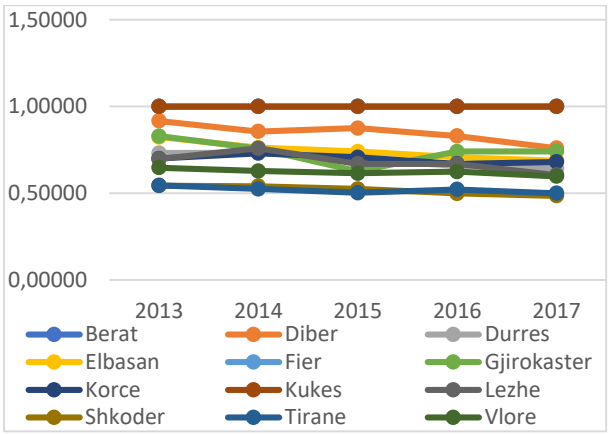
Figure 2. Cultivated area and production of main vegetables, 2013-2017

In the main vegetable production, Fieri and Tirana, which reach the highest levels of production, have experienced an increase in production during 2013-2017, followed by Korça, Shkodra and Berat, with production growth of 5 years, Durres and Elbasan with fluctuations in production over the years. Then the Diber and Lezhe counties come with light upgrades at the production level and Vlora with a slight increase in production. In the end, Gjirokastra and Kukes rank at almost constant levels of production. Korça is distinguished in the main vegetable production. Its production levels can be ranked after Tirana, which is not noticeable in the production of total vegetables.

On the other hand, the results presented in Table 2 are taken into account in the evaluation of the performance of the counties in the production of the main vegetables.

Table 2. Counties performance in production of main vegetables, 2013-2017 (the relevant graph).

County	Technical efficiency				
	2013	2014	2015	2016	2017
Berat	1.00000	1.00000	1.00000	1.00000	1.00000
Diber	0.91689	0.85564	0.87569	0.82980	0.76000
Durres	0.73136	0.73405	0.69414	0.65763	0.64243
Elbasan	0.82401	0.76259	0.74044	0.70768	0.68830
Fier	1.00000	1.00000	1.00000	1.00000	1.00000
Gjirokaster	0.82935	0.75859	0.62658	0.74106	0.74119
Korce	0.70008	0.73010	0.70713	0.66960	0.68038
Kukes	1.00000	1.00000	1.00000	1.00000	1.00000
Lezhe	0.69739	0.75936	0.66975	0.66850	0.60458
Shkoder	0.54447	0.54027	0.52422	0.49852	0.48523
Tirane	0.54484	0.52438	0.50291	0.52212	0.49999
Vlore	0.64783	0.62818	0.61522	0.62474	0.59772
Avarage	**0.78635**	**0.77443**	**0.74634**	**0.74330**	**0.72499**



As the highest and most stable counties throughout the period 2013-2017 are distinguished again the counties of Fier, Berat and Kukes, which are technically efficient in the production of main vegetables. As a matter of fact, Diber, Gjirokastra, Elbasan, Durres, Korca, Lezha, Vlora, Shkoder and Tirana are technically inefficient in the production of the main vegetables during the study period.

The charts below show the cultivated areas with second vegetables and the level of their production during the years 2013-2017, in our counties.

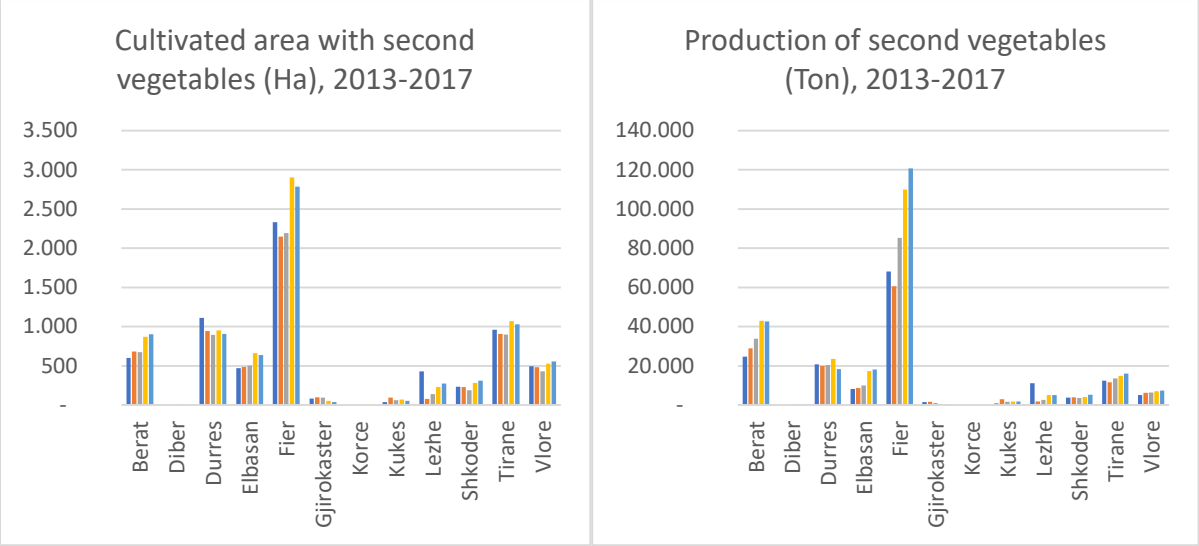


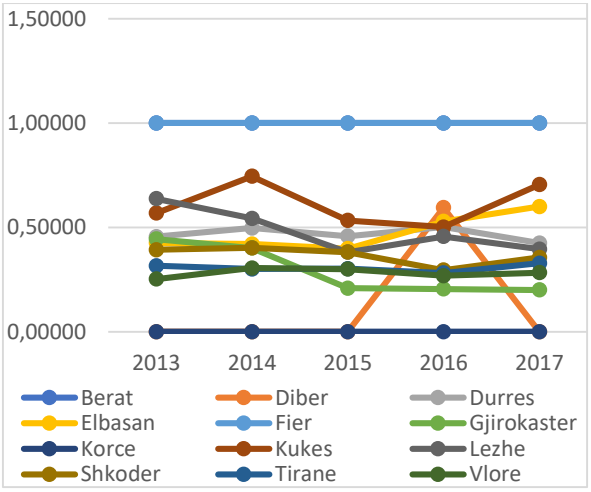
Figure 3. Cultivated area and production of second vegetables, 2013-2017.

In the second vegetable production, Fieri heads again, where, following a fall in 2014, marks a sharp increase in their level during 2015-2017, followed by Berat county with continuous growth in the first 4 years and approximately the same level in the last year, Tirana county with a decrease of production in the first year and ìncrease of production in the following years, Durres with a production decrease in the first and last year and then with Elbasan with slight growth tendencies. Then, Vlora county with easy growth of production, Shkodra and Lezha with decrease of production. Then Kukes county with production fluctuations, Gjirokastra with the rise initially and then decline in production, but always at low levels and Dibra with very low levels of production. Korca county does not produce second vegetables. Here, compared with the production of main vegetables, the situation for some of the country's counties is overthrown, such as Korca, Shkodra and Dibra.

From the evaluation of the performance of the counties related to the second vegetables production, the results given in Table 3 were taken.

Table 3. Counties performance in production of second vegetables, 2013-2017 (the relevant graph).

County	Technical efficiency				
	2013	2014	2015	2016	2017
Berat	1.00000	1.00000	1.00000	1.00000	1.00000
Diber	0.00000	0.00000	0.00000	0.59403	0.00000
Durres	0.45523	0.49696	0.45784	0.50140	0.42505
Elbasan	0.42513	0.42010	0.39754	0.52852	0.59948
Fier	1.00000	1.00000	1.00000	1.00000	1.00000
Gjirokaster	0.44422	0.40107	0.20856	0.20486	0.19990
Korce	0.00000	0.00000	0.00000	0.00000	0.00000
Kukes	0.56837	0.74452	0.53274	0.50170	0.70509
Lezhe	0.63716	0.54277	0.38122	0.45601	0.39538
Shkoder	0.39222	0.40191	0.38108	0.29490	0.35592
Tirane	0.31539	0.30047	0.30218	0.28144	0.32783
Vlore	0.25194	0.30487	0.29968	0.26784	0.28279
Avarage	**0.45747**	**0.46772**	**0.41340**	**0.46922**	**0.44095**



Here the situation is different from that of main vegetable production. Efficiency results are noticeably lower than in the case above. Fier and Berat counties are distinguished for the maximum level of relative technical efficiency and keep this level throughout the period 2013-

2017. Other counties result in lower values than technical efficiency. So, counties Lezha, Kukes, Durres, Gjirokaster, Elbasan, Shkoder, Tirana, Vlore, Diber and Korce are technically inefficient in the production of second vegetables.

The charts below show the cultivated area with vegetables in the greenhouses 2015-2017 and their production during the years 2013-2017 in the regions of our country.

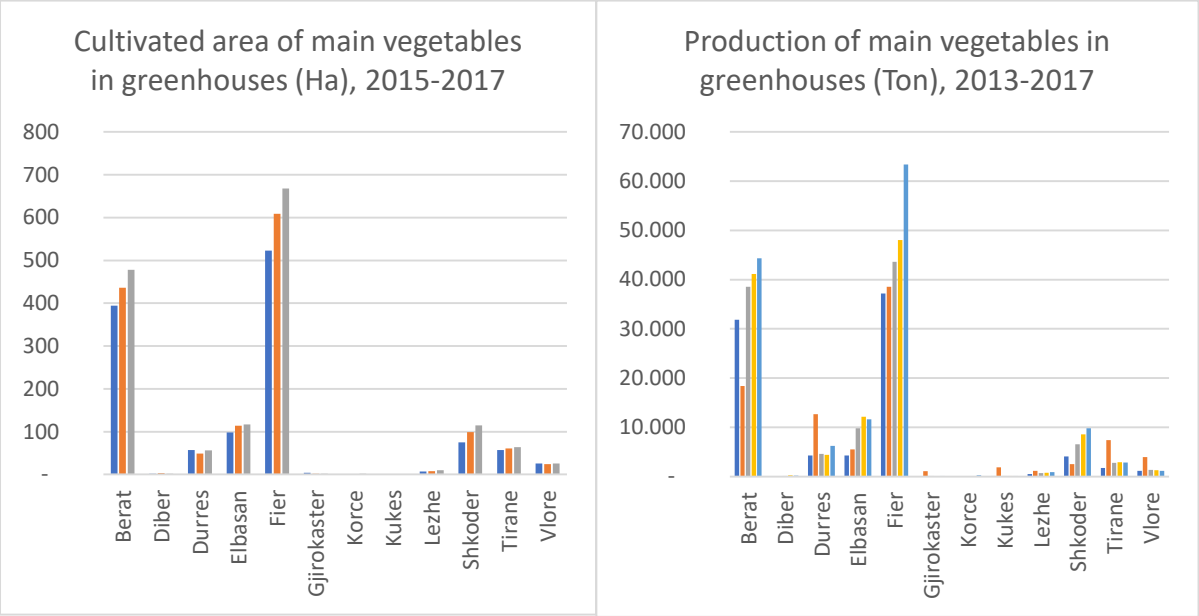
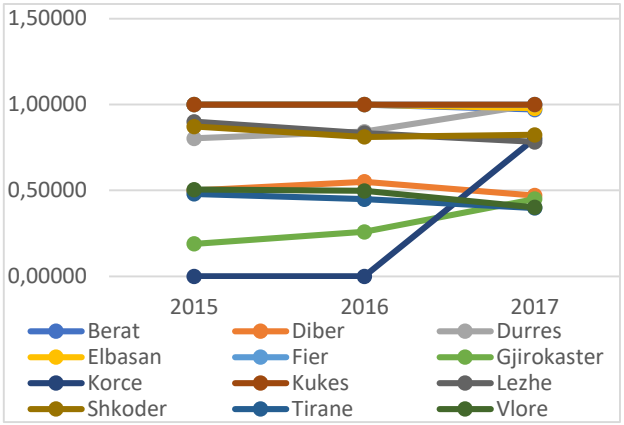


Figure 4. Cultivated area and production of main vegetables in greenhouses.

In the production of the main vegetables in the greenhouses Fier county is distinguished with continuous growth during 2013-2016 and especially a significant increase in 2017. It is also distinguished the Berat county which, after the production decline in 2014, has had a steady increase in production. Elbasan county has a slight growth of production until 2016, while in 2017 there has been a decline in production. Shkoder county, following a fall in the first year, has continued production growth. Than comes Durres county, which has a growth in production in 2014, followed by a second increase in 2017. Tirana county has a growth in production in 2014 and then fluctuates at lower production levels. Vlora county has a growth in production in 2014, followed by a continuous decline. Then come the counties of Kukes, Lezha and Korce. Kukes county has a growth in production in the first year and then maintains a constant production level. Lezhe county has production fluctuation, while the Korce county has produced for the first time in greenhouses only in 2017. In the end there are Diber and Gjirokastra counties. The Diber county has declined to level zero production in 2014, and then fluctuations in low production levels. The Gjirokastra county has fluctuations at the lowest levels of production. Graphs for Diber, Gjirokaster, Korce and Lezhe counties do not show their production levels, as they are quite low compared to other counties. The results presented in Table 4 are taken from the evaluation of the performance of the counties related to the production of the main vegetables in the greenhouses.

Table 4. Counties performance in production of main vegetables in greenhouses, 2015-2017 (the relevant graph)

County	Technical efficiency		
	2015	2016	2017
Berat	1.00000	1.00000	0.97129
Diber	0.49892	0.55033	0.47031
Durres	0.80341	0.84239	1.00000
Elbasan	1.00000	1.00000	0.97861
Fier	1.00000	1.00000	1.00000
Gjirokaster	0.18885	0.25899	0.45000
Korce	0.00000	0.00000	0.80122
Kukes	1.00000	1.00000	1.00000
Lezhe	0.89964	0.83379	0.78216
Shkoder	0.87248	0.81148	0.82352
Tirane	0.47968	0.44854	0.39798
Vlore	0.50476	0.49689	0.40149
Avarage	**0.68731**	**0.68687**	**0.75638**



In the production of the main vegetable in the greenouses, Fier and Kukes counties are technically efficient, followed by the counties of Durres, Berat, Korce, Elbasan, Lezhe, Shkoder, Diber, Gjirokaster, Vlore, Tirana, which are technically inefficient and exhibit lower levels in their performance.

DISCUSSION

The performance of the counties depends not only on the production produced by them but also on other indicators that affect it, such as the surface of the cultivated area for the production of vegetables. Therefore, the production levels should be faced with the results of the efficiency of the counties. At the same time, we support our discussion on the levels of production realized in the years 2013-2017 for the counties as well as their relative efficiency values during these years, given in Tables 1, 2, 3, 4.

Thus, the Fier county is distinguished for the highest level of vegetable production in the whole country. Main vegetables this county has produced up to 75% of total production and second vegetables about 25% of total vegetable production. It is distinguished from all counties even at the level of production of the main vegetables taken in the greenhouses, thus holding up to 22% of their production. Even in terms of performance, in all cases and at all times, it has been at the maximum level. So this county is efficient and always the production of vegetables is taken by maximally exploiting the cultivated land surface. Tirana county, which ranks second in the production of total vegetables in the country, about 87% of them are the main vegetables, and much less the second vegetables, about 13%. The level of main vegetables produced in the greenhouse from this county is small and it is only 2% of them. Conversely, the performance of the Tirana county in all cases and throughout the study period, compared to those of other counties, is rather poor by listing it in the penultimate or last place. This indicates that this county compared to other counties is inefficient, so it does not maximally utilize the cultivated area to reach the existing level of production. Berat county occupies the third place in the production of total vegetables. It produces about 70% of them as main vegetables and about 30% as second vegetables. But it differs in the production of the second vegetables being ranked after Fier and leaving behind even Tirana. It also differs in the production of main vegetables in the greenhouses, producing around 55% of their total level, where it also ranks after Fier leaving behind all other counties. While from the performance evaluation in all cases it is clear that the county of Berat is technically efficient reaching its maximum level, therefore it is efficient in utilizing the cultivated area in the production of vegetables in all cases, except in case of production of main vegetables in greenhouses in 2017, when there is a slight decrease from the maximum level. The counties of Durres, Elbasan and Shkoder are at average levels

of the production of total vegetables, following the above-mentioned main counties. The Durres county has made most of its production with main vegetables, up to 79%, and a smaller share of second vegetables, around 21%. In the greenhouses, a small amount of main vegetables is produced, up to 8% of them. The relative efficiency values in the total vegetable production in the main and second vegetables show inefficiencies in the production by not maximally utilizing the cultivated area in each of the cases. It distinguishes performance in vegetable production in greenhouses, where over the years there has been an increase in technical efficiency up to the maximum level, thus passing from technically inefficient to technically efficient in production. Elbasan, up to 90% of its production is in main vegetables and the rest in second vegetables. In the greenhouses, in different years, it has produced up to 17% in main vegetables. Efficiency values in total production and that of main vegetables show that it is always inefficient, so there is room for improvements with a view to maximizing the utilization of the cultivated area. The most notable inefficiency appeared in the second vegetables. Better situation occurs in the production in greenhouses, where the county reaches maximum values, though with a slight fall in the last year. Shkodra, too, most of the total production is in the main vegetables, approximately 95%, and less in the second vegetables (about 5%) and from the main vegetables up to 5% of them are produced in greenhouses. The relative efficiency values in the production of total vegetables and the production of main vegetables show inefficiencies in the use of the cultivated area. The situation is worse in the production of second vegetables. In the production of main vegetables in the greenhouses it seems inefficient but not as noticeable as in the cases above. Korce county, that follows them in the production of total vegetables, produces main vegetables only being ranked after Fier and Tirana and does not produce second vegetables. There is also no production in greenhouses in Korca county, with the exception of the last year, about 0.2% of the main vegetables. The values of the technical efficiency in the total vegetable and main vegetable production show the optimum utilization of the cultivated area. While in second vegetables it has 0 because it does not produce. In the greenhouses there is an increase of efficiency in the last year showing its potentials in production in the greenhouses. Diber, Lezha, Vlora counties follow Korca with their production levels. The Diber county marks a decline in the production of total vegetable, which are all main vegetables with the exception of 3 tonnes of second vegetables in 2017. From the main vegetables in the greenhouses it produces up to 0.03% of them. Dibra shows inefficiency in the production of total vegetables and main vegetables. In second vegetables inefficiency is even more noticeable. Even in the greenhouses it turns out to be inefficient. The Lezhe county marks a slight decrease in total vegetables. The main vegetables, by contrast, represent an increase, accounting for about 75% of total production. The rest are second vegetables (about 25%). In the greenhouses it produces up to 2% of the main vegetables. Lezha turns out to be inefficient in its performance in all cases. But the lower performance is in the production of second vegetables. Vlora county produces main vegetables up to 83% of them and second vegetables about 17%. Vlora is inefficient in the production of vegetables in all cases. Efficiency values in second vegetables are the indicator of its poor performance. Kukës and Gjirokastra have lower levels in the production of total vegetables. Kukes produces up to 93% of the total vegetables as main vegetables and and about 7% of total vegetables as second vegetables. But there are efforts to cultivate vegetables in greenhouses up to 1% of main vegetables. Kukes reaches the maximum value in its performance in total production and in main vegetables. This demonstrates technical efficiency in the production of vegetables by maximizing the cultivated area. It is inefficient in the second vegetables and efficient in the production of main vegetables in the greenhouses. Gjirokastra produces up to 97% of the total as main vegetables and about 3% as second vegetables. In the greenhouses the main vegetable production accounts for about 0.8% of them. Gjirokastra is inefficient in total production and

in main vegetables. Meanwhile, the performance is still lower in the production of second vegetables and in the production of main vegetables in the greenhouses.

CONCLUSIONS

In the assessment of the performance of the counties in the production of total vegetables, according to Table 1, during the period 2013-2017, about 25% of them reach the maximum value, so they are technically efficient, while about 75% of them are technically inefficient. During these five years there has been a slight decrease in the average level of county efficiency in the production of total vegetables. Even in the assessment of the performance of the counties in the production of the main vegetables, according to Table 2, it is noticed that during the period 2013-2017 about 25% of them are technically efficient, while about 75% of them are technically inefficient. During these years there has been a slight fall in the average level of the performance of counties in the production of main vegetables. The situation varies in the assessment of the performance of counties in the production of second vegetables. It is noted from Table 3 that during the period 2013-2017, approximately 17% of the counties reach the maximum level in their technical efficiency and about 83% of them are technically inefficient, therefore they do not exhibit optimal levels in the exploitation of the cultivated area in the production process. Counties performance in the production of second vegetables has been decreasing and increasing during these years. The performance is poorer than that displayed in the production of main vegetables. If we look at the performance of the counties in the production of the main vegetables in the years 2015-2017, according to Table 4, we notice that about 17% of the counties have the maximum value in the displayed performance, while about 83% of them are inefficient. A slight increase observed in the last year at the average level of county efficiency in the production of main vegetables in the greenhouses is to be mentioned.

As a conclusion, which is based on the indicators taken into consideration, counties that result technically efficient when evaluating their performance in vegetable production (total, main or second) have reached their level of production by minimizing the cultivated area. If they wanted to increase the production level, they would have to increase the amount of cultivated area with vegetables. While in any county and in any case we have a result of lower technical efficiency, there is room for improvement by making the best use of the area being cultivated for the production of vegetables. Improvement can be accomplished through θ and slacks or equivalent the improvement can be orientated by reference set. For year 2017, we provide reference sets and optimal lambdas for each county and for each occasion of the vegetable production discussed above, wich are shown in tables 5 and 6.

Table 5. Reference sets by type of production for 2017.

		Reference sets			
No	County	Total production of vegetables	Main production of vegetables	Second production of vegetables	Main production of vegetables in greenhouses
1	Berat	1	1	1	3,5
2	Diber	1,8	1,8	2	3,8
3	Durres	1,8	1,8	1,2	3
4	Elbasan	1,8	1,8	1,2	3,5
5	Fier	5	5	5	5
6	Gjirokaster	8	1,8	1,2	8
7	Korce	1,8	1,5	2	3,8
8	Kukes	8	8	1,2	8
9	Lezhe	1,8	1,8	1,2	3,8
10	Shkoder	1,8	1,5	1,2	3,5
11	Tirane	1,5	1,5	1,2	3,8
12	Vlore	1,8	1,8	1,2	3,8

Table 6. Optimal lambdas by type of production for 2017.

No	County	OPTIMAL LAMBDAS			
		Total production of vegetables	Main production of vegetables	Second production of vegetables	Main production of vegetables in greenhouses
1	Berat	$\lambda_1 = 1.000$	$\lambda_1 = 1.000$	$\lambda_1 = 1.000$	$\lambda_3 = 0.333$ $\lambda_5 = 0.667$
2	Diber	$\lambda_1 = 0.283$ $\lambda_8 = 0{,}717$	$\lambda_1 = 0.487$ $\lambda_8 = 0.513$	$\lambda_2 = 1.000$	$\lambda_3 = 0.001$ $\lambda_8 = 0.999$
3	Durres	$\lambda_1 = 0.718$ $\lambda_8 = 0.282$	$\lambda_1 = 0.912$ $\lambda_8 = 0.088$	$\lambda_1 = 0.428$ $\lambda_2 = 0.572$	$\lambda_3 = 1.000$
4	Elbasan	$\lambda_1 = 0.650$ $\lambda_8 = 0.350$	$\lambda_1 = 0.805$ $\lambda_8 = 0.195$	$\lambda_1 = 0.424$ $\lambda_2 = 0.576$	$\lambda_3 = 0.905$ $\lambda_5 = 0.095$
5	Fier	$\lambda_5 = 1.000$	$\lambda_5 = 1.000$	$\lambda_5 = 1.000$	$\lambda_5 = 1.000$
6	Gjirokaster	$\lambda_8 = 1.000$	$\lambda_1 = 0.018$ $\lambda_8 = 0.982$	$\lambda_1 = 0.008$ $\lambda_2 = 0.992$	$\lambda_8 = 1.000$
7	Korce	$\lambda_1 = 0.662$ $\lambda_8 = 0.338$	$\lambda_1 = 0.968$ $\lambda_5 = 0.032$	$\lambda_2 = 1.000$	$\lambda_3 = 0.013$ $\lambda_8 = 0.987$
8	Kukes	$\lambda_8 = 1.000$	$\lambda_8 = 1.000$	$\lambda_1 = 0.044$ $\lambda_2 = 0.956$	$\lambda_8 = 1.000$
9	Lezhe	$\lambda_1 = 0.270$ $\lambda_8 = 0.730$	$\lambda_1 = 0.387$ $\lambda_8 = 0.613$	$\lambda_1 = 0.121$ $\lambda_2 = 0.879$	$\lambda_3 = 0.121$ $\lambda_8 = 0.879$
10	Shkoder	$\lambda_1 = 0.659$ $\lambda_8 = 0.341$	$\lambda_1 = 0.995$ $\lambda_5 = 0.005$	$\lambda_1 = 0.122$ $\lambda_2 = 0.878$	$\lambda_3 = 0.937$ $\lambda_5 = 0.063$
11	Tirane	$\lambda_1 = 0.990$ $\lambda_5 = 0.010$	$\lambda_1 = 0.857$ $\lambda_5 = 0.143$	$\lambda_1 = 0.374$ $\lambda_2 = 0.626$	$\lambda_3 = 0.442$ $\lambda_8 = 0.558$
12	Vlore	$\lambda_1 = 0.223$ $\lambda_8 = 0.777$	$\lambda_1 = 0.277$ $\lambda_8 = 0.723$	$\lambda_1 = 0.174$ $\lambda_2 = 0.826$	$\lambda_3 = 0.170$ $\lambda_8 = 0.830$

To clarify the meaning of the reference set, we are referring to the example of the Tirana county, which reaches high levels in the production of vegetables ranking second after Fier, but in the performance evaluation it has resulted poorer than all other counties. According to Table 5, in the production of total vegetables the Tirana county has as its reference set the Berat and Fier districts, which are efficient and comparable with, according to which this county should project its improvement to technical efficiency. So if the cultivated area and production of vegetables of Berat and Fier counties are multiplied by the optimal lambdas given in Table 6,$\lambda_1 = 0.990$, $\lambda_5 = 0.010$, the Tirana county projection is reached at the efficient frontier. Following the same logic, in the production of main vegetables it is necessary to compare again with the counties of Fier and Berat, in the production of the second vegetables with the Berat and Diber counties and in the production of the main vegetables in greenhouses with Durres and Kukes counties, always keeping in mind the optimal lambdas given in table 6.

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NEW HABITATS OF *GONIOLIMON DALMATICUM* (C. PRESL) RCHB. F. IN BULGARIA

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Abstract

The conducted research was focused on monitoring three new habitats of Balkan endemic species *Goniolimon dalmaticum*, all three found in the Hadjidimovo Municipality, Blagoevgrad in 2011. The habitats are located near the villages Nova Lovcha, area "Polianite" and "Kosharite", and Gaitaninovo, area "Omaya". The habitats in "Kosharite" and "Omaya" occupy an area of about 2 ha and "Polianite" – 2.5 ha. The populations contain 2400, 3750, and 2850 plants, respectively. All three populations are very well developed, with one to three flowering stems per plant. Over the years no change was observed in the ratio of generative and vegetative plants within the populations. Generative plants dominated in the populations, which is an indicator of the population stability.

Keywords: *Protective, Endemic, Habitat, Population, Goniolimon dalmaticum*

INTRODUCTION

The wild species of the *Plumbaginaceae* family have valuable ornamental and commercial characteristics. The species with the highest ornamental value are of the genuses *Goniolimon* and *Limonium.* There are four species of the genus *Goniolimon* in the Bulgarian flora: *Goniolimon besserianum* (Schult. ex Rchb.) Kusn., *Goniolimon collinum* (Griseb.) Boiss., *Goniolimon dalmaticum* (C. Presl) Rchb. f., and *Goniolimon tataricum* (L.) Boiss. All species are of conservation significance and are listed in the Red Book of Bulgaria and Annex No. 3 of the Biological Diversity Act. *Goniolimon dalmaticum* is a Balkan endemic. According to the assessment made using the criteria of IUCN's *Red List of Threatened Plants* (Walter & Gillett (eds.), 1998.1997), on regional level the species is assigned to the category "Critically endangered species in the Bulgarian flora".

Due to their ornamental qualities, the wild species of the *Plumbaginaceae* family, genus *Goniolimon* are widely used in floriculture in various countries (Yanev, 1959; Anchev, 1982; Rizzotto, 1999). Research on the protected species of this family has been conducted by a number of authors in Bulgaria (Ivanova et al., 2008; Kaninski et al., 2000; Kaninski et al., 2008a; Kaninski et al., 2008b; Manolova et al., 2015).

Climate change over the last few years, pests as well as negative human impact are a threat to the preservation of the *Goniolimon dalmaticum* species.

Goniolimon dalmaticum is widespread not only in Bulgaria, but also in other countries on the Balkan Peninsula (Albania, Greece, Macedonia, Croatia, Montenegro, Serbia, European Turkey) (Goranova & Anchev, 2011; Buzurović et al., 2013; Crivelli, 1997).

The aim of this study was to monitor three new habitats of the Balkan endemic *Goniolimon dalmaticum* in Hadjidimovo Municipality, Blagoevgrad.

MATERIAL AND METHODS

The three new habitats of the endangered species *Goniolimon dalmaticum* found at the foot of Slavyanka Mountain in 2011 were studied during the period 2011 to 2013. The habitats "Polianite" and "Kosharite are near N. Lovcha, whilst the third habitat "Omaya" is located on the territory of Gaitaninovo, Hadjidimovo Municipality, Blagoevgrad.

Monitoring of the populations was carried out according to the methodology for monitoring of higher plants in two phenophases: full flowering and seed maturity (Baideman, 1954). The following habitats' characteristics were observed and analysed: size, slope, soil type, population density, and health status.

Biometric data were collected from 20 plants in eight fixed sites for each habitats, whereby the following two indicators were considered: height of flowering stems, number of flowering stems per plant (Lidanski, 1988).

The coefficient of variation (CV) for the indicators height of flowering stems during flowering and number of flowering stems per plant was determined with:

$$CV = \frac{\text{mx}100}{\text{M}} \%$$

CV – Coefficient of Variation;
M – Mean;
m – Standard deviation;
CV **–** up to 10% **–** Weak variation;

CV **–** 10-30% **–** Average variation;

CV – above 30% **–** Strong variation.

GPS coordinates and altitude were obtained for each habitat of *Goniolimon dalmaticum*.

RESULTS AND DISCUSSION

During the entire period of the study no change was observed in the size of the two newly found habitats of *G. dalmaticum* ("Kosharite" and "Omaya"). Their area amounts to approximately 2 ha. An increase in area was observed for the third habitat ("Polianite"). It expanded from 1.5 ha during the first year to 2.5 ha at the end of the study. The 1 ha increase in size of "Polianite" habitat was due to the population expanding to a greater area.

During the monitoring it was found that all three habitats are south facing and have brown forest soils with shallow capacity. The soils are stony, dry, and limy. The slope of the "Polianite" habitat ranges from 10° to 35°. For "Omaya" and "Kosharite" the slopes are between 3° and 30°, and between 5° and 10°, respectively.

Out of the three habitats, the average altitude of "Omaya" is the lowest one (649 m), followed by "Kosharite" (708 m) and "Polianite" (742 m).

Three newly found habitats of *G. dalmaticum* are located at approximately the same average altitude as two other habitats discovered by our team **–** "Zastavata" (631 m) and "Granichen punkt" (718 m), both near Nova Lovcha (Ivanova et al., 2008). Other authors (Anchev, 1982; Goranova and Anchev, 2011) have reported that this species can be found at an altitude of 1000 m.

The period of full flowering is virtually the same for all the three populations, the difference being merely one to two days. For seed maturing this difference ranges between two and four days (Table 1). This development probably results from the approximately equal altitude and southern exposure of the habitats.

Table 1. Phenological records for *G. dalmaticum* in the habitats "Kosharite", "Polianite", and "Omaya" (average for a period of three years (2011 – 2013)

Habitat	Full flowering	Seed maturity
"Kosharite"	12/6	28/8
"Polianite"	14/6	31/8
"Omaya"	12/6	26/8

The population of *G. dalmaticum* in the habitat "Polianite" near Nova Lovcha is numerous, amounting to approximately 2850 plants (Table 2), which are distributed relatively even throughout the area. The majority of the population consists either of groups of 2–5 individuals or of single plants. The number of plants in the population has increased by about 990 over the three-year period.

The population in the "Polianite" habitat is mixed and consists of the species *Goniolimon dalmaticum*, *Goniolimon tataricum*, and *Goniolimon collinum*. The predominant species in the population is *G. dalmaticum* – 70% of the total number of plants, followed by *G. tataricum* (20%) and *G. collinum* (10%). Over the years no change was observed with regards to the size ratio between species in the population. With 0.114 plants per m^2 the density of the species is good; in some areas it reaches 2–3 plants per m^2.

During the years of study population size of *G. dalmaticum* in the habitat "Kosharite" totals roughly 2400 plants (Table 2), distributed relatively even throughout the area. The density of the species is good – 0.225 plants per m^2, reaching 3–5 plants per m^2 in single areas.

The population of the species *G. dalmaticum* in the habitat "Omaya" near Gaitaninovo consists of 3750 plants (Table 2). The plants are mostly single and in groups of 3–4 plants. With regards to population density no change was detected over the three years of study. Population density is good – 0.19 plants per m^2, reaching 2–5 plants per m^2 in some areas. The population consists only of the species *G. dalmaticum*.

Table 2. Biometrical data on *G. dalmaticum* in the habitats "Kosharite", "Polianite", and "Omaya" (average for a period of three years (2011 – 2013)

Habitat	Total number of plants in the population (no.)	*Plant height, cm*		Number of flowering stems per plant (no.)	
		M ± m	CV %	M ± m	CV %
"Kosharite"	2400	13.82 ± 7.3	52.82	1.6 ± 0.55	34.37
"Polianite"	2850	10.52 ± 4.5	42.78	1.95 ± 0.93	47.69
"Omaya"	3750	18.35 ± 6.35	34.60	2.24 ± 0.86	30.39

Note: M – mean; ±m – standard deviation; CV – coefficient of variation.

A variation concerning plant height, number of flowering stems per plant, and height during the full flowering has been observed over the years (Table 2). The greatest height was registered for plants of the *G. dalmaticum* population in "Omaya", ranging between 18.35 cm and 23.5 cm. The lowest figures in this respect were recorded for plants in the habitat "Polianite", which have an average height of 10.52 cm.

In all three populations, the number of flowering stems per plant ranges from 1 to 3 (Table 2), whereby there were single plants (0.5%) with 4 flowering stems per plant in the habitat "Omaya" in the last year of the study. There are differences between the three populations with

regards to the percentage ratio in the number of plants with one, two and three flowering stems. In the habitat "Omaya" the plants with 3 flowering stems prevail (47.5%), followed by those with 1 flowering stem (32.8%) and with 2 flowering stems (19.7%). In the habitat "Polianite" mostly plants with 1 flowering stem are found (52.3%), followed by those with 2 flowering stems (35.0%) and with 3 flowering stems (12.7%). In the habitat "Kosharite" the number of plants with one, two and three flowering is approximately equal with 35.7%, 34.0%, and 30.3%, respectively.

The high values of the coefficient of variation (Table 2) for the indicators plant height and number of flowering stems per plant during full flowering in all three populations show that populations are highly uneven under natural conditions. This is probably due to the more unfavourable soil and climate conditions as well as to the different age and structure of the individual plants within the population in the habitat.

During the years of the study no change in the ratio of generative and vegetative plants within the populations was observed. Generative plants dominate in all three habitats, which is an indicator of the stability of the populations (Table 3).

Table 3. Ratio of generative and vegetative plants of the species *G. dalmaticum* in the habitats "Kosharite", "Polianite", and "Omaya" (average for a period of three years (2011–2013)

Habitat/ Species	Percent (%)		Number of reproductive plants in the population per m^2		
	Vegetative plants (no.)	Generative plants(no.)	Gegetative plants(no.)	Generative plants(no.)	Total
Habitat "Polianite"					
G. dalmaticum	25	75	0.02	0.06	0.080
G. tataricum	20	80	0.005	0.018	0.023
G. collinum	40	60	0.007	0.008	0.011
Habitat "Kosharite"					
G. dalmaticum	*25*	*75*	*0.056*	*0.169*	*0.225*
Habitat"Omaya"					
G. dalmaticum	45	55	0.09	0.10	0.19

Monitoring during the first year show that the populations of *G. dalmaticum* in the three habitats have an excellent health status. No damage from diseases or by pests on vegetative and generative organs was observed. Except for the population in the habitat "Omaya", in the second and third year of study, damage was detected for the populations in the two other habitats. Leaves were damaged by leaf-mining moths. There were also nibble marks of unknown origin on unripe seeds. For the population in "Kosharite", 2012 saw a 4% increase in damage, which then went up to 8% in the last year of study. As for the habitat "Polianite", about 50% of the plants of all three *Goniolimon* species experienced seed damage in the second year of study, whereas the third year saw a significant reduction in the percentage of damaged seeds (-15%).

Changes in health status require annual monitoring of the populations in both habitats during flowering and seed formation in order to establish the extent of damage for preservation purposes.

CONCLUSION

Initial data about the population of the protected species *Goniolimon dalmaticum* in three newly found habitats "Kosharite", "Polianite", and "Omaya" at the foot of Slavyanka Mountain, Hadjidimovo Municipality, Blagoevgrad, were obtained.

Populations of the species *Goniolimon dalmaticum* in all three habitats are numerous. The plants are well developed with 1 to 3 flowering stems per plant.

Generative plants dominated in all three habitats, which is an indicator of the stability of the populations.

In the habitats "Kosharite" and "Polianite", damages by leaf-mining moth on the leaves as well as nibble marks of unknown origin on unripe seed were detected. These damages require annual monitoring of the populations in both habitats during the phases flowering and seed formation in order to determine the extent of the damage for preservation purposes.

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CO-PRECIPITATED MAGNETITE PROPERTIES IN WASTEWATERS PHOSPHATE ADSORPTION AND MICROBIAL CHARGE REDUCTION

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ABSTRACT

In this paper are presented experimental results of synthetic magnetite adsorption capacity study for phosphorus removal from wastewaters. The magnetite used is produced by co-precipitation synthesis. First, the optimal precipitation conditions, such as NaOH addition rate, the presence of inert media, and the drying temperature are identified. After that, adsorption characteristics in synthetic and real wastewaters are evaluated. It is concluded that magnetite manifest better characteristics if it is produced in an inert media by fast addition of NaOH in the Fe(II)/Fe(III) mixture and dried at 60°C. Phosphorus adsorption in synthetic waters takes place after Langmuir isotherm. The situation differs in real wastewaters where, considering the competing ions, the adsorption takes place after Freundlich isotherm with equation constants k=13.12 and 1/n=2.9037. The presence of competing ions also seems to lead to weaker capacities of magnetite to adsorb phosphorous. It is also shown a microbial reduction in the presence of magnetite, especially coliform bacteria, but also yeasts and moulds colonies.

Keywords: *Magnetite, Co-precipitation synthesis, Adsorption capacity, Phosphate removal, Microbial load reduction*

INTRODUCTION

It is known that natural magnetite ($FeO \cdot Fe_2O_3$) has very good adsorption capacities of a variety of aqueous substances. It has been widely used to separate chemical, physical and biological substances (Boyd at al., 1986).

Due to its novel properties, there has been numerous efforts in producing synthetic magnetite with higher efficiency, capable of being used in treating wastewaters produced by mines (Navratil and Akin, 2009), metal finishing industries (Oskay, 2003; Yuan et al., 2010; Mayo et al., 2007), oil industry (Moniwa et al., 2010) etc. Magnetite has also been used for color and turbidity removal (Anderson et al., 1982), bacteria cells removal (Mac Rae and Evans, 1984), phosphorus removal (Yao-Jen Tu at al., 2015; Abo Markeb et al, 2016) etc.

There are a variety of methods for producing synthetic magnetite such as classical synthesis by co-precipitation, reactions in constrained environments, hydrothermal and high-temperature reactions, sol-gel reactions, polyol methods, flow injection syntheses, electrochemical methods, aerosol/vapor methods, and sonolysis (Laurent, 2008). The co-precipitation technique is probably the simplest and most efficient chemical pathway. Iron oxides (either Fe_3O_4 or γFe_2O_3) are usually prepared by aging ferrous and ferric salts solutions with a stoichiometric ratio of 2:1 (Fe3+/Fe2+) in a non-oxidizing oxygen aqueous medium, at a pH between 8 and 14. It is important to consider that magnetite (Fe3O4) is not very stable and is sensitive to oxidation. It is transformed into maghemite (γFe2O3) in the presence of oxygen and acidic conditions. The main advantage of the co-precipitation process is that a large amount of nanoparticles can be synthesized. However, the control of particle size distribution

is limited. The size and shape of the nanoparticles can be tailored with relative success by adjusting pH, ionic strength, temperature, nature of the salts (perchlorates, chlorides, sulfates, and nitrates), FeII/FeIII concentration ratio or flow rate of added chemicals. The addition of chelating organic anions (carboxylate ions, such as citric, gluconic, or oleic acid) or polymer surface complexing agents (dextran, carboxydextran, starch, or polyvinyl alcohol) during the formation of magnetite can help to control the size of the nanoparticles (Laurent, 2008).

In this paper there are presented the experimental results of co-precipitated magnetite adsorption characteristics for orthophosphate and microbial removal from synthetic and diary wastewaters.

MATERIALS AND METHODS

Magnetite production

Synthetic magnetite is produced through chemical co-precipitation method of Fe(II)/Fe(III) salts solutions, at a ratio of 0.5, like that of natural magnetite. Ferrous and ferric stock solution is obtained using $FeCl_2*4H_2O$ and $FeCl_3$ (3%) reagents. Stock solution of 10M NaOH is used to create the alkali conditions (Kim at al., 2001). A solution of 0.01M HCl is prepared for surface neutralization, while a solution of 2% sodium oleate is used as a surfactant solution for coating of magnetite particles (Kim at al., 2001). The inert reaction medium is assured by bubbling N_2 gas in the reaction vessel. All the chemicals are of reagent grade.

Aqueous dispersion of magnetic particles was prepared by alkalinizing an aqueous mixture of ferric and ferrous salts with NaOH at room temperature. For that, first 2.28 g $FeCl_2*4H_2O$ dissolved in 100 ml distilled water is mechanically mixed with 1 ml of $FeCl_3$ (3%) solution. A certain volume of NaOH is added drop-wise (at a certain flow rate), until pH 10 is reached. After 30 min of stirring the solution is subjected to an external magnetic field and the precipitated powder is isolated. The magnetite powder is washed four times with distilled water and one time with 0.01M HCl to neutralize the anionic charge on the particle surface. The cationic particles are separated by decantation in the presence of the magnetic field and washed three more times with distilled water prior to coating. Coating is carried out using sodium oleate solution under vigorous mechanical stirring for 30 min at 90°C (Kim at al, 2001). After coating, the particles are separated by the surfactant and washed for tree times with distilled water to remove the excessed surfactant adsorbed physically on the particle surface. All the main synthesis steps are carried out in the presence of N_2 gas to avoid possible oxygen contamination during the operations. Three operating conditions: (i) the flow rate of NaOH, (ii) the presence of inert gas, and (iii) drying temperature of magnetite particles are considered for different synthesis experiments.

Batch experiments for orthophosphate adsorption on magnetite

All magnetite water treatment experiments are realized after the same protocol. In a series of chemical flasks, 40 ml of phosphate solutions are treated with 0.1 g magnetite, under mechanical stirring. After a certain contact time, they are subjected to filtration and the filtrate is analyzed for phosphate content. Two scenarios have been investigated: (i) synthetic phosphate wastewater and (ii) diary industry wastewater. The synthetic wastewater samples, of different phosphate concentration, are prepared using 200 mg/l KH_2PO_4 stock solution.

Aqueous orthophosphate spectrophotometric determination method (APHA, 1998).

The reagents used are: ammonium molibdate solution (9.5 g of (NH4)6Mo7O24·4H2O dissolved in 100 mL water); 4.5M sulfuric acid; ascorbic acid solution (7 g C6H8O6 diluted in 100 mL water); potassium antimonile tartrate solution (3,25 g of K(SbO)C4H4O6·1/2H2O

diluted in 100 mL water); mixed reagent (45 mL of ammonium molibdate solution is added in 200 mL sulfuric acid and 5 mL of potassium antimonile tartrate solution).

In 20 ml of water sample is added 0.5 ml of ascorbic acid, 1 ml of combined reactive and water till 25 mL volume. After 15 minutes the absorption value in the wave length 880 nm is determined. Measurement are performed using UV 1200 spectrophotometer.

Experimental method of determining the water sample microbial load

Sample dilution: 10 ml of waste water sample is added in 90 ml of sterile water. The method used is that of general and selective field coverage (coliform, lactic bacteria, yeasts and moulds). After incubation in respective conditions, the colonies are counted after 2-7 days. To evaluate magnetite effect in microbial loading reduction, this method is applied to waste water samples before and after the addition of magnetite.

RESULTS

The aim of this work is the evaluation of a) magnetite production operating conditions and b) magnetite capacity in adsorbing phosphate and reducing microbial load. Two scenarios have been taken in consideration: i) synthetic waters and ii) real diary wastewaters.

Magnetite production operating conditions

Three operating conditions have been taken in consideration: NaOH flow rate, the presence of inert medium and drying temperature of magnetite particles. They are determined by comparing the phosphate adsorption efficiency of magnetite produced at different conditions. 10ppm KH_2PO_4 solutions have been used for the purpose. The magnetite-solution treatment time is 4 hours. The experimental results are shown in table 1.

Table 1. Co-precipitated magnetite characteristics produced at different conditions.

Sample	NaOH flow rate, L/h	Presence of N_2	Drying temperature, °C	Magnetite color	Orthophosphate adsorption efficiency, %
M1	3.6	-	60	Brown	10
M2	3.6	+	60	Black	98
M3	0.9	+	60	Black	30
M4	3.6	+	110	Chocolate brown	8

Comparing the adsorption characteristics of M1 and M2 samples, it can be seen that the inert reaction medium is a crucial factor that influences adsorption efficiency. In the absence of N_2 gas, magnetite particles are of brown color, rather than black (the color of real magnetite), showing that the oxygen present in solution has caused oxidation of ferrous species. In fact, adsorption efficiency decreases from 98% for M2 to 10% for M1.

Another important parameter that influences adsorption effectiveness is particle size of the adsorptive media. In this case particle size depends on the rate that NaOH is added to Fe(II) - Fe(III) solution. Comparing the samples M2 and M3 it can be concluded that fast addition of NaOH (M2) yield to higher phosphate adsorption efficiency. Magnetite crystallization time is much shorter and thus much more crystals are formed with smaller size. The specific surface area of magnetite particles produced by fast addition is expected to be greater than that produced by slow addition. Taking this in consideration, all the other experiments are carried out with magnetite produced in the presence of N_2 and by fast addition of NaOH.

Comparing the samples M2 and M4, it seems that the drying temperature of magnetite particles is another parameter that influences magnetite adsorption characteristics. The most appropriate drying temperature, between the two values taken in consideration, is 60°C. By increasing the temperature, the magnetite particles color changes from black to brown which means that there has been some changes in their structure. In fact, even the adsorption efficiency decreases from 98% to 8%. After (Tang et al., 2002), in the temperatures around 25°C magnetite is oxidizing very slowly to maghemite (c-Fe2O3). If temperature increases the oxidation goes till hematite. This is why we conducted the drying process for a short period of time (1 hour).

Orthophosphate adsorption capacity of magnetite

The aim of this experiments set is to evaluate adsorption isotherm. For that, first it is evaluated the time required to reach adsorption equilibrium. The experimental results are shown in figure 1. Six parallel tests are conducted for this purpose. 40 ml of 10 ppm aqueous KH_2PO_4 sample is treated with 0.1 g magnetite, at room temperature, 20 °C, for contact time, respectively 1, 2, 3, 4, 6, and 12 hours.

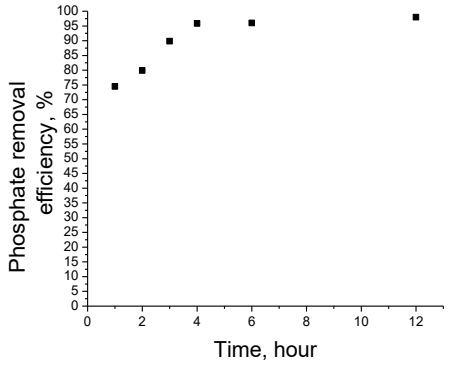

Figure 1. The change of phosphate removal efficiency by adsorption on magnetite over contact time.

As can be seen, the percentage of adsorbed phosphorus increases as the treatment time increases up to 4 hours. After that, the percentage of phosphorus removed is almost constant. This means that the adsorption equilibrium is reached for 4 hours. All the following experiments are accomplished for contact time over 4 hours.

In order to determine the adsorption isotherm, six parallel treatment tests of 40 ml KH_2PO_4 aquatic solutions, with concentration 1, 5, 7, 10, 15, and 20 mg/l respectively, are performed. Each of them are treated with 0.1 g magnetite, at room temperature, 20 °C, for contact time 4 hours. The experimental adsorption results are presented at figure 2 and 3. The maximum amount of phosphorus removal achieved is 0.14 g/g magnetite.

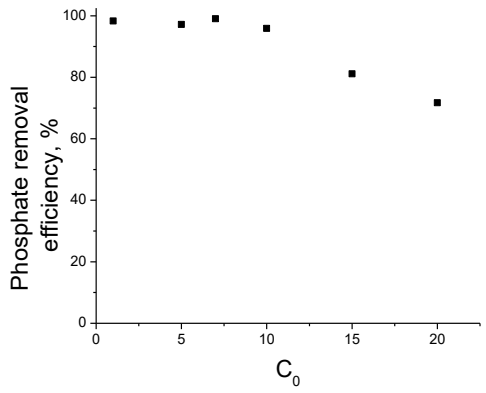

Figure 2. Phosphate removal efficiency over initial concentration, in mg/l.

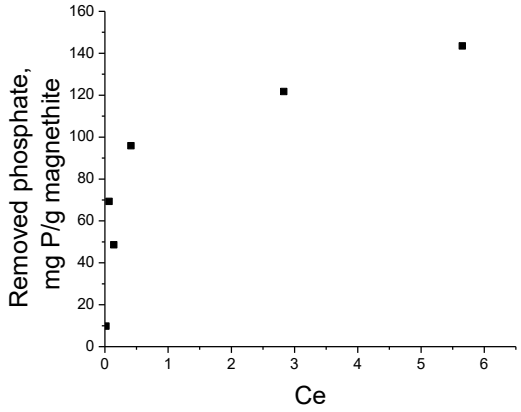

Figure 3. Adsorption isotherm of phosphate in magnetite. C_e is the equilibrium concentration, mg/l.

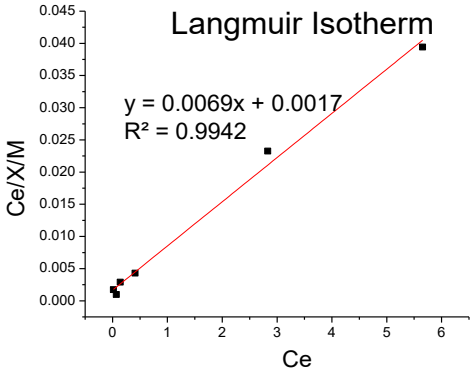

Figure 4. Langmuir isotherm linearization of phosphate adsorption in magnetite. X/M is the amount of orthophosphate adsorbed per amount of magnetite, in mg/g. C_e is the equilibrium concentration, mg/l.

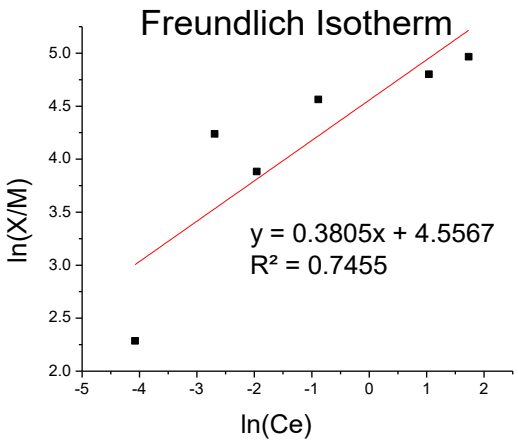

Figure 5. Freundlich isotherm linearization of phosphate adsorption in magnetite. X/M is the amount of orthophosphate adsorbed per amount of magnetite, in mg/g. C_e is the equilibrium concentration, mg/l.

By linearization after Langmuir, figure 4, and Freundlich, figure 5, it is concluded that adsorption takes place after the Langmuir isotherm (R^2=0.9942), with isotherm equation coefficient a=0.14492 g P adsorbed/g magnetite and b=4.06.

It should be noted that all the above conclusions are derived from experiments in synthetic waters. The behavior of magnetite to real wastewaters is expected to differ. Consequently, to evaluate the influence of competing ions in the process, all the adsorption experiments are performed on some real diary wastewaters. The experimental results of the adsorption isotherm are presented in figure 6, 7. The phosphate removal efficiency decreases to 70%.

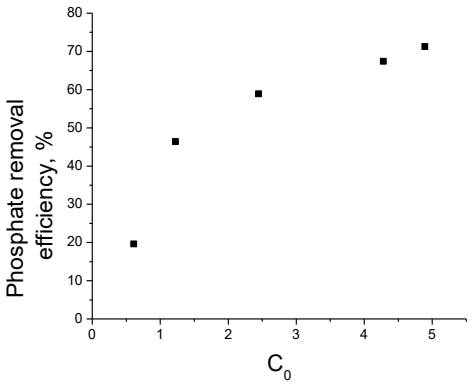

Figure 6. Phosphate removal efficiency over initial concentration, in mg/l.

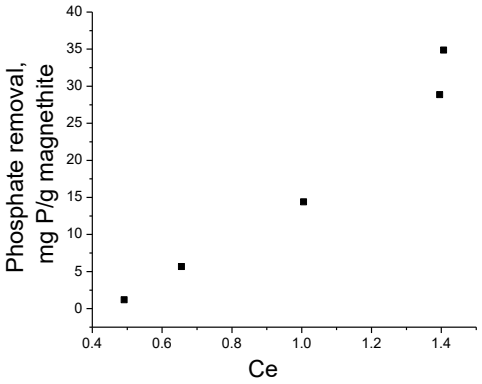

Figure 7. Adsorption isotherm of phosphate in magnetite. C_e is the equilibrium concentration, mg/l.

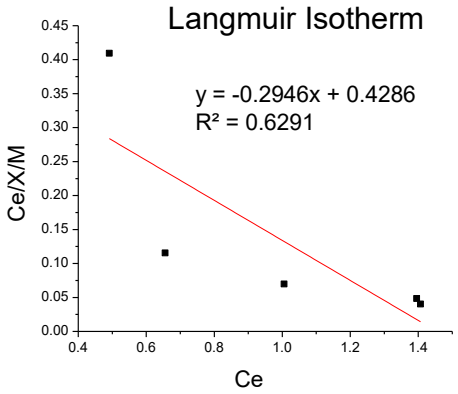

Figure 8. Langmuir isotherm linearization of phosphate adsorption in magnetite. X/M is the amount of orthophosphate adsorbed per amount of magnetite, in mg/g. C_e is the equilibrium concentration, mg/l.

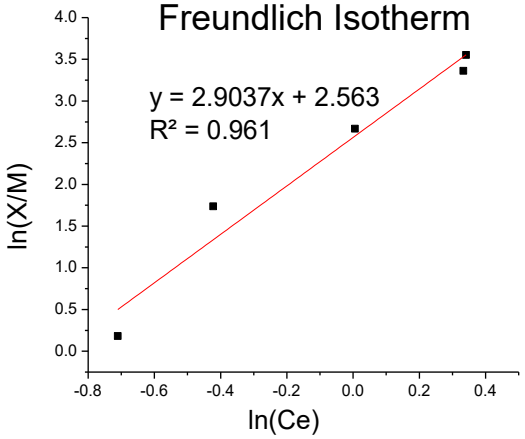

Figure 9. Freundlich isotherm linearization of phosphate adsorption in magnetite. X/M is the amount of orthophosphate adsorbed per amount of magnetite, in mg/g. C_e is the equilibrium concentration, mg/l.

By linearization after Langmuir, figure 8, and Freundlich, figure 9, it is concluded that adsorption in such dairy wastewaters takes place after the Freundlich isotherm (R^2=0.961), with isotherm equation coefficient k=13.12, and 1/n=2.9037.

Microbial load reduction

In addition, real dairy wastewaters are also used to investigate magnetite ability in removing microorganisms. The experimental results for microbial load of magnetite untreated and treated water samples are presented, respectively, in table 2 and 3.

As seen, the total charge of non-treated diary waste water corresponds to $4*10^6$ cfu/ml mesophilic bacteria. It is shown the presence *Fusarium spp.* as white colonies in the Czapek media and also coliform bacteria in Mc Concey media, whose presence may be related to the cleaning water in the dairy industry.

It is important to emphasize that there is no presence of coliform bacteria in Mrs selective media, but we have faced a number of colonies very similar to moulds which may correspond to *Sphaerotilus natans*. This strain can be grown in the presence of high content of lactose,

organic acids and low percentage of phosphorus and in specific conditions may also be pathogenic.

Table 2. Microbiological results for the magnetite non-treated diary waste water

	Cfu/ml			
Media	Dilution 1	Dilution 2	Dilution 3	Dilution 4
PCA	Non cauntable	Non cauntable	Non cauntable	$4*10^6$
Mrs	$20*10^1$	$2*10^2$	-	-
Mc	$2*10^3$	$95*10^2$	$13*10^3$	$3*10^4$
Czapek	$30*10^1$	$4*10^2$	-	-

Table 3. Microbiological results after treatment with magnetite of the diary waste water

	Cfu/ml			
Media	Dilution 1	Dilution 2	Dilution 3	Dilution 4
PCA	$55*10^1$	-	-	$1*10^4$
Mrs	-	-	-	-
Mc	-	-	-	-
Czapek	-	-	-	-

It is obvious the reduction of the microbiological charge from $4*10^6$ cfu/ml to $1*10^4$ cfu/ml after the sample treatment. It is not shown the presence of coliform bacteria in Mc media. There is also seen no presence of yeasts and moulds in respective PDA and Czapek media. As in microscopic views, the only microbiological charge that could be grown in this conditions, correspond to bacteria of cylindrical shapes. This bacteria can also be identified in further studies.

CONCLUSIONS

The experimental results of synthetic and real diary wastewater treatment by synthetic magnetite concluded that this is an excellent method to decrease phosphate and microbial content in industrial wastewaters. The magnetite produced by co-precipitation method through fast addition of NaOH, in an inert reaction media and dried at 60°C showed high efficiency in removing phosphates from diary wastewaters by 70%. In fact, its adsorption ability was higher for pure orthophosphate aqueous solutions, 98%. The presence of other ions in water seem to have influenced the process by conducting competitive reactions. Adsorption takes place after the Freundlich isotherm with equation constants k=13.12 and 1/n=2.9037. It was also shown that magnetite is highly efficient in reducing wastewaters microbial load. The results were excellent especially for coliform bacteria, yeasts and moulds colonies.

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PERFORMANCES OF NEW INDUSTRIAL TOMATO CULTIVARS (*LYCOPERSICUM ESCULENTUM*) IN THE GHARB REGION OF MOROCCO

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ABSTRACT

Considering the importance of the cultivars performance in improving productivity, comparative study of ten new industrial tomato cultivars was conducted at the experimental field of Sidi Alla Tazi in the Gharb region of Morocco, for their phenological and production parameters. The objective of this experiment is to study the agronomic performance of 10 Tomato cultivars in order to seek out the most adapted and high yield potential in the Gharb agro-climatic conditions. The cultivars trial was transplanted in April, with dual lines and led by fertigation. The tested plant material consisted of cultivars with fixed growth. The adopted trial is a randomized block with four replications. Obtained results have statistically identified four cultivars; Num 0058 (120 t/ha), Artix (118 t/ha), Mariflor (117 t/ha) and Riotinto (116t/ha), which differ by the best morphological, agronomic and technological criteria as: height, vegetative port, Leaf Area Index (LAI), yield, synchronized maturity, precocity and Brix. These cultivars expressed the highest yields and the best brix have shown the best performance of growth and development. The lowest yield was obtained by Heinz 2710 control (100 t/ha). In viewpoint of precocity, cultivars Num 0058 and NPT 63 are the earliest.

Keywords: *Performance, Cultivars, Brix, Industrial Tomato, Morocco*

INTRODUCTION

Two main types of tomatoes (*Lycopersicum esculentum*) exist, composed by indeterminate growth tomato and the growth fixed which has a bushy port, growth stops after the formation of a limited number of fruit-bearing clusters; it is the "dwarf" or so-called industrial tomato. The elongate form of fruit is characterized by a high dry matter, a brix of around 5.5 and the pH is slightly acid. It is important to notify that the new hybrid cultivars are known by a high pulp rate and without any seeds.

Globally, the major producers of industrial tomatoes according to the International Association Mediterranean Tomato (AMITOM, 2010[1]) are; USA (12.6 million tonnes), China (6.2 million tonnes) and Italy (5 million tonnes). In Morocco, the area reserved to this crop has experienced a constant evolution in the various regions where irrigation is possible (Atherton, 1986 quoted by Chleyah 2001). The production is currently concentrated in the Gharb and Loukkos regions on an area of about 5,000 ha and over 75% of the sowings exists in Gharb region (ORMVAG, 2008).

These crops present only 13% of sown areas, but they contribute to 54% of the total value of crop production (Green Morocco Plan 2008). Tomato sector provides 120 working days per Ha and ensures the continued operation of an emerging processing industry. However, average yields are still low and unstable (29-54 t / ha) and there is an inter-annual variability yields of cultivated areas (Krira and El hasnaoui, 2007).

The major constraints for the development of this sector are technical and concern others aspects related to market. The main part of production is converted into concentrated and derivatives by one of the important aggregation companies; Les Conserves de Meknes (LCM-Aicha) that convert more than 110,000 tonnes per year (Essafi and Krira, 2015).

In this context, this study aims to contribute to the improvement of productivity by comparing ten new cultivars in order to propose the best ones to the farmers. This study was conducted in collaboration between INRA and Les Conserves de Meknes (LCM)-Aicha.

MATERIAL AND METHODS

Plant Material

The plant material used is composed of ten cultivars with determinate growth. All these cultivars namely; Num 0058, NPT 63, Num 0001, Num0051, Mariflor, Riotinto, Artix, NPT 65 and NPT 64, are produced by Syngenta and Nunhems companies. Seeds of cultivars object of this experiment are sown in a peat substrate in trays cells. Morphological and agronomic characteristics are not known except Heinz 2710 variety used as control. Transplanting plants in soil blocks was completed in April.

Soil Characteristics

The experiment was installed on a heavy soil in the experimental area of Sidi Allal Tazi (INRA). Results of physicochemical analysis show that the superficial horizons consist of alluvium depositing are rich of silt. The soil texture is clayey loam moderately provided organic matter (2.98%) and very rich in potassium (546.45 ppm). pH (7.69) is slightly alkaline, favorable to the installation of tomato. Trial was conducted in fertigation for a target yield of 100 t/ha. Fertilizers units used are; 130U NO2, 97U P2O5, 264U K2O and 12U Mg associated with foliar fertilizers and biostimulants taking into account the soil fertility level.

Experimental setup

The experimental model is a randomized complete block with four replications. The basic plot with an area of 36 m^2 consists of three twinned lines spaced of 2m. The plant stand is made of about 25,396 plants per hectare. Each elementary plot or each cultivars stage magnification fruit, three plants are selected at random on the middle line of each plot. In total, we analyzed 120 plants for vegetative characters as following:

a) Height at the maximum growth (cm);
b) LAI or Leaf Area Index (LAI): The measurements were performed using a LAI meter Licoorn 2000;
c) Vegetative port matches the covering of the variety (cm);
d) Precocity is evaluated by a rating scale of 1 to 6 for five successive visits at intervals of 3 to 7 days from the stage pre-flowering. A variety is considered flowering when 50% of plants are in flowering step. 1: very early, 2 early, 3: medium early, 4: fairly late, 5 late, 6: very late.

e) Yield and its components, the early bloom stage to grow fruit, we followed the evolution of yield components of the plants selected in each unit plot by counting the branches, the number of clusters,
number of flowers and number of fruits per plant.

At harvest, the productions were weighed and characters qualitative have been noted.

f)Caliber, a sample of 12 fruits is picked at random from a sample of the harvest of each individual parcel (variety). At the laboratory, the sample is weighed, fruit shape is determined by the length of the superficial circumference of the plant. The number of lodges was also noted after dissection of the fruit.

g)Technological quality, the sample of fruit was crushed and analyzed in the laboratory for Brix, pH and NaCl content.

Statistical analysis

For each measured character as agronomic, technological and morphological, we have proceeded to the analysis of variance (ANOVA) by a single classification criterion. When a significant difference was found between cultivars for one character, the ANOVA is completed by Dunnett test for the comparison of means and for identifying cultivars that differ significantly from the control. For each character, the variation coefficient is used to assess levels of variation of averages observed between cultivars.

RESULTS AND DISCUSSION

Phenological parameters

The variance analysis of measured quantitative and qualitative characteristics, grouped in Table 1 revealed a high significant HS difference for the height character and the LAI character respectively (P≤0.0044**) and (P≤0.0043**) and very HS for the vegetative shape character (P≤0.0005***). While the other characters are not significant, indicating existence of morphological diversity among the cultivars tested but with values of variation coefficients indicating a low variation for all characters respectively; 6.03%, 8.73% and 6.7%. We mention therefore that the morphological characters are less variables.

Comparison of heights average of different cultivars, compared with the control, showed that cultivars such as Artix (76.66 cm; a), Num 0058 (72.91 cm; ba) and Riotinto (72.91cm; ba) give a remarkable growth in height greater than that of Heinz 2710 control (69.58 cm; bc), against an average height of all cultivars (68.91 cm) with a variation coefficient of 8.78% (Table 1). Indeed, this result justifies their adaptation to agro-ecological conditions of the region. The LSD test (6.03) and a threshold P= 0.05 allowed to classify cultivars in 3 homogeneous groups. The first consists of Artix cultivar with an average height of 76.66 cm followed by the second group of cultivars Num0058 and Riotinto having an average of 72.91 cm and a last group of other cultivars including Heinz 2710 control, not exceeding 70 cm (Figure 1).

Table 1. ANOVA of observed parameters

Variation ssources		Height (cm)	Leaf Area Index (LAI)	Vegetative shape (cm)	Yield (T/ha)	Fruit weight (g)
	Num 0058	72,91 ± 2,9ba	5.24±0.68ed	2.24± 0.31a	119.98 ±21.73a	85.08±9.14
	NPT 63	67.08±6.43bc	5.69±1.20becd	1.92±023 cb	114.44±34.59ba	91.47±5.25
	Num 0001	65.41±4.16c	5.17±1.12e	1.96 ±0.13b	104.75±27.89ba	107.01±9.70
	Num0051	65.41±5.16c	6.42±0.51ba	1.76±0.14 c	101.31 ±39.36b	104.80±12.65
	Mariflor	66.24±4.78c	5.66±0.80ecd	1.83±0.22 cb	117.47±25.98 a	77.38±8.63
	Riotinto	72.91±10.66ba	6.80±0.88a	1.84± 0.26cb	115.56±31.5 ba	88.75±5.96
	Artix	76.66±3.60a	6.01±1.02bc	1.90±0.13 cb	118.35. ±29.75a	94.44±6.59
	NPT 65	67.49± 5.18bc	5.91±1.04becd	1.80± 0.25cb	111.69±33.71b	103.66±10.43
	NPT 64	65.41± 2.09c	6.00 ±0.87bc	1.76± 0.21c	101.93 ±31.08b	89.42±7.47
	Heinz (Control)	69.58±4.16bc	5.98±0.75bcd	1.90±0.24cb	100.76±32.30b	99.90±9.46
	Fobs	3.64	3.65	5.00	2.08	1.19
	P	<0.0044(HS)	0.0043(HS)	0.0005(THS)	0.0686(NS)	0.3140(NS)
	CV%	6.03%	8.73%	6.70%	9.60%	29.51%

(For each character, values with the same letter are statistically equal according to Dunnett's test)

Comparing the averages for the leaf area index (LAI) was leading to estimate the biomass directly dependent on the photosynthesis that takes place in the plant stand. The ability of the latter to intercept the incident radiation depends on this index. The density of leaf system has consequences on the health status of the culture and the reduction in evapotranspiration. This comparison shows that cultivars: Riotinto (6.80; a), Num0051 (6.42; ba), Artix (6.01; bc) and NPT 64 (6.00; bc) have shown greater leaf area index in comparison to Heinz 2710 control (5.98; bcd) and therefore these cultivars have a denser biomass (Table 4). It appears that the planting in twin rows, density doubles and leaf area increases. This results in an increase of the incident radiation, which also depends on this density. LSD test (0.74) at threshold □ = 0.05 has shown the existence of more homogeneous groups. The first consists of the Riotinto cultivar that comes out on top with an average of 6.80 followed by the Num 0051 (6.42), Artix, NPT64 and other cultivars that overlap each other between 5.17 and 6 (Figure 2).

Comparing the averages for the vegetative shape character which refers to the foliage, shows that cultivars Num 0058 (2.24; a), Num 0001 (1.96; b) and NPT63 (1.92; cb) have a vegetative volume significantly higher than other cultivars including Heinz 2710 control (1.90; cb). These cultivars tend to better display their vegetative port unlike other cultivars overlapping between vegetative ports semi-spread and erect. The illustration of vegetative volumes depending on the variety is shown in Figure 3.

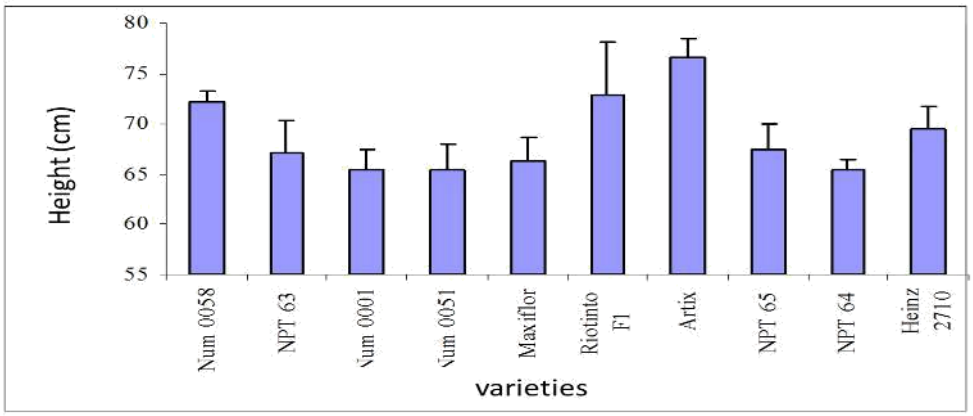

Figure1. Importance of plants height according to cultivars (cm)

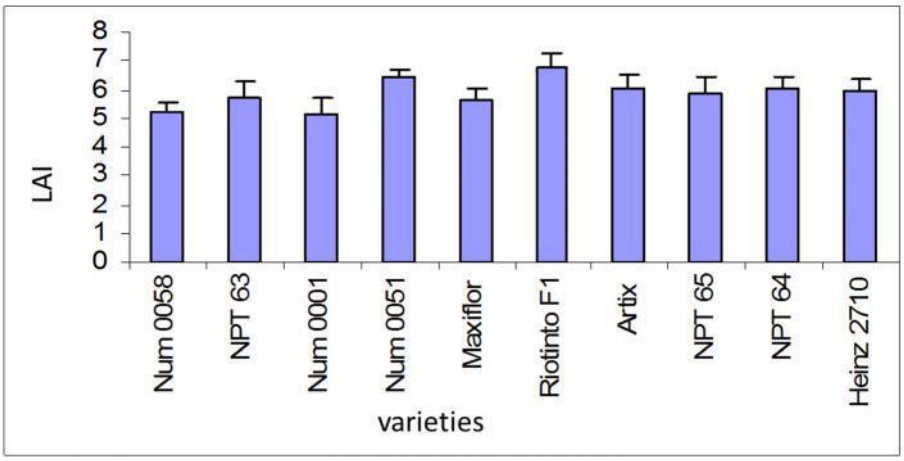

Figure 2. Importance of Leaf Area Index (LAI) according to cultivars

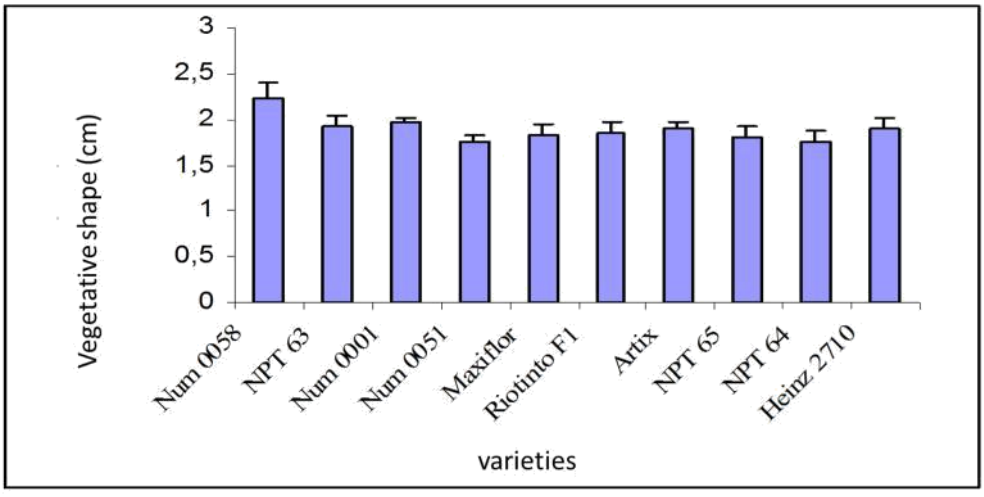

Figure 3. Variability of vegetative shape according to cultivars

LSD test (0.18) with threshold □ = 0.05 allowed to classify the cultivars in 4 homogeneous groups. The first one consists of the variety Num 0058 that ranks first with an average of 2.24 cm followed by the second group of the variety Num 0001 (1.96 cm). These cultivars tend to better display their vegetative port. An intermediate group of cultivars: NPT63, Heinz 2710, Artix, Riotinto, Mariflor and NPT 65 whose averages overlap between them and with values between

1.80 and 1.92 cm. The last group is composed by cultivars of NPT 64, Num 0051 and with those of vegetative volume was 1.76 cm only. These two cultivars have an erect vegetative port type.

Yield Components

Data on yield components (number of flower clusters / plant, number of flowers / plant, number of fruits / plant and yield) were submitted to principal component analysis (PCA) for discriminating cultivars and look for correlations that might exist between the various components and performance (Tables 2 and 3).

Table 2. Computing data yield components

Cultivars	N° of variety	Nb. of flower cluster	Nb. of flowers	Nb. of fruits	Yield t/ha
Num 0058	V1	43	295	140	120
NPT 63	V2	31	109	50	114
Num 0001	V3	34	103	85	105
Num0051	V4	24	103	41	101
Mariflor	V5	36	190	71	117
Riotinto	V6	47	220	117	116
Artix	V7	42	167	65	118
NPT 65	V8	55	149	135	112
NPT 64	V9	22	124	44	102
Heinz 2710	V10	31	140	68	100
Average		36	159.50	76.60	111
Type-Ecart		10.32	61.18	36.76	7.72

According to the above analyze, we classify the cultivars on three homogeneous groups, the first one consists of the folowing cultivars as Num 0058, Mariflor, Riotinto and Artix. The best positive correlation is mentioned between Yield and the Flowers number (r^2=0.75). This constitute good illustration for the best yield (Table 3).

Table 3. Correlations R^2

	y1 (N.Branch)	y2 (N.Clust)	y3 (N.flowr)	y4 (N.fruit)	y5 (Yield)
x1 (N.Branch)	**1.000**	0.643	0.724	0,896	0,484
x2 (N.Clust)		**1.000**	0.502	0.847	0.649
x3 (N.flowr)			**1.000**	0.709	**0.747**
x4 (N.fruit)				**1.000**	0,622
x5 (Yield)					**1.000**

Number of flower cluster

The number of flower clusters per plant varies widely between cultivars, it varies from 22 to 55 (Figure 4). The best performances were recorded by the following cultivars: NPT 65, Riotinto, Num 0058 and Artix. The principal component analysis for all components measured yields reveals a positive correlation between the number of flower clusters and other yield components like number of flowers, fruit number and yield. This relationship is confirmed between number of flower clusters and number of flowers, also between fruit number and yield that establishes a significant correlation respectively as following; r (x2, y3) > 0.50, r (x2, y4)> 0.84, r (x2, y5)> 0.64. Therefore, this component explains strongly the performance. Num 0058, Riotinto, Mariflor and Artix cultivars have confirmed an abundant flowering compared to the rest of genotypes.

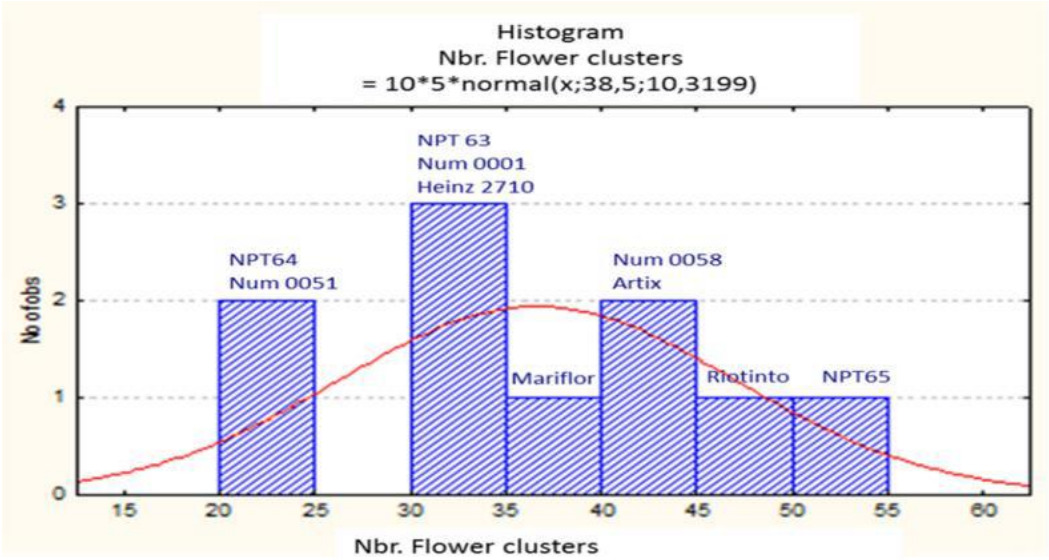

Figure 4. Distribution of homogeneous groups according to flowers

Clusters 2.2 Number of flowers per plant

The number of flowers per plant varies between cultivars; it changes from 103 to 295. The best performances were recorded by the cultivars: Num 0058, Riotinto, Mariflor, Artix and that showed superiority over the rest of the cultivars having abundant flowering compared to the rest of cultivars. The correlation between the number of flowers and the yield is significant (r> 0.74). The significance test of the correlation coefficient explained that the number of flowers determines greatly the yield parameter, which is obvious. Figure 5 shows the importance of cultivars depending on the number of flowers.

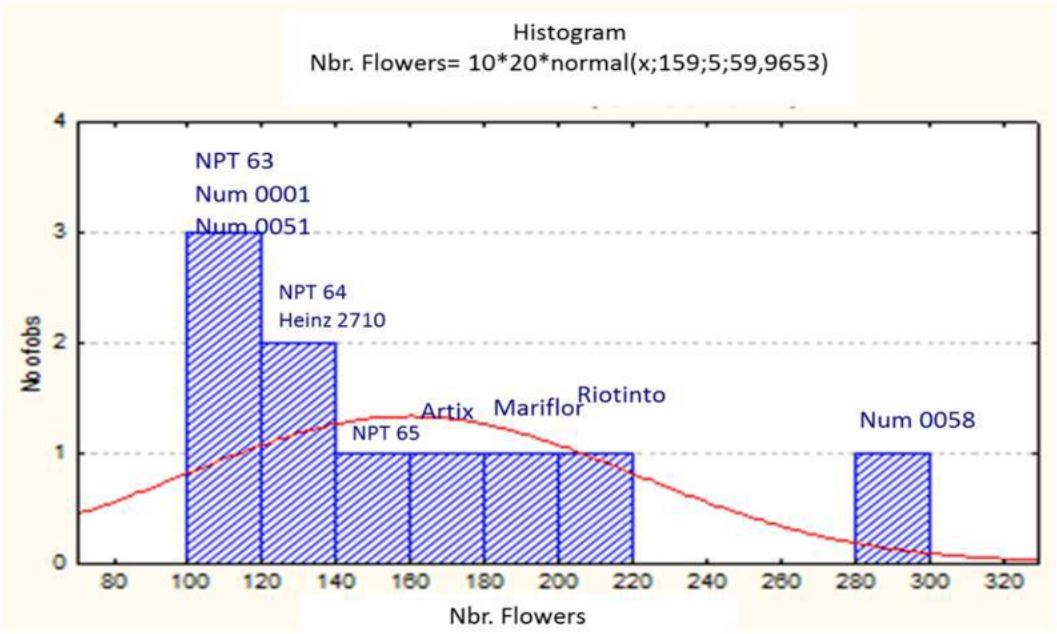

Figure 5. Cultivars rating according to the flowers number

Number of fruits per plant

The number of fruits per plant varies from 44 to 140 according to the variety. Cultivars NPT 65, Num 0058, Riotinto and Artix are shown remarkable regarding to the number of flower cluster per plant which ranged from 22 to 55 depending on the variety. At harvest the counts of fruit number per plant shows that the number of fruits produced represents only half of the formed flowers number. The correlation between the number of fruits and yield was significant (r> 0.62) and therefore this component explains very well the cultivars performance. While the percentage of aborted flowers exceeds fairly 50%. Figure 6 shows the arrangement of cultivars based on the number of fruits produced. According to the control variety, the Num 0058, NPT65, Riotinto, Mariflor, Num0001 and Artix have given the best Number of fruit. The highest fruits number was obtained by the Num0058, NPT65 and Rotinto cultivars.

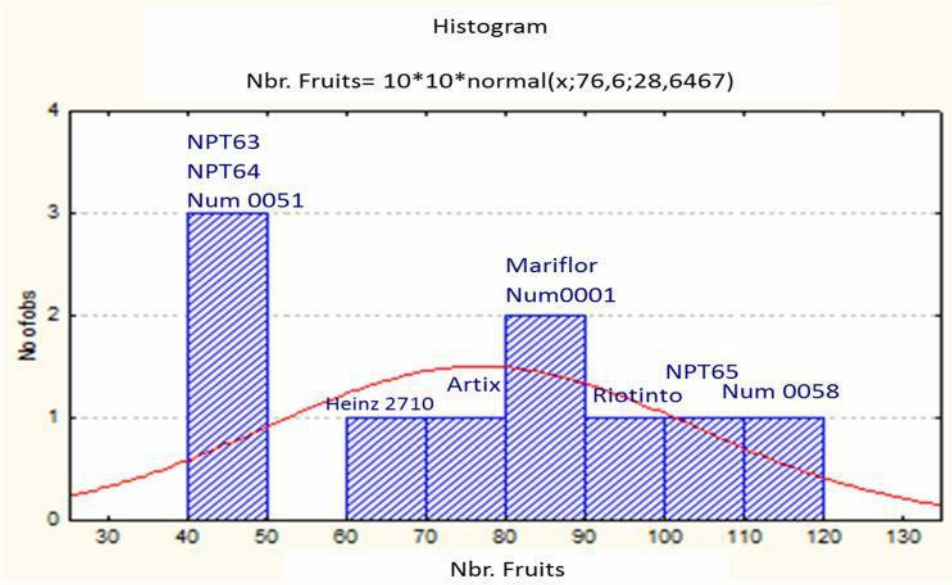

Figure 6. Cultivars rating according to fruits number

Yields

The threshold = 0.05, ANOVA revealed no significant difference. LSD test has three potentially interesting cultivars that are specially Num 0058, Artix and Mariflor. The highest yield was obtained by the cultivars like Num 0058 (120 t / ha), Artix (118 t / ha), Mariflor (117 t / ha) and Riotinto (116 t/ha), but the lowest was obtained by Heinz 2710 control (100 t / ha). The average yield of all other cultivars is of about 110.62 t / ha with a variation coefficient of 9.6%. (Figure 7).

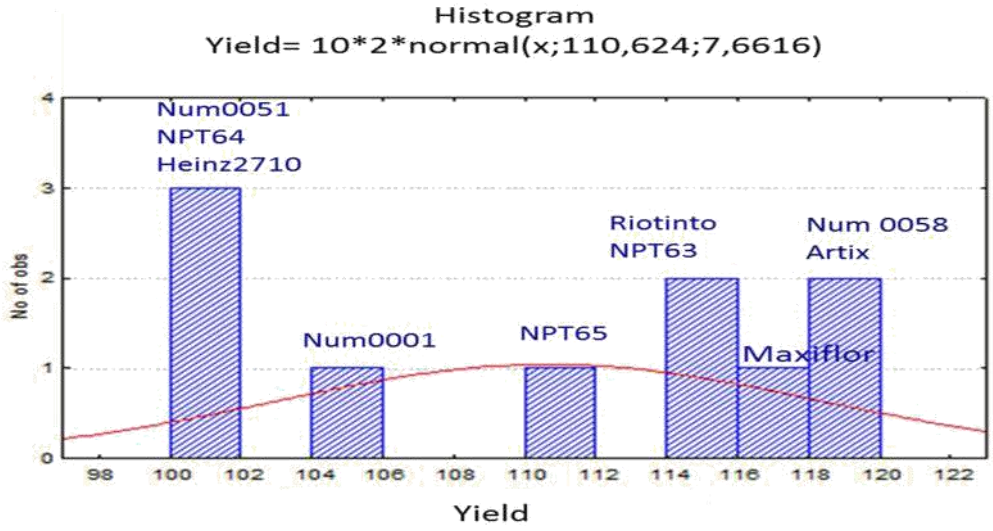

Figure 7. Rating of homogeneous groups according to yields

Note that the recorded performance levels (Table 2) range from 100 to 120 tonnes per hectare. These yields are very satisfactory compared to other similar experiments carried out in the region with other cultivars. The Heinz 2710 control gives only 90 tonnes / ha. Moreover, cultivars Petoseed, Sun 6200, and Heinz 9661 have given yields comprise between 70 to 80 t/ha, while Boss, Sun 6235 and Heinz 8704 have given yields from 55 to 70 t / ha (El Atir et al, 2001). We thought that it is useful to make a classification for these cultivars in 3 homogeneous groups which are illustrated in the histograms in Figure 7. The first consists of cultivars Num 0058, Artix, and Mariflor who gives the best yields, respectively 120, 118 and 117 t/ha. Then come the cultivars Riotinto F1, NPT 63, NPT 65 and Num 0001 with yields of 116, 114, 112 and 105 t / ha and other similar variety as Heinz 2710 control who gives yield of about 100 t/ha.

Precocity, inter varietal maturity, size and technological quality

Monitoring rating flowering has determined the flowering period of the different cultivars. This period has a duration of 17 days. The evolution of flowering percentage allowed to a classification according to a gradient in the number of flowers, which gives an idea of the inter-varietal precocity (Table 4). Thus, cultivars like NPT 63, Num 0058, Num 0001 and Mariflor are in advanced phase of nearly 15 days compared to other cultivars. This aspect match partially with some cultivars that are found with almost (20%) for their crops with after 15 days from the first harvest

Two harvests were conducted to observe if the cultivars expressing a grouped maturity. The first harvest was performed by 98 days after sow and the second after 15 days. Thus, compared to Heinz2710 control, the others cultivars like Mariflor, Num 0058 and Num 0001 tend to have a grouped maturity and expressed more than 90% of their production in the first harvest (Table 5).

able 4. Rating of flowering percentage according to studied cultivars

Obser./dates/cultivars	jours	Num 0058	NPT 63	Num 0001	Num0051	Mariflor	Riotinto	Artix	NPT 65	NPT 64	Heinz 2710
pré-flowering (%) (3/06/05)	0	3	4	1	-	2	1	1	1	-	1
Start- flowering (%) (6/06/05)	3	30	30	20	5	10	5	3	2	2	1
Flowering (%) (10/06/05)	7	40	45	30	10	25	10	10	8	8	10
Extended flowering % (13/06/05)	10	45	50	35	10	40	15	12	12	12	15
Flow-blooming % (20/06/05)	17	70	70	65	45	60	50	40	40	40	45
Total	17	188	199	151	70	132	81	66	63	62	72
Scale rating		**2**	**1**	**3**	**6**	**4**	**5**	**6**	**6**	**6**	**6**

Table 5. Proportion of harvest in the parcelar global production

Varities	1st. harvest (04/08/05) (%)	2nd. harvest (20/08/05) (%)
Num 0058	87,8	12,1
NPT 63	86,8	13,1
Num 0001	87,2	12,7
Num0051	80,9	19,0
Mariflor	90,1	9,8
Riotinto F1	82,9	17,0
Artix	81,2	18,7
NPT 65	86,9	13,0
NPT 64	86,4	13,5
Heinz 2710	88,4	11,5

We have conducted two crops to observe if the cultivars express particular precocity. The percentage of the first harvest in the overall production ranged from 80.9 to 90.1% depending on the variety. Genotypes like Mariflor, Heinz, Num 0058 and Num 0001 tend to have a grouped maturity compared to other cultivars and expressed almost 90% of their production to the first

harvest (Table 5). The group of intermediate cultivars such as NPT 63, NPT 64 and NPT 65 showed a maturity relatively less grouped. However, Num 0051, Riotinto and Artix cultivars presented 20% of their products 15 days after the first harvest. These cultivars have a relatively grouped maturity than other cultivars. The crop was transplanted by April 28 and the first harvest was established on August 4, this is equal to a cycle of 98 days.

The analysis of variance of fruit weight showed that no significant difference (P> 5%) between cultivars. The assessment of the form by the combination of the length and diameter of each fruit variety showed the existence of the rounded shape for the variety Num 0001, the long rounded, and flattened for Num 0051cultivar and Heinz 2710variety. The rest of the cultivars have a long flat shape.

Moreover, the number of lodges has been 3 for genotypes such as Num 0058, Num 0001, Mariflor, Riotinto F1, NPT 63 and Heinz 2710, when he was only 2 for cultivars like Num 0051, Artix, NPT 65 and NPT 64. However, the average of fruit weight is of around 77 to 107 g/fruit, with no significant difference between all genotypes.

Brix is the most important criterion of the quality in tomato industry. It denotes the degree of dry matter, which determines the performance for processing. All cultivars have shown a brix above 5 except one genotype as NPT 65 (4.8). Cultivars like Num 0051, Mariflor and Artix expressed the best brix between 5.2 and 5.4. Other cultivars have a satisfactory brix.

The NaCl content is relatively the same for all cultivars. It is of the order of 0.06 to 0.08%. Also, the pH of the juice from all the other cultivars is relatively the same (Table 6)

Table 6. Results of technological analyzes

Cultivars	pH	°degree of Brix	NaCl content
Num 0058 (V1)	4,18	5	0,08
NPT 63	4,02	5	0,08
Num 0001	4,09	5	0,08
Num0051 (V4)	4,06	5,4	0,08
Mariflor	4,24	5,2	0,09
Riotinto F1	4,22	5	0,08
Artix	4 ,22	5,2	0,07
NPT 65	4,20	4,8	0,08
NPT 64	4,20	5	0,06
Heinz 2710 (control)	4,08	5	0,08

Agronomic and morphological characteristics of cultivars

According to the results of the parameters mentioned in Table 7, it is clear the existence of 3 cultivars that seem most appropriate and present the best performance. These cultivars are Num 0058, Artix and Mariflor. These cultivars have expressed the best yields and the best brix. They also expressed the best growth and development performance (best height, good vegetative port and the number of the highest lodges except the variety Artix (2 boxes). Concerning precocity, cultivars Num 0058 and NPT 63 are the best ones, while the cultivars Num 0001, Riotinto F1 and Artix are later. In viewpoint of disease resistance,the Num 0058 and NPT 63 cultivars were free of diseases and

physiological mosaic unlike cultivars Mariflor and Artix which presented some mosaic branches. We also observed that the Num 0058 variety presented a less dense foliage than other cultivars.

Table 7. Agricultural Features of the studied cultivars

Cultivars	Height (cm)	Veget. Shape (m)	LAI	Precocity	**Yield** (t/ha)	Nbr. lodges	**Brix**	Diseases physio.	Mosaique	Foliar density
Num 0058	72.9	2.2	5.2	2	**120**	3	**5**	-	-	clair
NPT 63	67.0	1.9	5.6	1	114	3	5	-	-	very dense
Num 0001	65.4	1.9	5.1	3	105	3	5	+	-	dense
Num0051	65.4	1.7	6.4	5	101	2	5.4	+	2	very dense
Mariflor	66.2	1.8	5.6	4	**117**	3	**5.2**	+	1	dense
Riotinto F1	72.9	1.8	6.8	5	116	3	5	+	-	dense
Artix	76.6	1.9	6.0	6	**118**	2	**5.2**	+	-	dense
NPT 65	67.4	1.8	5.9	4	112	2	4.8	-	-	dense
NPT 64	65.4	1.7	6.0	6	102	2	5	+	2	very dense
Heinz 2710	69.5	1.9	5.9	5	101	3	5	-	-	very dense

CONCLUSIONS AND RECOMMENDATIONS

Gharb region is currently the local production of tomato industry in Morocco. The behavior study of new industrial tomato cultivars in the conditions of Gharb has giving interesting results. Obtained results have identified four cultivars statistically distinguished by the best morphological and agronomic technology criteria such as height, vegetative shape, LAI, yield, early maturity and Brix. These cultivars are specially Num 0058 (120 t/ha), Artix (118 t/ha), Mariflor (117 t/ha) and Riotinto (116 t/ha).These cultivars expressed the highest yields and the best brix. They also expressed the best growth, the good development performance and the number of lodges. Note that these yields are above the regional average does not exceed 60 t/ha.

In viewpoint of precocity, cultivars like Num 0058 and NPT 63 are the earliest, while Num 0001, Riotinto and Artix cultivars are later. Concerning disease resistance cultivars Num 0058 and NPT 63 were free of diseases and physiological mosaic unlike Mariflor cultivar has shown twigs with mosaic in few plants. We also observed that the Num 0058 variety with a less dense foliage than other cultivars has given the high level of yield. Adoption of these cultivars identified remains a promising project that could have a positive effect on increasing productivity in the irrigated perimeter of Gharb. For exchanging information about the methods and techniques of production, these cultivars have been appreciated by Moroccan and foreign professionals. Although, industrial tomato is an opportunity for diversification of incomes for small farmers in the region. Improving productivity remains dependent on an integrated production program including the selection of improved and adapted cultivars and the mastery of a reasoned technical and less expensive. It is recommended to better sowing early culture when the agro-ecological conditions do not present significant risks.

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PRACTICAL USE OF *PUCCINIA TRITICINA* ERIKS. ISOLATES FROM THE STATE COLLECTION OF PHYTOPATHOGENIC MICROORGANISMS OF THE ALL-RUSSIAN RESEARCH INSTITUTE OF PHYTOPATHOLOGY (ARRIP)

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ABSTRACT

The collection of isolates of leaf rust agent *P. triticina* exists in ARRIP more 50 years. Now all isolates of leaf rust agent are included in the State Collection of Phytopathogenic Microorganisms created in ARRIP in 1996. Research associates of institute study the genetic structure of leaf rust populations, select monopustule isolates, determine the virulence genes and transfer material to the State Collection. The main requirements for transfer of isolates to the Collection are viability, the known origin, the studied virulence and genetic stability. Currently the Collection contains more 1500 isolates of *P. triticina* from all wheat-growing areas in Russia. The genetic potential of the leaf rust collection is very various; there are the isolates with different spectrum of virulence genes including ones with unique infrequent virulence genes. In the State collection there is widely used the method of low-temperature storage of live biological objects, enabling the restoration of their biological functions after defrosting. Every year the Collection is replenished by the new leaf rust isolates. The Catalog of strains and Methodical recommendations for storage are placed on the institute website. According to requests of the external organizations and ARRIP laboratories the Collection takes from storage, multiplies and transfers to consumers the required isolates. Collection isolates are used to study the strategies for the breeding of rust resistant wheat cultivars, specificity of pathogen populations and the racial composition in different areas of wheat cultivation, to determine the effective resistance genes of wheat to regional populations of *P. triticina* and for creation of simulated infectious background in experimental rust nurseries.

Keywords: *State Collection, Puccinia triticina, Isolates, Virulence, Wheat, Resistant cultivars*

INTRODUCTION

Leaf rust is one of the most widespread and harmful wheat diseases in all regions of wheat cultivation in the world, including Russia. Leaf rust epidemics cause yield losses up to 50% (Sanin, 2016). During a long period, one of the reasons of brown rust epiphytoties on wheat in Russia was uncontrolled growing of varieties with the same efficient genes of race-specific resistance. Creation and cultivation of resistant to *P. triticina* wheat cultivars is environmentally favorable and safe way of fight against the disease (Smirnova et al., 1991).

One of the main factors of emergence of new leaf rust virulent races and the increase their frequencies is natural selection occurring in fungus populations under the influence of varieties with race-specific resistance (Kolmer, 1996; Singh et al., 2005). Annual variations of frequency of occurrence of brown rust races depend on not only composition of the cultivated wheat varieties, but also the weather conditions (Long et al., 1998). Mutations and recombination

variability of genetic material and spores migration have a great importance for the formation of the genetic structure of fungus populations (Park et al., 2001).

Monitoring virulence of *P. triticina* populations is the basis for the study of genes dynamics on wheat crops and requires the creation of a collection, allowing to preserve the genetic material of different years, to trace the dynamics of virulence genes in regions of Russia, to determine the role of cultivated wheat varieties in the emergence and spread of pathogen races (Kovalenko et al., 2012).

Need of continuous use of different fungus isolates for immunologic evaluations of new wheat cultivars formed a basis for creation of a collection of *P. triticina* isolates. The collection of isolates of leaf rust agent *P. triticina* exists in ARRIP more 50 years. Now all isolates of leaf rust agent are included in the State Collection of Phytopathogenic Microorganisms created in ARRIP in 1996. Research associates of institute study the genetic structure of leaf rust populations, select monopustule isolates, determine the virulence genes and transfer material to the State Collection. The main requirements for transfer of isolates to the Collection are viability, the known origin, the studied virulence and genetic stability (Kovalenko et al., 2002; Makarov et al., 2009).

MATERIALS AND METHODS

Currently the collection contains more than 1500 isolates of *Puccinia triticina* Eriks. from all areas of wheat cultivation in Russia, but also from Belarus, Ukraine, Moldova, Armenia, Georgia, Kyrgyzstan, Kazakhstan, Uzbekistan, Tajikistan. The genetic potential of the collection is very various; there are the isolates with unique virulence genes. A large number of fungus isolates with a wide range of virulence allows keeping the diversity of natural leaf rust populations in the collection.

In the ARRIP collection the widespread method of the low-temperature storage of live biological objects giving the chance of restitution of their biological functions after defrosting is used (Zhemchuzhina et al., 2015). Since 2006, all collection isolates of brown rust are placed on storage in cryogenic test tubes in the REVCO freezers at a temperature -80°C. Biomaterial is stored as previously propagated on the susceptible wheat cultivar urediniospores of each isolate in the amount of 4-5 mg.

When material is requested for work or at periodic planned inspections of viability and virulence urediniospores bring out of an anabiosis by heating them in open glass test tubes in the thermostat at 45°C within 5 minutes.

The viability of rust isolates is determined by germination of the spores applied on thin layer of 2% agar on a microscope slide and by disease symptoms on inoculated by the spores' suspension wheat seedlings. Taken for checkup in 2017 isolates stored in freezer since 2006 - 2011 revealed their high viability without the loss of virulence properties.

RESULTS AND DISCUSSION

During conducted in 2005-2017 investigations there was proved the presence of the same 30 genes virulence in leaf rust populations from different regions of the Russian Federation. These were the following genes: *pp 1, 2a, 2b, 2c, 3a, 3bg, 3ka, 9, 10, 11, 14a, 14b, 15, 16, 17, 18, 20, 21, 23, 26, 27 + 31, 30, 32, 33, 36, 39, 40, 46, B.* Annually there were dominated the leaf rust isolates with virulence genes *pp 1, 2b, 2c, 3a, 3bg, 3ka, 10, 14a, 14b, 17, 18, 21, 23, 27 + 31, 30, 32, 33, 39, 40, B.* There were no revealed genes *pp24, 29, 41, 42, 45, 47, 51, 53*.

Need of expansion of territories in which it is necessary to select fungus isolates is explained by the fact that the drift with air streams of new, including potentially dangerous clones, is possible. Studying of virulence of brown rust populations in such areas allows to monitor emergence of potentially dangerous genes for the cultivated wheat varieties and to predict their distribution.

Virulence genes *p19* and *p46* were revealed in some collection isolates collected from 2006 to 2010. These genes were identified in isolates collected from the Middle Volga in 2006, the Central-Chernozem and the North Caucasus regions in 2008, and the Central region in 2009.

In recent years the collection was replenished by leaf rust isolates, virulent to the wheat varieties with the *Lr9* resistance gene. In 2010 isolates *P. triticina* with the complementary virulence gene were allocated from samples of spring wheat varieties Chernyava and Chernyava 13 in the Omsk region. The same gene was revealed in leaf rust isolates in the Central region in 2013, when Moscovskaya 24 cultivar began to be grown up. The frequency of occurrence isolates possessed p9 gene has reached 76% in the Central, and 44% in the North Caucasus regions.

It testifies that the virulent genes of leaf rust capable to overcome resistance of cultivated previously resistant wheat varieties have appeared there. Leaf rust isolates with the specified genes are also stored in the collection.

The phenotypes possessed virulence genes *p24, p38, p41, p42, p45, p47* are especially dangerous. Currently these phenotypes weren't detected in Russia, and leaf rust isolates possessed these genes are absent in the State collection.

The collection is also important for the selection of resistant to leaf rust wheat varieties, above all, for the creation of infectious backgrounds necessary for the testing of wheat varieties.

To create of artificial infectious backgrounds in the nursery for the evaluation of wheat varieties researchers select from the collection *P. triticina* isolates with the virulence genes reflecting a gene pool of local populations where the tests are conducted. For example, in 2015 isolates 782-3 and 784-1 were selected for the tests in the Central region, 776-11 and 780-15 in the North Caucasus, 786-3 and 786-6 in Middle Volga region (table 1).

There for the inoculums were used spore mixtures of collection leaf rust isolates with the maximum number of virulence genes. Artificial populations were created from equal parts of the spores of each isolate, and they included 32 or more virulence genes corresponding to the gene virulence pool of natural *P. triticina* populations.

Collection leaf rust isolates with known virulence genes can be taken for postulation resistance genes of wheat varieties by use of methods of phytopathologic testing and molecular marking (Dyck, 1993; Maleeva et al., 2003).

The method of phytopathologic testing gives the chance to determine the resistance genes of wheat varieties or their combinations without using hybridization. According to the theory "gene for gene" each resistance gene of the plant corresponds to the virulence gene of parasite (Person, 1959). In the case of a combination of the dominant allele of the virulence gene and the dominant allele of the wheat resistance gene resistance is observed. If the corresponding genes are in the homozygous recessive stated the plant is susceptible. The use of collection isolates of leaf rust marked by different virulence genes allows identifying the resistance genes in the studied varieties and samples of wheat.

Table 1. *P. triticina* isolates identified in 2015 on wheat crops of the Central, North Caucasian and Middle Volga regions of the Russian Federation and selected for the tests in 2016

Code	Virulence genes	Race	Origin
782-3	*1, 2a, 2b, 2c, 3a, 3bg, 3ka, 10, 11, 14a, 14b, 15, 17, 18, 19, 21, 25, 26, 27+31, 30, 32, 33, 38, 39, 40, 46, B*	TCTT	Central region, Moscow distr., winter wheat, cv. Lgovskaya
781-4	*1, 2a, 2c, 3a, 3bg, 3ka, 9, 10, 11, 14a, 14b, 15, 17, 18, 20, 25, 27+31, 30, 32, 33, 38, 39, 40, B*	TLTT	Central region, Moscow distr., winter wheat, cv. Moscovskaya 39
776-11	*1, 2a, 2b, 2c, 3a, 3bg, 3ka, 9, 10, 14a, 14b, 15, 16, 17, 18, 19, 20, 21, 23, 25, 26, 27+31, 30, 32, 33, 38, 39, 40, 46, B*	TRPT	North Caucasus, Stavropol distr., winter wheat, cv. Severodonetskaya
780-15	*1, 2b, 2c, 3a, 3ka, 10, 11, 14a, 14b, 18, 25, 26, 27+31, 30, 32, 33, 39, 40, 44, B*	PCPT	North Caucasus, Stavropol distr., winter wheat
786-3	*1, 3a, 3bg, 3ka, 9, 10, 14a, 14b, 15, 17, 18, 19, 20, 21, 25, 27+31, 30, 32, 33, 39, 40, 44, B*	MLPT	Middle Volga region, Penza distr., winter wheat
786-6	*1, 2b, 2c, 3a, 3bg, 3ka, 10, 11, 14a, 14b, 16, 17, 18, 20, 21, 23, 25, 26, 27+31, 30, 32, 33, 39, 40, 46, B*	PHTT	Middle Volga region, Penza distr., winter wheat
776-4	*1, 2a, 2b, 2c, 3a, 3bg, 3ka, 9, 10, 14a, 15, 17, 18, 19, 20, 25, 26, 27+31, 32, 33, 39, 40, 46, B*	TMPT	North Caucasus, Stavropol distr., winter wheat, cv.Severodonetskaya
781-1	*1, 2b, 2c, 3a, 3bg, 3ka, 9, 10, 11, 14a, 14b, 17, 18, 21, 25, 26, 27+31, 28, 30, 32, 33, 38, 39, 40, B*	PLTT	Central region, Moscow distr., winter wheat, cv. Moscovskaya 39

Table 2 shows the results of postulating resistance genes *Lr9, Lr26, Lr46, Lr20, Lr23* in domestically produced wheat varieties with the help of 10 isolates-testers of *P. triticina*.

Table 2. Reactions of plants to infection by the collection isolates-testers of *P. triticina* marked by different virulence genes, and the presumed *Lr*-genes in spring wheat varieties

Varieties and monogenic lines of wheat	**Isolates-testers of *P. triticina***										**Presumed *Lr*-genes**
	593-8	**689-4**	**621-5**	**628-8**	**721-11**	**720-5**	**676-1**	**730-13**	**718-1**	**721-9**	
Nemchinovskaya 17	-	-	-	-	+	-	-	+	-	-	*Lr9*
Nemchinovskaya 24	-	-	-	-	+	-	-	+	-	-	*Lr9*
Tertsiya	-	-	-	-	+	-	-	+	-	-	*Lr9*
Lr9	-	-	-	-	+	-	-	+	-	-	*Lr9*
Donshina	+	+	+	+	+	+	+	+	-	+	*Lr26*
Snezhana	+	+	+	+	+	+	+	+	+	+	*Lr26*
Lr26	+	+	+	+	-	+	+	-	-	-	*Lr26*
Gene	-	-	+	-	-	+	+	-	-	+	*Lr46+++*
Lr46	-	+	+	-	-	+	+	-	+	+	*Lr46*
Doka	+	+	-	-	-	-	-	-	-	-	*Lr20+Lr23+*
Lr20	+	+	-	+	-	-	+	+	-	-	*Lr20*
Lr23	+	+	+	-	+	+	+	-	+	+	*Lr23*

- \+ susceptibility to isolate-tester,
- \- resistance to isolate-tester.

For the period from 2006 to 2017 more than 800 varieties of *Triticum aestivum sp. aestivum* and *Triticum turgidum sp. durum* of Russian and foreign breeding were studied by method of phytopathologic testing, and for these researches there were used leaf rust isolates from State collection with different spectrum of virulence genes. The single *Lr*-genes and their various combinations were identified in these wheat varieties. According to the frequency of occurrence of resistance genes in wheat varieties of different origin it is possible to estimate similarities and differences of genotypes. For example, genes *Lr10, Lr26, Lr23, Lr16, Lr11* were the most common in the cultivars of Russian breeding. In the samples from the US collection there prevailed genes *Lr14b, Lr16, Lr21, Lr23, Lr36*. Genes *Lr10, Lr14b, Lr16, Lr21* were the most common ones in Mexican samples.

CONCLUSION

Thus the leaf rust isolates stored in the State collection are required to use by a wide range of specialists. Collection isolates are used to study the strategies for the breeding of rust resistant wheat, specificity of pathogen populations, the racial composition of leaf rust in

different areas of wheat cultivation, and also to determine the effective resistance genes of wheat against regional populations of *P. triticina*.

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PRECISION LIVESTOCK FARMING APPROACH TO MEASURE THE LIVE WEIGHT OF ANIMALS

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ABSTRACT

The accurate estimation of the animal body weight is important to provide some information about the slaughter weight, growth, feeding level, uniformity, treatment doses and feed conversion efficiency. Between the many methods used for live-weight determination, a weighing scale is considered as a reference method (gold standard). In addition to that, farmers sometimes rely on visual observations to determine the animal live weight. However, it is a subjective method due to accuracy depends on farmers' experience. Traditionally, the animal live weight is predicted by manual weighing scale with a random sample of animals. Conventional method of animal weighing usually includes the basic procedure of penning a group of animals or catching and weighing these animals individually. This traditional method is time consuming, labour intensive, open for human errors and stressful for animals and farmers. Therefore, there is a huge need a novel technique. These days, the potential of computer and digital imaging system offer a novel way to predict the animal live-weight by detecting animal body dimensions with a non-intrusive way. Although the precision of live weight prediction depends on different factors, the development of automated monitoring systems for weighing animal is feasible. Due to the important correlation between body size and weight of animals, image monitoring and processing systems with Precision Livestock Farming (PLF) approach can detect the main sizes and the shape of animals. Furthermore, by combining these relations, animal live weight can be predicted accurately. There is a lot of advantage of this technique like non-contact measurements, fast and labour saving. Further studies should focus on the control of image quality, position of the camera and 3D cameras.

Keywords: *PLF, Automatic weighing, Image analysis,*

INTRODUCTION

Weight is one of the most important point in animal rearing. Animal's daily gain can be assessed everyday through getting animal's weight (Doeschl-Wilson et al., 2004). Feed efficiency can also be defined by automatic feeders (Huang et al., 2012).

Then animals in average weight can be reared separately to fulfil the industry standard. In traditional method, animals have to be removed to weighing scales. This method needs too many time and labour. It needs at least three or more farmer to work 4-6 minutes for every animal (Brandl and Jorgensen, 1996). This method causes too many problems to animals like sudden death. Feed uptake of animals is less at the weighing day than to other days (Augspurger and Ellis, 2002). Some producers used automatic weighing sensors in the feeders to

autpmatically measure the weigh of animals during their life span. These sensors are expensive and easy to be destroyed by animals. Because of too many problems of this method, the non-invasive and non-intrusive measurement methods attracted attentions to measure animal weight. Image processing were proposed to have many practice in livestock farming (Deshazer et al., 1988). Using image processing technology, some sizes of animals can be calculated. Image processing technique presents many advantages, like non-contact, fast and labor saving. In this manuscript, the practice of image processing technique in predicting the live animal weight has been reviewed.

Image Processing System

A complete image processing technology primarily includes the following features: light source, digital camera, different kind of lenses, computer, image analysis platform, image processing algorithm. The check system is not necessary in animal weight prediction system, and the only output is the weight of animal information. Therefore this manuscript summary as the following points: location of digital camera, lighting and image analysis method.

Location of Digital Cameras

Digital cameras were set at the top and the side of animals to define the accurate sizes of animals. The top view camera is used to define the animal back area and the side view camera is used to measure animal's body height (Yan et al., 2006). For the protection of digital cameras, most of the researchers prefer to use top view camera instead of a side view. The vision obtained from the top camera provide the information about the body width and length of animals. Top view camera mostly fixed exactly the top of the feeder or drinker to be easily record animals without moving. High quality images can be obtained when a digital cam was fixed exactly on the top of feeding station (Schofield et al., 1999). To record high quality images, digital cameras can also be fixed top of the drinker (Minagawa and Murakami, 2001). Because, animal's body are not moving when it is drinking.

Light Sources

Light source is one of the most important parts of image processing system. A good light condition increases the contrast among animal and the surface of the barn. It is also simplify image calculations, by increasing the speed and robustness of system. The light sources plays a very important role o define body size of animals. Different colour lights were tested to find the most appropriate one for the image processing (Minagawa et al., 2003). The red light was found as more useful when compared to other lights (green and blue). Another study was performed to define the most useful light for animal weight estimation system (Fu et al., 2009). Different kind of light sources like leds, halogens and fluorescent lamps, were compared. Based on the results of their research, the fluorescent lamp was found as the best lamp for uniform light.

Image Analysis

It was known that some animal's size have high correlation by their body weight. Animal's weight was measured by animal volume multiplying the animal's density. However, animal's sizes is not regular and difficult to measure the weight of animals. For example, in the study of Fu et al. (2006) the pig's body length were used to build a model with pig weight. The correlation of the number of pixel of animal back surface with body weight was investigated. The average weight error was below than 5%. In another study, this error was under 2% (Schofield et al., 1999). Field experiments showed that the prediction of animal weight is 0.77% (Fu, 2011). It was found that body width have less correlation with weight, than body length.

CONCLUSION

The aim of this research was to investigate the potential of image processing to estimate live animal weight. Even though the accuracy of live weight prediction depends on different variables, the results of many research showed that the application of an imaging system to measure the animal weight is possible. Further studies should focus on image quality by, changing the camera position lighting situations. However, it should be investigated that how the prediction system combine with automatic feeders and drinkers for maximizing economic benefits of farmers.

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QUALITY ASSURANCE AND PREVENTIVE MEASURES, A PRE-CONDITION FOR FOOD SAFETY GUARANTY

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ABSTRACT

Globalization of the food supply has led to the rapid and widespread international distribution of foods. Throughout the world the incidence of foodborne diseases is increasing and international food trade is disrupted by frequent disputes over food safety and quality requirements. An estimated 600 million – almost 1 in 10 people in the world fall ill after eating contaminated food and 420 000 die every year (DALYs). Safe food supplies support national economies, trade and tourism, contributes to food and nutrition security, and underpins sustainable development. It is also important to note that addressing the risk of foodborne disease goes beyond the public health worker. Ultimately it requires the implementation of a well functioning and integrated food control system which needs collaboration among all the components of a food control system, including food law and regulations, food control management, inspection services, epidemiological and food monitoring (laboratory services) and education of and communication with the consumer. Food quality assurance, on the other side, imposes for food manufacturing to comply standards on their products. Dietary changes in global society, restriction on importing several foods from other countries, lack of consumer food knowledge, allergies from various food ingredients, economic crisis, etc., do affect the food consumption and food industries. Food production companies may try to reduce production costs by mixing in low-quality materials, which may damage people's health. Due to the lack of knowledge and awareness, it is very difficult for consumers to distinguish between healthy and unhealthy food. Given to private sector the possibility to increase profits at all costs, food safety protection must be guided by strong government regulations. Actually, those regulations and government policy, influenced by industry, do create favorable rules that decrease the cost of business by putting consumers at risk. Regulators act more in reactive manner than preventive one, using voluntary product recalls to respond to major food safety scandals rather than addressing the underlying problems. It is up to each actor in the food production and distribution chain to take all steps to make sure that products placed on the market are free of all risks to consumers 'health. The aim of this paper is to discuss factors affecting food safety, the implementation of food quality standards and highlight significant findings on quality assurance and preventive measures in food industry, for better effective intervention strategies against food-related diseases and the benefits to consumers globally.

Keywords: *Food Safety, Quality assurance, Food-borne diseases, Food chain*

INTRODUCTION

Food borne diseases have been an issue for all societies since the beginning of humanity. The types, severity and impacts of these illnesses have changed through the ages and are still diverse across regions, countries and communities. A food borne disease can be defined as a disease commonly transmitted through ingested food. Food borne diseases comprise a broad group of illnesses, and may be caused by microbial pathogens, parasites, chemical contaminants and biotoxins.

Globalization means food products can move easily from country to country, widening the scope of food safety problems. Global food production has rapidly been consolidated in recent decades with some troubling results. The takeover of the food industry by a few multinational corporations has radically shifted the focus of food production. Increased competition and the imposition of the corporate structure have together led to a general preference for profitability over quality. Unfortunately cutting corners has become common practice across all aspects of food production – from waste management to the quality of animal feed, to the technical training that farm and food workers receive. The consequences have largely come to the cost of consumers and the safety of their food.

People have the right to expect the food they eat to be safe and suitable for consumption. Foodborne illness and foodborne injury are at best unpleasant; at worst they can be fatal. Foodborne disease outbreaks involving agents such as *Escherichia coli*, *Salmonella* and chemical contaminants highlight problems with food safety and increase public anxiety that modern farming systems, food processing and marketing do not provide adequate safeguards for public health. Factors which contribute to potential hazards in foods include improper agricultural practices; poor hygiene at all stages of the food chain; lack of preventive controls in food processing and preparation operations; misuse of chemicals; contaminated raw materials, ingredients and water; inadequate or improper storage, etc. Specific concerns about food hazards have usually focused on microbiological hazards, pesticide residues, misuse of food additives, chemical contaminants, including biological toxins, adulterations. This list has been further extended to cover genetically modified organisms, allergens, veterinary drugs residues and growth promoting hormones used in the production of animal products.

Outbreaks of foodborne illness can damage trade and tourism, and lead to loss of earnings, unemployment and litigation. Food spoilage is wasteful, costly and can adversely affect trade and consumer confidence. International food trade and foreign travel are increasing bringing important social and economic benefits. But this also makes the spread of illness around the world easier. An estimated 600 million – almost 1 in 10 people in the world fall ill after eating contaminated food and 420 000 die every year (DALYs). Eating habits too, have undergone major changes in many countries over the last two decades and new food production, preparation and distribution techniques have developed to reflect this. Effective food control therefore, is vital to avoid the adverse human health and economic consequences of food borne illness, food borne injury and food spoilage. Consumers expect protection from hazards occurring along the entire food chain, often described as the farm – to – table continuum. Protection will only occur if all sectors in the chain operate in an integrated way and food control system address all stages of this chain (FAO, 2010).

Human health surveillance of food borne diseases in Albania is led by the Public Health Institute within the Ministry of Health, which collates data supplied by regional departments of public health. An early warning surveillance system operates across all of Albania (similar to the system that operates in Serbia and Macedonia (Valenciano et al., 2004), and the case definitions are the same as for syndrome surveillance under the International Health Regulations. Key indicators of food borne disease are the annual rates of reported gastrointestinal illness (approximately 56 000 cases per year, approximately 2 000 cases per 100 000 population) and cases reported as food poisoning (approximately 2800 cases per year, approximately 100 cases per 100 000 population). Food poisoning cases are reported on the basis of assessment by physicians from primary health care, as well as hospitalized cases. Surveillance for parasitic or viral infections is not routine, apart from infection with *Entamoeba histolytica* (WHO 2015).

Cross-sectional studies of faecal samples for viral and parasitic infections have been carried out (Fabiana et al., 2007; Sejdini et al., 2011). Access to health care is limited, particularly in rural areas. A lack of awareness of entitlements, and informal payment systems, mean that 20–30% of people cannot access primary health care (UNICEF). Another section of the Ministry of Health, the Department of Health and Environment, is responsible for general hygiene and sanitation across all businesses, including food-related businesses. The Ministry of Agriculture, Food and Consumer Protection Food Safety Directorate includes the National Food Authority (NFA), which is

responsible for official control, risk assessment, and communication. Official control involves the inspection of food production hygiene, and certification of hazard analysis critical control point (HACCP)-based systems.

Implementation of food law and quality standards

The development of relevant and enforceable food laws and regulations is an essential component of a modern food control system. Food law has traditionally consisted of legal definitions of unsafe food, and the prescription of enforcement tools for removing unsafe food from commerce and punishing responsible parties after the fact. It has generally not provided food control agencies with a clear mandate and authority to prevent food safety problems. The result has been food safety programs that are reactive and enforcement-oriented rather than preventive and holistic in their approach to reducing the risk of food borne illness.

In addition to legislation governments need updated food standards. In recent years, many highly prescriptive standards have been replaced by horizontal standards that address the broad issues involved in achieving food safety objectives. While horizontal standards are a viable approach to delivering food safety goals, they require a food chain that is highly controlled and supplied with good data on food safety risks and risk management strategies and as such may not be feasible for many developing countries. The importance of food safety standard may stand in the definition. According to Giovannucci and Reardon (2001), standards have been defined as parameters that segregate similar products into categories and describe them with consistent terminology that can be commonly understood by market participants. Much of the current literature agreed that worldwide food industries applied Good Agricultural Practices (GAPs), Hazards Analysis of Critical Control Points (HACCPs) and International Organization for Standardization (ISO) as benchmark of food quality system in food safety management (Lamuka, 2014).

In preparing food regulations and standards countries should take full advantage of Codex standards and food safety lessons learned in other countries. Taking into account the experiences in other countries while tailoring the information, concepts and requirements to the national context is the only sure way to develop a modern regulatory framework that will both satisfy national needs and meet the demands of the SPS Agreement and trading partners (FAO/WHO 2010).
Henson and Loader (2001) has revealed that outdated laws, lack of knowledge in sharing limited coordination between organizations handling food safety issues; includes funding of national research institutes and the lack of awareness for standards and quality may affect developing countries lack the resources to effectively participate in international trade. World Health Organization (2015) reported that food safety legislation in many developing countries is not in line with international requirement.

A study conducted by Athukorala and Jayasuriya (2003) reached a different approach, which is standards typically much higher than those existing in developing countries, and often difficult and costly to meet, but they are also subject to frequent changes. Changes are caused by scientific knowledge about health hazards, improvements in food processing technology and highly income-elastic consumer preferences for higher safety standards. Lamuka (2014) pointed out that the core responsibility of ensuring food safety in the food supply chain is government dependability.

Adoption of food safety standards is found to lead to higher export sales, revenues, and incomes. In some cases, it is found to lead to adoption of improved technology, greater efficiency in production or marketing, higher labor income, or improved health through reduced on-farm exposure to hazards. Thus, one summary view of the studies is that they generally support the "standards as catalysts" model posed by Jaffee and Henson (2004). That is, standards tend to serve as catalysts for improved processes and products, leading to capture of greater value added for successful export industries.

Quality assurance and preventive measures

The objective of reduced risk can be achieved most effectively by the principle of prevention throughout the production, processing and marketing chain. To achieve maximum consumer protection it is essential that safety and quality be built into food products from production through to consumption. This calls for a comprehensive and integrated farm-to-table approach in which the producer, processor, transporter, vendor, and consumer all play a vital role in ensuring food safety and quality.

It is impossible to provide adequate protection to the consumer by merely sampling and analyzing the final product. The introduction of preventive measures at all stages of the food production and distribution chain, rather than only inspection and rejection at the final stage, makes better economic sense, because unsuitable products can be identified earlier along the chain. The more economic and effective strategy is to entrust food producers and operators with primary responsibility for food safety and quality. Government regulators are then responsible for auditing performance of the food system through monitoring and surveillance activities and for enforcing legal and regulatory requirements.

Food hazards and quality loss may occur at a variety of points in the food chain, and it is difficult and expensive to test for their presence. A well structured, preventive approach that controls processes is the preferred method for improving food safety and quality. Many but not all potential food hazards can be controlled along the food chain through the application of good practices i.e. good agricultural practices (GAP), good manufacturing practices (GMP), and good hygienic practices (GHP).

An important preventative approach that may be applied at all stages in the production, processing and handling of food products involves the Hazard Analysis Critical Control Point system (HACCP). The principles of HACCP have been formalized by the Codex Committee on Food Hygiene (Codex Alimentarius 1997), and provide a systematic structure to the identification and control of food borne hazards. Governments should recognize the application of a HACCP approach by the food industry as a fundamental tool for improving the safety of food.

Lamuka (2014) has listed emphasizing on food safety regulations in trade, introduction of stringent food safety standard, reorientation of food quality techniques toward preventive management and a shift by regulatory agencies toward process-based standards may become an opportunity to the developing countries, mainly in agricultural commodity export markets and domestic food sector. The existence of the private standard has been said to harmonize the public and private safety standard (Unnevehr, 2014). Several recent studies have begun to examine the importance of private standards in food production. As public and private food safety and quality standard has been written and presented in different approach, still the importance to implement in food industry is a high requirement.

Effective intervention strategies against food related diseases

The preparation of a national food control strategy enables the country to develop an integrated, coherent, effective and dynamic food control system, and to determine priorities which ensure consumer protection and promote the country's economic development. Such a strategy should provide better coherence in situations where there are several food control agencies involved with no existing national policy or overall coordinating mechanism. In such cases, it prevents confusion, duplication of effort, inefficiencies in performance, and wastage of resources.

Devising strategies for food control with clearly defined objectives is not simple, and the identification of priorities for public investment in food control can be a challenging task. The strategy should be based on multi-sectoral inputs and focus on the need for food security, and consumer protection from unsafe adulterated or misbranded food. At the same time it should take into consideration the economic interests of the country in regard to export/import trade, the development of the food industry, and the interests of farmers and food producers. Strategies should

use a risk based approach to determine priorities for action. Areas for voluntary compliance and mandatory action should be clearly identified, and timeframes determined. The need for human resource development and strengthening of infrastructure such as laboratories should be also considered (FAO/WHO 2010).

Certain types of food control interventions require large fixed capital investments in equipment and human resources. While it is easier to justify these costs for larger enterprises, imposing such costs on smaller firms who may coexist with larger enterprises may not be appropriate. Therefore the gradual phasing in of such interventions is desirable. For example, countries may allow small enterprises longer periods of time to introduce HACCP. The strategy will be influenced by the country's stage of development, the size of its economy, and the level of sophistication of its food industry.

The researcher alleged that the building of facilities to improve quality regulations and the building of government structures to ensure quality and safety of products are key points of attention. EU countries with regard to food safety and quality have well-established industry standards and are now focusing on communication of quality and safety aspects to consumers. Emerging economy countries are in the phase of implementation and harmonization of food quality and safety standards, while developing countries are still struggling with the establishment of the right conditions to enforce food quality and safety of their products.

Albania has some level of preparation in food safety, veterinary and phytosanitary policy. It made some progress in implementing relevant policies in the food safety and veterinary sectors. In the coming year, Albania should in particular update the relevant legislation to approximate it with the latest EU legislation on official controls, animal health and plant health; and enforce food safety rules, including official controls, import conditions and controls, and maximum pesticide residue levels (Commission staff working document Albania 2018 Report).

CONCLUSION

Food safety is an essential public health issue for all countries. Food born diseases due to microbial pathogens, biotoxins and chemical contaminants in food represent serious threats to the health of thousands of millions of people. Food born diseases not only sign significantly affect people's health and well-being, but they also have economic consequences for individuals, families, communities, businesses and countries.

Food production, processing, and marketing systems are complex. In many developing countries they are also highly fragmented and dependent upon a large number of small producers. While this may have socioeconomic benefits, as large quantities of food pass through a multitude of food handlers and middlemen, the risk of exposing food to unhygienic environment, contamination and adulteration increases.

The objective of reduced risk can be achieved most effectively by the principle of prevention throughout the production, processing and marketing chain. The introduction of preventive approaches such as HACCP have resulted in industry taking greater responsibility for and control of food safety risks. Such an integrated approach facilitates improved consumer protection, effectively stimulates agriculture and the food processing industry, and promotes domestic and international food trade.

The development of relevant and enforceable food laws and regulations is an essential component of a modern food control system. Food control agencies should address the specific training needs of their food inspectors and laboratory analysts as a high priority. These activities provide an important means of building food control expertise and skills in all interested parties, the thereby serve an essential preventive function.

Adoption of food safety standards is found to lead to higher export sales, revenues, and incomes. Standards tend to serve as catalysts for improved processes and products, leading to capture of greater value added for successful export industries.

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GENETIC DISTANCE OF NEW BULGARIAN DURUM WHEAT VARIETIES AND BREEDING LINES OF FCI-CHIRPAN, BULGARIA

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ABSTRACT

The durum wheat breeding program in Field crops institute FCI-Chirpan started in 1928. In the last ten years, a number of varieties have been created to meet modern cultural requirements. Genetic distance is of great importance for the success of the combining breeding. In the study are included 13 varieties created over the last 10 years and 8 of the Institute's best advanced durum wheat breeding lines. The field experiment was conducted in experimental field of FCI-Chirpan during 2015-2017 year. A randomized block design in four replicated was used with a trial plot size of 15 m^2. A standard farming technology for durum wheat in the country was applied. Tre traits: grain yield, plant height, heading date, test weight, 1000 kernel weight, protein content, wet gluten and vitreousness were observed. The means of three-year trials for all studied traits were calculated and genetic differences between genotypes were found. The coefficients of variation (CV%) obtained for the individual traits show that the heading date and grain yields are the most variable. The correlation analysis determines significant coeficients for 9 out of 28 possible combinations of traits. The multivariate methods for determining the genetic distance between the involved genotypes in the study were used. According to the dendrogram of the cluster analysis, the distances between the four main groups formed are determined. The applied graphical PC analysis shows the interrelations between the traits and the grouping of genotypes in terms of the genetic distance between them. These methods can help for faster breeding progress using the established genetic distance as the basis for deploying thebreeding program.

Keywords: *Durum wheat, Genetic distance, Breeding, Economic traits*

INTRODUCTION

In the last ten years more than ten varieties of durum wheat was created in Field crops institute - Chirpan, Bulgaria. The breeding program includes crosses of varieties (parents) from all over the world and a selection in segregating generations. Increasing genetic diversity allows for reliable sources of variation in the future. By determining the genetic distance, a proper selection of the parental forms can be made, to achive serious progress on the yield potential of the recombinant genotypes (Islam, 2004). The estimation of the genetic distance between genotypes can be based on the phenotypic manifestation of quantitative and qualitative traits (Souza and Sorells, 1991). Most often, genetic distance is measured as a phenotypic distance (Arriel et al., 2007; Gashaw et al., 2007a; Debnath et al., 2008; Kabir et al., 2009). It is assumed that if the genotypes are different phenotypically in many traits they are also genetically distant by their genomes. Some researchers have widely applied cluster analysis and PC analysis methods to determine genetic distance in breeding (Bhatt, 1970; Carves et al., 1987; Mohammadi and Prassana, 2003; Eivazi et al., 2007). The results of cluster analysis and PC analysis may show partial differences between them, furthermore the separation by cluster analysis gives the best estimate of the genetic distance of the genotypes Khodadadi et al.,

(2011). The PC analysis takes into account the first two components and this results in some distortion of the results Fotokian et al., (2002) and Siahbidi et al., (2013). Fang et al., (1996) applied a cluster analysis of 120 genotypes of durum wheat, defining five clusters based on morphological and economic qualities. Narouee R., (2006) determines with cluster analysis the genetic distances of local wheat varieties based on theyr morphological traits. The authors Talebi et al., (2010) tested 24 genotypes of durum wheat to determine their genetic distances by important yield-related traits and using cluster analysis to define three clusters. Hashjin et al., (2014) examine 116 genotypes from several different regions to determine the genetic distance between them. The results show highly significant genetic diversity between genotypes and the participants are divided into three clusters. Authors suggest that the parents from divergent clusters can be used for hybridization to isolate useful recombinants in the segregating generations in breeding programs for improvement of durum wheat.

The aim of our study is to determine the genetic distance between 21 Bulgarian durum wheat varieties and breeding lines created in FCI-Chirpan based on 8 key agronomic traits using multivariate methods.

MATERIAL AND METHODS

In the study are included 13 varieties created over the last 10 years and 8 of the Institute's best advanced durum wheat breeding lines. The field experiment was conducted in experimental field of FCI-Chirpan, Bulgaria during 2015-2017 year. A randomized block design with four replications was used with a trial plot size of 15 m^2. A standard farming technology for durum wheat in the country was applied. Tre traits: grain yield - GY (kg/da), plant height – H (cm), heading date – HD (m. may), test weight – TW (kg/hl), thousand kernel weight – TKW (g), protein content – PR (%), wet gluten content – GL (%) and general vitreousness – VIT (%) were observed. The results were processed through ANOVA (for 3 year results), variation analysis, correlation analysis, cluster analysis and PC analysis over the three year average data. For the statistical treatment of data is using package program Statistica 10 (StatSoft inc). The hierarchical cluster analysis was performed using the Ward's method by Ward (1963).

RESULTS AND DISCUSSION

The results of the variation analysis by traits are presented in Table 1. The table shows the mean values (M) with their standard errors (± m) and the coefficients of variation (CV%). There is evidence of genetic diversity in the studied trait. According to the coefficients of variation the most varying traits are grain yield(GY) (9.94%) and heading date (HD) (10.12%), and the lowest varying - test weight(TW) (2.21%) and vitreousness (1.69%). The other traits occupy an intermediate position. It can be seen that the coefficients of variation are relatively low in the studied traits. Therefore, the breeding process that was led was directed to a particular model of a variety ideal. There is a certain tendency to increase the early maturity of genotypes, which corresponds to the meteorological conditions in the areal of study.

Table 2 presents the two-factor ANOVA by traits and years. The results presented significant differences between genotypes for all traits. The differences in years of cultivation are well significant for all the studied traits and show the significant influence of the conditions of the years on their expression. The genotype-environment interaction indicates that half of the traits: grain yield (GY), heading date (HD), wet gluten (GL) and vitreousness (VIT) are well significant (tab.2). This causes a certain reaction in the conduction of the breeding process. When conducting the breeding process for traits associated with the conditions of the years,

observation should prolonged for more years or phenotypic stability of the individual genotypes - to be determined by any of the conventional methods by Kang (1998).

Table 1. Mean values (M), standard error (± m) and coefficients of variation (CV%) over 3 seasons (2015, 2016, 2017) for 8 traits of 21 genotypes new Bulgarian durum wheats

Genotype	GY (kg/da)	H (cm)	HD (m. May)	TW (kg/hl)	TKW (g)	PR (%)	GL (%)	VIT (%)
Zvezditsa	372.13	92.0	13.00	73.38	43.16	15.44	33.86	95.40
IPK Deni	411.86	97.3	11.66	74.71	43.10	14.94	29.36	95.60
Deyana	419.03	98.0	11.00	76.06	44.36	15.00	30.23	92.56
Tserera	432.70	92.6	12.66	78.00	45.13	14.30	27.56	94.53
Predel	451.62	91.3	14.00	74.56	42.83	14.44	30.73	95.60
IPK Elbrus	457.16	95.0	14.00	75.17	42.90	14.06	27.26	94.40
Viktoria	462.46	102.0	13.66	75.74	43.60	14.69	30.60	95.46
Trakiets	463.63	100.3	15.33	76.63	43.16	14.59	31.50	96.36
D-7763	479.96	104.0	14.66	79.05	48.73	13.69	25.96	93.40
Kehlibar	482.46	98.3	15.33	77.35	43.96	14.58	28.03	96.53
Raylidur	491.36	95.0	14.00	73.81	73.70	13.77	26.06	95.60
D-8040	500.20	97.3	15.00	78.97	45.03	13.97	25.93	94.40
D-8036	504.86	103.0	13.66	78.03	45.76	13.74	30.46	92.46
Heliks	518.13	99.3	11.66	77.87	52.33	13.69	28.90	95.80
D-8243	518.20	97.0	12.66	75.32	50.56	14.23	31.93	97.06
D-8091	518.60	99.6	16.00	76.77	47.63	13.19	26.33	91.16
Saya	520.63	97.6	15.33	75.72	43.33	14.41	30.76	96.80
D-8031	527.06	98.3	12.33	77.32	51.66	13.97	29.33	94.96
Reyadur	533.36	102.0	15.33	75.99	46.10	14.01	27.13	96.93
D-8148	543.36	107.6	14.66	78.46	48.26	13.68	30.43	93.13
D-8159	559.60	98.0	13.33	78.57	48.90	13.62	29.03	95.33
M	484.20	98.35	13.78	76.54	45.91	14.19	29.11	94.92
±m	10.5	0.87	0.3	0.36	0.66	0.12	0.47	0.35
CV%	9.94	4.08	10.12	2.21	6.66	3.88	7.55	1.69

Table 2. ANOVA of traits by genotypes and years

No	Trait	Source of variance		
		Genotypes G	Environments E	Interaction GxE
1	Grain yield	**	**	**
2	Heading date	*	**	*
3	Plant height	**	**	n.s.
4	1000 kernel weight	**	**	n.s.
5	Test weight	**	**	n.s.
6	Wet gluten	*	**	**
7	Grain protein content	**	**	n.s.
8	Vitreousness	**	**	**

* - $P \leq 0.05$; ** - $P \leq 0.01$; n.s. – no significant

The established correlation coefficients between the studied traits are presented in Table 3. The table shows significant positive correlations coefficients for grain yeild (GY) with: plant height (H) (r = 0.53 *), test weight (TW) (r = 0.52 *) and tousand kernel weight (TKW) (r = 0.63 **). Dogan R., (2009) and Gashaw et al., (2007) reported same significant and positive correlation between grain yield and thousand kernel weight. The positive correlation between grain yield (GY) and plant height (H) is due to the fact that all genotypes tested are from the group of medium plant height. Authors Nofouzil et al., (2008) also reported significant and positive correlation between grain yield and plant height. On the other hand, there is a very well significant negative correlation (r = -0.80 ***) between grain yield (GY) and grain protein (PR). We have established a well significant positive correlation (r = 0.56 **) of plant height (H) with the test weight (TW), which once again explains the relationship of plant height with yield. The test weight (TW) is in a positive correlation with the tousand kernel weight (TKW) (r = 0.53 *) and negative with grain protein content (PR) (r = -0.57 **). The thousand kernel weight (TKW) is also found to be negative in relation to grain protein content (PR) (r = -0.59 **). Expected, very well correlation occurs between protein (PR) and wet gluten (GL) in the grain (r = 0.61 **). There is no significant corelations with heading date and vitreousness.

Table 3. Correlation coefficients between studied traits

Trait	GY	H	HD	TW	TKW	PR	GL	VIT
GY	1	0.53*	0.39	0.52*	0.63**	-0.80***	-0.29	0.02
H		1	0.29	0.56**	0.37	-0.42	-0.11	-0.30
HD			1	0.17	-0.22	-0.36	-0.32	-0.01
TW				1	0.53*	-0.57**	-0.38	-037
TKW					1	-0.59**	-0.11	-0.11
PR						1	0.61**	0.38
GL							1	0.27
VIT								1

* - $P \leq 0.05$; ** - $P \leq 0.01$; *** - $P \leq 0.001$

The results of the hierarchical cluster analysis are presented in Figure 1. The cluster analysis was performed on the average data of the three crop years. The data is standardized in order to align the scale of the traits and obtain a more accurate assessment (Siahbidi et al., 2013). The dendrogram shows two big clasters in the first level of division. The bigger claster covers exclusively the certified varieties of the institute. In the second level of distance, this group is divided into two subclasters, which include the varieties from the two breeding branches developed at the institute - combining breeding and experimental mutagenesis. In the smaller main claster are all advanced durum wheat lines included in the experiment. This group also includes the new one variety of the Institute - Heliks (certified 2017) which falls in a subgroup with two of the most promising breeding lines. Therefore, it can be pointed out that the Institute's breeding program leads to the production of relatively diverse genotypes of durum wheat. In conducting the breeding process, we should take into account the genetic distance of the genotypes involved in the combining breeding. To achieve faster success, it is necessary to combine genetically closer parents. In order to achieve greater breeding advances in agronomical traits, it is necessary to cross genetically more remote parents (from different clusters). The authors Khodadadi et al. (2011) reached the same conclusion in the breeding strategy.

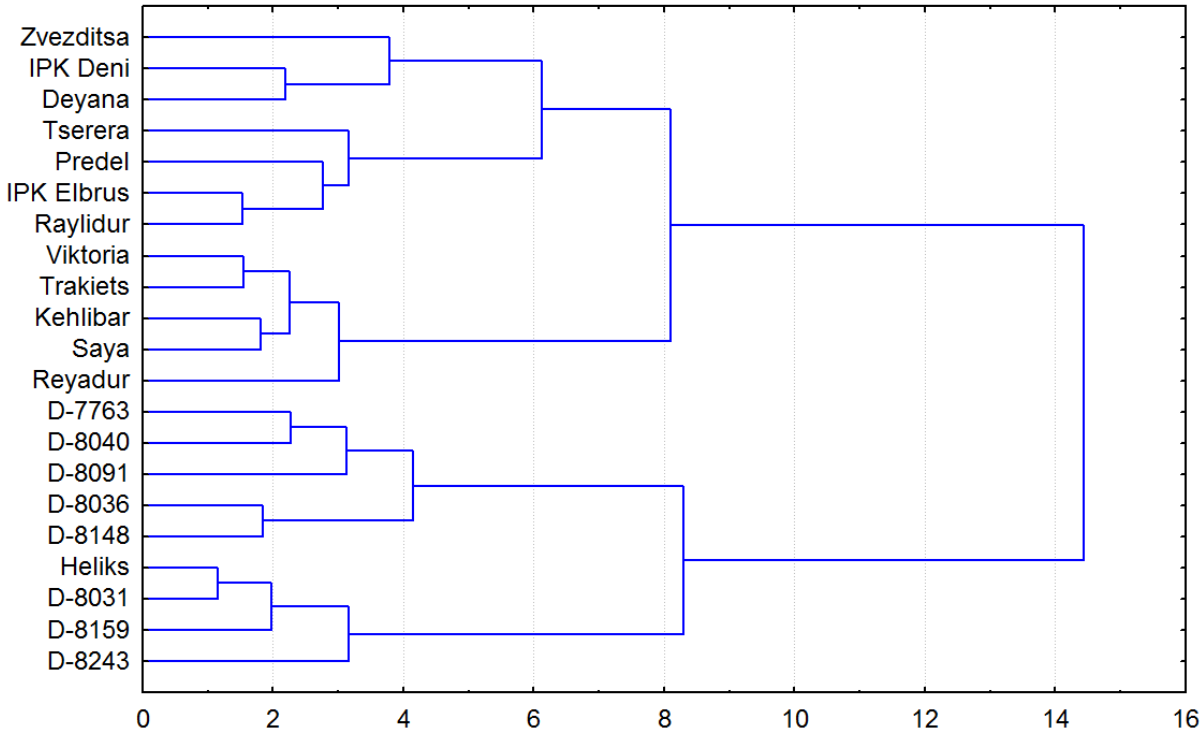

Figure 1. Dendrogram of 21 genotypes of Bulgarian durum wheat by 8 economic traits.

The PC analysis is represented graphically in Figure 2 and Figure 3. The two main components PC-1 and PC-2 account for 62.28% of the total variation for all genotypic traits. This variation is large enough to perform the analysis. Figure 2 shows the points and vectors of the traits investigated in the experiment. From the angles between the vectors of the traits, can be explained the correlation between them. From the magnitude of the angle we can judge the magnitude of the correlation. The smaller the angle, the stronger the correlation between the traits is. At an angle of 90 degrees the correlation is zero. At angles greater than 90 degrees, the correlation is negative, increasing with the increase in the angle. According to the corners of Figure 2, the correlation relationships are close to the correlation coefficients in Table 3. This figure 2 should be viewed in conjunction with Figure 3. Figure 3 shows the distribution of genotype points in the coordinate system the PC-1 to PC-2. According to the quadrant in which the genotyping points and passing vectors of the traits are located, Figure 2 can be judged for the corresponding stronger influence of the particular trait on the genotype. Figure 3 shows a significant similarity between the genotype distribution in the PC analysis (Figure 2) and the data from dendrogram (Figure 1). The authors Golabadi and Arzani., (2003) suggest that cluster analysis was similar to factor analysis in grouping the characters. In general, it can be assumed that the separation by cluster analysis gives the best estimate of the genetic distance of the genotypes. Therefore, in studying genetic diversity related to genetic proximity and distance, it is preferable to use cluster analysis (Bhatt., 1970; Carves et al., 1987; Fang et al., 1996; Khodadadi et al., 2011; Siahbidi et al., 2013).

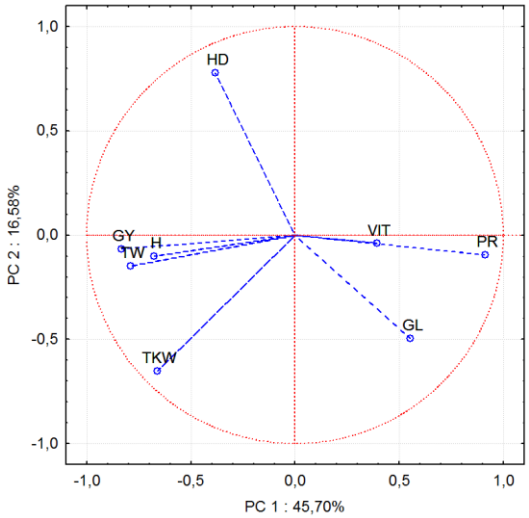

Figure 2. PC - analysis of traits

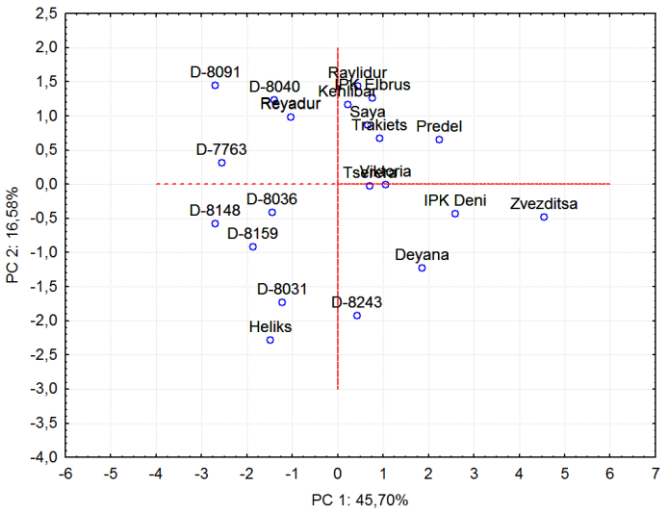

Figure 3. PC – analysis of genotypes

CONCLUSIONS

When conducting the breeding process for traits associated with the conditions of the years, observation should be prolonged for more years or the phenotypic stability of the individual genotypes - to be determined by any of the conventional methods.

Established correlation relationships between the studied traits can be successfully used in the breeding of durum wheat.

Using of cluster analysis provides a reliable estimation of the genetic distance of genotypes in our breeding program. The established genetic distance of genotypes allows optimization of the breeding process when choosing its strategy.

Despite the possibility of PC analysis to show clustering of genotypes by genetic proximity and distance, it is preferable to apply cluster analysis for this purpose because its more precision.

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SORGHUM CROP, AN ALTERNATIVE FOR DOBROGEA FARMERS IN THE CONTEXT OF CLIMATE CHANGES

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ABSTRACT

Dobrogea is the most drought area of Romania (average 1961-2016 :464 mm rainfall precipitation). Climate change in recent years has accentuated this phenomenon .For farmers from this area sorghum crop is a solution.At Sport Agra in Amzacea, in the last few years there have been experimented new sorghum crop technologies designed to face the current climate changes. These technologies include the following elements: changing the sowing epoch with one month before the usual period recommended by classical technologies; (beginning of April in order to benefit from the soil's humidity la 4-5 cm depth boosting the germination process); choosing early hybrids in order to avoid the drought season which starts in June; applying adequate crop protection treatments, with pre-emergent and post-emergent herbicides and last generation insecticides. The agricultural crops in this area are not irrigated, so the farmer proposed a new technology, with the sowing of the crops earlier. This way the plants will benefit from the moisture from the soil accumulated in the winter.The obtained production from sorghum crop was over 10t/ha for most of the varieties tested.

Keywords: *Sorghums, Climate changes, Technologies*

INTRODUCTION

The crop taking into study is sorghum, which is recommended for these arid areas; called the camel of crops due to its drought resistance (Amsalu Ayana and col.1998), sorghum requires the following technological elements:

Selecting early hybrids to overcome the drought periods that occur between the 5-10th of June until the 20-25th of August. There are recommended hybrids with shorter vegetation period (Poschiscanu et al., 1015).

Sorghum sowing is recommended between 20th April and 10th May (Trotus et al. 2015) ensuring a minimum of 120-140 kg / ha of nitrogen (Owen, 1967), treatment of seeds before sowing with chemicals containing thiamethoxam, pre-emergence herbicide with Guardian 2l / ha and post-emergence with Ceredin Super 40 SL 1 l/ha (Micheal et al., 2005).

The results from comparative crops in a 2-year dynamics have demonstrated sorghum crops with outstanding yields of over 10 t/ha.

The agricultural crops in this area are not irrigated, so the farmer proposed a new technology, with the sowing of the two crops earlier by about a month. This way the plants will benefit from the moisture from the soil accumulated in the winter.

MATERIALS AND METHODS

Experimental plots were placed at S.C. SPORT AGRA S.R.L. Amzacea, Constanța. The experience was situated on a land belonging to the South Dobroudja plateau, represented by cambic chernoziom, with a profile deeper than other chernozioms, a blackish-brown soil of 40-50 cm thickness, medium texture (Demeter, 2009). The content of nutrients was: mobile P index - 72; N index - 4; Humus - 3.11; K index - 200; Neutral pH - 7.2. The climate is deeply temperate continental, with an average annual temperature of 10.7-12.12°C, with a high temperature between June and August. Meteorological data are presented in Tables 1 and 2. Sowing was carried out on April.

Table 1. Precipitation and temperature during 2016 growing vegetation season (Valul lui Traian Station, Constanta)

	Month								
	Jan.	Febr.	March	Apr	May	June	July	Aug.	
Periods	The growing season 2016: Precipitation (mm) for 10-day periods								Sum
1-10	0	12.0	10.0	0	60.0	3.5	56.0	4.0	145.5
11-20	95.0	18.5	19.0	0	21.0	20.0	0	0	173.5
21-30	15.0	0	15.0	20.0	16.0	0	0	0	66.0
Sum	110.0	30.5	44.0	20.0	97.0	23.5	56.0	4.0	385.0
	Average 1961-2010 : monthly values of precipitation (mm)								Sum
	27.7	24.0	29.1	31.8	37.7	47.1	38.9	37.4	273.7
	The growing season 2016: Mean air (°C) for 10-day periods								Mean
1-10	2.5	4.1	6.8	10.3	13.9	19.8	22.6	23.2	12.9
11-20	4.8	5.2	7.9	12.9	16.8	21.4	24.2	22.6	14.57
21-30	4.3	5.4	10.2	13.5	18.7	22.1	23.8	21.4	14.92
Mean	3.9	4.9	8.3	12.2	16.5	21.1	23.5	22.4	14.1
	Average 1961-2010 : monthly values of mean air temperature (°C)								Mean
	0.4	0.9	4.4	9.7	15.3	19.4	21.9	16.9	12.12

Table 2. Precipitation during 2017 growing vegetation season (Valul lui Traian Station, Constanta)

	Month								
	Jan.	Febr.	March	Apr	May	June	July	Aug.	
Periods	The growing season 2016: Precipitation (mm) for 10-day periods								Sum
1-10	60	5.0	4.0	0	13.0	18.0	9.0	0	109.0
11-20	10	13.5	31.0	35.0	12.0	6.0	0	0	107.5
21-30	0	2.0	5.0	6.0	2.0	4.0	92.0	6.0	117.0
Sum	70.0	20.5	40.0	41.0	27.0	28.5	101.0	6.0	333.5
	Average 1961-2010 : monthly values of precipitation (mm)								Sum
	27.7	24.0	29.1	31.8	37.7	47.1	38.9	37.4	273.7

RESULTS AND DISCUSSIONS

As written in Table 1, year 2016, provided higher amount of rainfall between May and June, 100 mm higher than the multiannual average. These precipitations favored the development of sorghum crops. Regarding the sorghum crop, the main technological links pursued by the research team consisted of the following: Choosing early hybrids to overcome the burning periods that occur between June 5 through August 20-25, Recommendation of shorter vegetation hybrids, Sowing the sorghum between April 20th-May 10th according to classical technology (Trotus et col 2015.), Provide a minimum of 120-140 kg / ha of nitrogen, Treatment of seeds before sowing with chemicals containing thiamethoxam to combat pests in the early stages of vegetation, Pre-emergence herbicide with Guardian 2l / ha and post-emergence with Ceredin Super 40 SL 1 l/ha

The experiments were carried out in 2016 on 6 hybrids, as shown in Table 3. Most of the hybrids were sown one month earlier (9 April) compared to the classic technology recommended by specialists (Trotus et al. 2015) and EURALIS. Hybrid Arkanciel was sown and in recommended (May 14). Table 10 shows data regarding sorghum productivity consisting in very high yields of about 10-11 tons / ha for most hybrids, due to the change of the sowing date which the plants benefit from the moisture accumulated in the soil during the winter and also avoid the drought crashes begin in June. It can be seen in the Arkanciel hybrid a production increase of 2212 kg/ha, obtained by its earlier sowing.

The data obtained in the experimental year 2017 are presented in Table 4. The sowing took place this year on April 4, and the hybrid Arkanciel was sown on 4 May. From the data presented, it can be seen that this year, through earlier sowing, large production increases of over 10000 kg / ha were obtained. This year, with the Arkanciel hybrid, an increase of 3436 kg/ha was obtained. Tables 3 present the data on the technical sheet of sorghum culture on the two plots. Experiments in plots 1 were made on 2195 sqm. Treatment of seed prior to sowing was performed with chemicals containing thiamethoxam. Pre-emergence herbicide was carried out with Frontier forte 1l/ha and post-emergence with Ceredin Super 40 SL 1 l / ha. Figures 3 are referred to: - Experimental field-sorghum. All of these data show that sorghum is a valuable alternative crop for this dry area.

Table 3. Demonstrative plots for sorghum crop - Amzacea 2016

Hybrid	Pre-emergent plant.	Surface sqm	Seeds /ha	Sowing date	Emergence date	Yields kg / ha
ES Arfrio	Wheat	2195	230000	9 April	18 April	10013
ES Aqulion	Wheat	2195	230000	9 April	18 April	12340
ES Alize	Wheat	2195	230000	9 April	18 April	11785
Arack	Wheat	2195	230000	9 April	18 April	11919
Arkanciel	Wheat	2195	230000	9 April	18 April	10022
Arkanciel	Wheat	2195	230000	2 May	14 May	7810
ES Foehn	Wheat	2195	230000	9 April	18 April	8601

Table 4. Demonstrative plots for sorghum crop - Amzacea 2017

Hybrid	Pre-emergent plant.	Surface sqm	Seeds /ha	Sowing date	Emergence date	Yields kg / ha
Euralis	Wheat	2195	220000	4 April	14 April	10439
Foehn	Wheat	2195	220000	4 April	14 April	11504
Arkanciel	Wheat	2195	220000	4 April	14 April	10336
Arkanciel	Wheat	2195	220000	4 May	16 May	6900
Albanus	Wheat	2195	220000	4 April	14 April	10130
Typhon	Wheat	2195	220000	4 April	14 April	8859
Armorik	Wheat	2195	220000	4 April	14 April	10645

Figure1. Experimental field-sorghum

Figure 2. Sorghum harvest

CONCLUSIONS

At Sport Agro Amzacea, there have been experimented in the last few years new and improved sorghum crop technologies in order to adapt to the new climate changes. These technologies comprise the following technological elements:

Selecting early hybrids to overcome the drought periods that occur between the 5-10th of June until the 20-25th of August. There are recommended hybrids with shorter vegetation period.

Changing the sowing age - the hybrids were sown one month earlier (4 and 9 April)

The results from comparative crops in a 2-year dynamics have demonstrated sorghum crops with outstanding yields of over 10 t/ha.

The agricultural crops in this area are not irrigated, so the farmer proposed a new technology, with the sowing of the two crops earlier by about a month. This way the plants will benefit from the moisture from the soil accumulated in the winter.

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THE INFLUENCE OF LEAF LITTER ON THE DISTRIBUTION OF AQUATIC MACROBENTHIC FAUNA IN TUNCA RIVER (EDIRNE/TURKEY)

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ABSTRACT

The study was carried out in Tunca River that rises and is mostly located in Bulgaria and only a part of it is located in the European part of Turkey. With Arda and Ergene River, Tunca River constitutes a portion of Meriç basin that is one of the largest river systems in East Balkan Basin. The length of this river is 384 km-long and its basin area is 7.884 km^2. Tunca River 12 km along forms the border with Turkey-Bulgaria. Then flowing for a while inside Turkey (approximately 30 km) missed with Meriç River in the South-west of Edirne. Our experiment was designed to comparison potential differences on colonisation of macrobenthic fauna in various leaf packages. Tree localities were chosen on the river and 4 different leaves of trees found in the environment and artificial boxwood were used to take samples. Macrobenthic samples were taken between May 2012 – October 2012. 20 kg sacks of potatoes were used while packaging of leaves and a total of 25 packs were put in localities. The collected samples were kept in 70% alcohol and brought to the laboratory and they were sorted and identified to the lowest possible taxonomic level under a stereomicroscope. As a result, various invertebrate groups were detected in leaf packs. These groups: Chironomidae larvae, Chironomidae pupae, Oligochaeta, Gastropoda, Tabanidae, Odonata, Ephemeroptera, Tricoptera, Plecoptera, Isopoda, Hirudinae, Cructacea, Tipulidae, Lumbricidae. Then ANOVA test was used for analysis of macrobenthic fauna according to dates, stations and leaf various and 0.05 α statistical significance was used for all tests. When there was a meaningful difference, the reason was revealed by the Tukey test. The number of organisms in artificial boxwood was found to be the most intense. This result has shown that leaf packages are for protection purposes.

Keywords: *Macrobentic organism, Leaf litter, Tunca River, Edirne*

INTRODUCTION

According to relative analyses, benthic invertebrates performed both natural and artificial colonies in rock and leaf packs (Sylvestre and Bailey, 2005; Haapala et al., 2003). One of the major carbon sources of rivers is the leaves that are poured from trees and reach the water. Dried leaves are indispensable carbon source, especially for the mountainsides. Along with the deterioration of the leaf, the chemicals it contains include lignin, phenolic compounds, nutrients in the tan and carbon, affecting the nutrient content in the water (Hunter, 2003; Welsh, 2007). It has been proven that the activities of certain benthic invertebrates are enhanced by the amount of decaying leaves. The activities of leaf decay chemistry and of macroinvertebrates are interdependent. Because the composition of the leaf litter is the most important factor in the

colonization of the macroinvertebrates. Besides, it can be effective in forming both leaf decay and colony in environmental conditions (Costa and Melo, 2008).

Tree rashes play a balancing role in river ecosystems (Heede, 1972, Nelson, 2000). The main source of dissolved organic and inorganic nutrients and particulate matter is caused from high places and from riverside trees. This rashes are the basis of the food cycle. Rashes falling from the tree change the geomorphology of the river; thus, creates new habitats for some taxa. Accumulation and degradation of leaf rashes provide habitat source and food for aquatic microorganism and macroinvertebrates (Petersen and Cummins, 1974). The relationship between the macroinvertebrates groups and characterization of plant litter species accumulated in rivers is important to understand the distribution of macroinvertebrates communities and processes at the ecosystem level.

Although there are many faunistic and limnological studies in the Bulgarian part of the river (Dimitrov, 1972; Uzunov, 1980; Russev et al., 1984; Javena and Russev, 1985), In the Turkey part of the river there are only two graduate theses (Kavaz, 1997; Öterler, 2003), two phytoplanctonic studies (Öterler et al., 2003 a; 2004), two faunistic studies (Kirgiz et al., 2005; Camur-Elipek et al., 2006) and one studies colonization of leaf packs of Chironomidae larvae (Özkan, 2018).

In this study, we aimed to determine leaf package preferences of aquatic macrobenthic fauna and the advantages of leaf packages in faunistic study.

MATERIALS AND METHODS

This study was designed to compare the potential differences in the colonization of aquatic macrobenthic fauna in various leaf packs. Study was carried out between May and October 2012 at three stations located in the Tunca River (Figure 1). The first station was Suakacağı Village, the second station was Değirmenyeni Village, and the third station was Tunca Barracks (Edirne) (Figure 1).

Then *Juglans regia* L. (walnut), *Morus alba* L. (mulberry), *Ulmus leavis* Pall. (elm) leaves were collected because of the widespread presence around the river. In addition, *Buxus* sp. (artificial boxwood) and *Platanus orientalis* L. (dried plane leaf) poured from plane trees in the autumn of the previous year were collected. The leaves were then filled into 20 kg potato bags. 5 leaf packages which consisted of 5 different kinds of leaves were prepared for each station. Totally 25 packages were prepared (Figure 2).

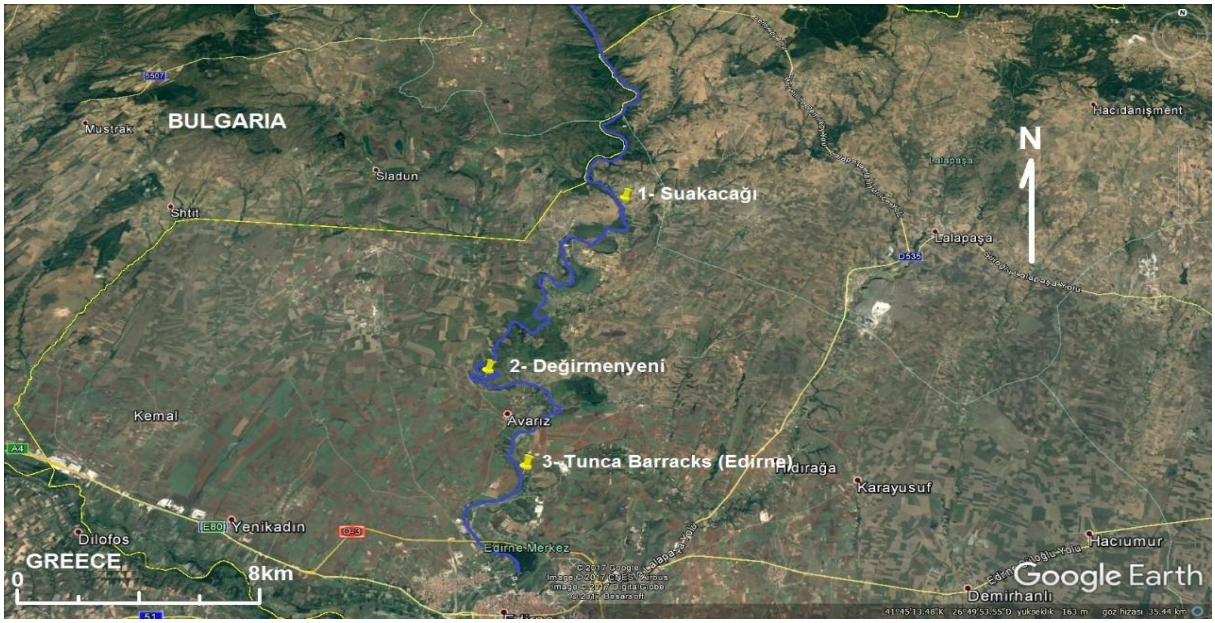

Figure 1. Tunca River sampling stations: 1. Suakacağı Village (465409.00 D, 4632519.00 K, 47m); 2. Değirmeniyeni Village (461067.00 D, 4623425.00 K, 40 m); 3. Trakya University Tunca Barracks (Edirne) (462965.63 D, 4619414.88 K, 37m)

Packages were regularly placed on the benthic region of the river (Figure 3). Within the following 5 months, a series of leaf packs were collected from sampling stations once a month. They were washed in sieves of different aperture sizes (Nominal Aperture -0,600µ, 300µ and 1,18mm) and the macrobenthic organism were picked up with some pointed forceps (Figure 4). They were fixed and stored in tubes containing 70% ethyl alcohol. Samples collected from leaf packs were moved to the laboratory, placed in petri dishes under a stereo microscope (Olympus SZ51), cleaned from the mud and grouped.

Then ANOVA test was used for total organism analysis in leaf packages according to dates, stations and leaf types. A statistical significance of 0.05 α was used in all tests. When there was a significant difference, the Tukey test results show why the difference was caused.

Figure 2. Preparation of leaf packs

Figure 3. Station 2 (Leaving packages to land)

Figure 4. Station 3 (collection of organisms from leaf packs)

RESULTS

The analysis of aquatic macrobenthic fauna according to different dates, stations and leaf kinds (Table 1, 2, 3, 4) are as follows:

Table 1. Variability analysis of aquatic macrobenthic fauna according to dates

	Sum of squares	**df**	**Mean square**	**F**	**Sig.**
Between Groups	96894,853	4	24223,713	,568	,687
Within Groups	2987529,333	70	42678,990		
Total	3084424,187	74			

*****The mean difference is significant at the 0.05 level.

There was no difference between the aquatic macrobenthic fauna according to dates (Table 1).

Table 2. Variability analysis of aquatic macrobenthic fauna according to stations

	Sum of squares	**df**	**Mean square**	**F**	**Sig.**
Between Groups	21477.307	2	10738.653	.252	.778
Within Groups	3062946.880	72	42540.929		
Total	3084424.187	74			

*****The mean difference is significant at the 0.05 level.

There was no difference between the aquatic macrobenthic fauna according to stations (Table 3).

Table 3. Variability analysis of aquatic macrobenthic fauna according to leaf kinds

	Sum of squares	**df**	**Mean square**	**F**	**Sig.**
Between Groups	736483.520	4	184120.880	5.489	.001
Within Groups	2347940.667	70	33542.010		
Total	3084424.187	74			

*****The mean difference is significant at the 0.05 level.

There was difference between the aquatic macrobenthic fauna according to leaf kinds (Table 3).

Table 4. Multiple comparisons analysis of aquatic macrobenthic fauna differences according to leaf types

I) Leaf types	**(J) Leaf types**	**Mean difference (I-J)**	**Std. error**	**Sig.**	**95% Confidence Interval**	
					Lower bound	**Upper bound**
Buxus sp.	*Juglans regia*	251.20000(*)	66.87502	.003	63.9397	438.4603
	Platanus orientalis	209.46667(*)	66.87502	.021	22.2064	396.7269
	Morus alba	269.86667(*)	66.87502	.001	82.6064	457.1269
	Ulmus leavis	240.86667(*)	66.87502	.005	53.6064	428.1269
Juglans regia	*Buxus* sp.	-251.20000(*)	66.87502	.003	-438.4603	-63.9397
	Platanus orientalis	-41.73333	66.87502	.971	-228.9936	145.5269
	Morus alba	18.66667	66.87502	.999	-168.5936	205.9269
	Ulmus leavis	-10.33333	66.87502	1.000	-197.5936	176.9269
Platanus orientalis	*Buxus* sp.	-209.46667(*)	66.87502	.021	-396.7269	-22.2064
	Juglans regia	41.73333	66.87502	.971	-145.5269	228.9936
	Morus alba	60.40000	66.87502	.895	-126.8603	247.6603
	Ulmus leavis	31.40000	66.87502	.990	-155.8603	218.6603
Morus alba	*Buxus* sp.	-269.86667(*)	66.87502	.001	-457.1269	-82.6064
	Juglans regia	-18.66667	66.87502	.999	-205.9269	168.5936
	Platanus orientalis	-60.40000	66.87502	.895	-247.6603	126.8603
	Ulmus leavis	-29.00000	66.87502	.992	-216.2603	158.2603
Ulmus leavis	*Buxus* sp.	-240.86667(*)	66.87502	.005	-428.1269	-53.6064
	Juglans regia	10.33333	66.87502	1.000	-176.9269	197.5936
	Platanus orientalis	-31.40000	66.87502	.990	-218.6603	155.8603
	Morus alba	29.00000	66.87502	.992	-158.2603	216.2603

*****The mean difference is significant at the 0.05 level.

According to Table 4, whether or not there is any difference in colonization of aquatic macrobenthic fauna according to leaf types was examined by ANOVA test and found to be different according to the results ($p<0.05$). This difference is due to artificial *Buxus* sp. the source is shown with multiple comparative test results.

DISCUSSION AND CONCLUSION

In the study, Chironomidae larvae and pupae, Oligochaeta Tricoptera, Ephemeroptera, Plecoptera, Odonata, Isopoda, Crustacea, Gastropoda, Hirudinae, Lumbricidae, Culicidae, Tabanidae and Tipulidae macroinvertebrate groups were detected. The organisms were diagnosed to the extent possible. There were Amphipoda, Hemiptera, Coleoptera, Bivalvia and Nematoda organisms in the Tunca River which were not included in this study by Çamur-Elipek (2006).

In this study carried out in the Tunca River, were found to be most intensively in artificial boxwood (3825 organisms), although the number of organisms was generally high all leaf packs. This shown that organismsuse leaf packaging for protection rather than feed. At least the organism was found in mulberry leaf (331 organisms) and we can link this to the gradual decrease of mulberry leaves due to rapid decomposition. Walnut (606 organisms), elm (765 organisms) and dry plane (1229 organisms) leaves were less dissociated. This indicated that leaf packs can be preferred in stream fauna studies.

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THE ROLE OF PRECISION FARMING IN PEST MANAGEMENT AND CROP YIELD

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ABSTRACT

Farmers encounter weeds, insects and diseases as major pests during crop cultivation. Although, there are different kind of pest management methods like cultural, mechanical, and biological, farmers continue to rely upon chemical method for its easy handling, greater efficacy and quick results. Nevertheless, the over application of pesticides leads to chemical residues in soil and crop. However, Precision Farming (PF) includes the best useful technologies to make farm management decisions precisely by obtaining the information about specific conditions. Precision farming uses Global Positioning System (GPS), Geographic Information System (GIS), and Remote Sensing (RS) technologies to help farmers for their management decisions. The main purposes of precision farming are to increase production efficiency and quality, efficient chemical use, energy conservation and protection of soil and water. The use of precision farming can provide so many benefits like the diagnose, the crop and field problems, improved equipment efficiency by using better scheduling and improved varietal choices like crop rotation. In addition, it can be used for crop conditions, and required inputs, to reduce the potential for leaching and runoff. Precision farming helps the farmers to use crop inputs such as tillage, fertilizers, pesticides and irrigation more effectively for greater crop yield and quality, without polluting the environment. However, the most important reasons for non-adoption of precision farming are the lack of finance, credit facilities and the lack of knowledge about precision farming technologies. Therefore, farmers should trained adequately to be able to monitor the dynamics of pests and to take right decision when it required.

Keywords: *PLF, Pest management, Image analysis*

INTRODUCTION

Pest Management Strategies

Agricultural pesticides are dangerous for surface and groundwater quality of the world. Precision farming technologies can help to protect water quality by reducing pesticides that farmers use. Precision farming technologies provides other options to the farmers to use different kind of pest management methods. For example, cultural methods, biological controls, and other alternatives to traditional method (chemical managements) can be used.

Pest Management

Pest management is to manage weeds, animals, insects and diseases that cause some damage on plants. Management of the pests with precision farming technologies based on the use of different tools and methods to reduce the effects of pests on plants in order to fulfil industry standard. Precision farming for pest management is used to support following aims: Increase crop quantity and quality, drop to negative effects of chemical pest controls on soil, water, air, plant and animal resources, and also for humans. During crop cultivation, the major pests are the insects, disease and weeds. Even though, there are different kinds of pest management methods like cultural, mechanical and biological, unfortunately the farmers still prefer to use chemical method for its high efficacy and rapid results. Nevertheless, over use of pesticides causes some problems in soil water and produce. In that point, precision farming plays an important role to use pesticide at micro-level.

Precision Farming

Precision farming is a novel management method using newer technologies and approaches to manage farming operations for the aim of increasing the yield and quality of plants (Biswas et al., 2008). Precision Farming (PF) uses the novel technologies to obtain the required information to decide farm management methods that fit the specific conditions for each product. Precision Farming includes Global positioning system, Geographic Information System, and Remote Sensing to record data for more informed management applications.

Example of Precision Farming Applications

The main purposes of precision farming are to increase production efficiency and quality, by using efficient chemical, in regarding to soil and ground water protection (Biswas et al., 2008). Most precision farming techniques have focused on the inputs to increase crop yields (Srinivasan, 2006). Over 70% of the research efforts were focussed on nutrients, seeding rates, irrigation etc. (Lambert and Lowenberg- DeBoer 2000). For example, the potential profitability of nitrogen application with precision farming method in corn was increased from 11 to 72 dollars per hectare according to the uniform method (Malzer et al., 1996). In another research performed by Koch et al. (2003), reported that the net return per hectare was increased from $12 to $35 using another precision farming method when compared to the traditional way. In another research, pesticide usage was reduced 18% while keeping yield and fibre quality at same level (Fridgen et al., 2003).

Precision Pest Management

Precision pest management is a novel management method which uses pesticides at micro-level by considering to the existing population of pests. It can also be defined as the technology for increasing crop yield while dropping environmental problems to the world (Khosla, 2001). Precision farming consist of Geographical information system, Remote sensing, Farmer and Global positioning system (Sharma et al., 2005).

Global Positioning System (GPS)

All the applications of precision agriculture needs positioning knowledge and it can be obtained from the GPS. GPS was first developed and used by the US army. The accuracy of GPS is less than 5m, (Sharma et al., 2005). For agricultural treatments, GPS was used for variable-rate-input applications and data collection during agricultural operations (Morgan and Ess, 1997). Lange (1996) reported that trees, buildings, steep and slopes can block a GPS signal.

Geographic Information System

A GIS consist of a computer, algorithms, and softwares designed to make easy the analysis of recorded data that can help to take important management decisions (Aronoff, 1989). A Geographic Information System for agriculture includes different information from different sources like GPS-based yield maps, pest or pathogen scouting data, aerial photography and satellite images. All data are shown on top of a field map, allowing layers to analyse the crop health and maturity. When the basic map is in place, the farmer can collect all data about different variables like weather, insect and weed problems to get correct information about the current status of the plants.

Remote Sensing

Remote Sensing is the method for the detection or identification of an object or landscape without contact sensor (Frazier et al., 1997). Agricultural applications of RS commonly include detection of electromagnetic energy like light and heat. Sensors can measure energy which cannot be detected by human vision. Digital images for remote sensing can be gathered from either an o satellite or from an airplane fly-over. RS was used for different kinds of applications such as detection of crop stress; diseases of plants, weeds, soils, detection for problematic situations like broken drainage tiles or crop injury during harvesting.

Farmer

Precision farming is information, technology and knowledge based practice. Thus, farm managers or stockmen have to be trained adequately so that they can use the technology to monitor their plants by taking right decision before too late (Biswas et al., 2008).

Conclusions

Precision farming technologies gives opportunity to the farmers to more effectively use plant inputs including tillage methods, fertilizers, pesticides management and irrigation schedule. Precision farming ensures to use all inputs more effectively and this provides greater crop yield and quality, without polluting to the world. Geographic Information System, Geographic positioning System, and Remote Sensing can help farmers to reduce risk. Farmers who use precision farming technologies have numerous types of data to take immediate management actions.

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THE SOLUBILITY OF TURKEY OAK (*QUERCUS CERRIS* L.) AND HUNGARIAN OAK (QUERCUS FRAINETTO TEN) IN COLD WATER

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ABSTRACT

Albania has an area of 28,748 km2 where forests cover occupies an area of 1.5 million hectares. Coppice forests dominated by oaks occupy an area of 623,799 hectares, where the occupied surface by oaks goes to 132,910 ha with a standing volume of 6.5 million cubic meters. Oaks forests are relatively young, and 82% of them have an age from 1-30 years. There are many types of oak in Albania, but the species that have the greater spread and the largest area are: Turkey oak (*Quercus cerris* L) and Hungarian oak (*Quercus frainetto* Ten). The study was carried out in six sites along longitudinal gradient. Three stem discs from the bole R1, middle R2 and top R3 of the stem were taken from each tree. Extraction conducted at 23 ± 2°C with constant mixing for 48 hours. The main components of wood parts, soluble in water, consist of carbohydrates, proteins, and inorganic salts. Average values for solubility of stem wood in cold water, in disks at the base of the trunk (R1), for the analyzed samples of Turkey oak trees (*Q. cerris*), from all stations resulted Mean of SCW % = 7.0 ± 1.83% and for the analyzed samples of Hungarian oak trees (*Q. frainetto*), resulted Mean of SCW= 8.96 ± 2.68%. Results for solubility in cold water SCW (%), in samples of Turkey oak, are grouped in narrower margins and lower limit values than in the samples of Hungarian oak. There was no correlation found between solubility in cold water SCW (%) and variables such as the cutting diameter, age and annual rings width TRW, for samples of both species. Solubility in cold water, SCW % the disks at the base were significantly higher than those in R2 disk and fallen further towards the top gasket.

Keywords: *Hungarian oak, Turkey oak, Wood solubility, Wood extractives, Carbohydrate*.

INTRODUCTION

Albania has an area of 28,748 km^2 where forest cover occupies an area of 1.5 million hectares. Coppice forests dominated by oaks occupy an area of 623,799 hectares. The genus *Quercus* is one of the most important clades of woody angiosperms in the Central and Western Europe, in terms of species diversity, ecological dominance, and economic value (NIXON KC 2006). The genus *Quercus* in Albania is mainly represented by Turkey oak (*Quercus cerris* L.), Italian oak (*Quercus frainetto* Ten.), pedunculate oak (*Quercus robur* L) and sessile oak (*Quercus petraea* Liebl.). *Q. cerris* and *Q. frainetto* are the most widespread species in Albania, covering 132910 ha (30.8 % of overall forest area) (ANFI 2004). In Albania, there are few

studies related to this species focused on silvicultural aspects (DIDA M 2003), but less investigated for the chemical composition and in particular for theirs extractives content.

Besides the main structural component of the cell wall (cellulose, hemicelluloses and lignin) in wood was found a large group of compounds (which in oak constitutes a substantial part) called extractives. Wood extractives are non-structural compounds of low molecular weight that can relatively easily be separated from the wood using different solvents. This group of substances, which have different physical properties, chemical and biological weapons presents numerous incentives to use forest products. They include primary metabolites, which usually are described as substances that are basic chemical units of plant living cells, such as nucleic acids, proteins and polysaccharides, and secondary metabolites which often are defined to be anything else that body produces but it is not a structural compound (polysaccharide or lignin). Wood extractives may darken the heartwood and give species such their characteristic colour. Extractives of most wood species range approximately from 2 to 10% of dry matter, in some cases (mostly in tropical species) this content can increase up to 20% or more of dry matter (Yang et al., 2003).

The term "extractive" commonly refers to the organic compounds only (Fengel & Wegener 1989), although it can also refer to inorganic materials. An important aspect of extractives is their solubility, and with regard to analysis it is useful to distinguish between substances that are soluble in water or in organic solvents.

The structure, composition and content of extractives vary greatly between species and even within a single tree, where they are generally more abundant in the bark, branches and knots. The distribution of extractives within the tree stem vary greatly, with changes radially and vertically on the one hand (DeBell et al., 1999; Gartner et al., 1999; Gierlinger and Wimmer, 2004), and different cell locations (Hillis, 1971) and positions within earlywood and latewood (Côté et al., 1966) on the other.

Factors of these varieties include: differences within a tree, tree age, growth rate, soil and climate in the region, and most importantly, genetic differences between different species. The extractives play a role in protecting the plant from disease and pests, as well as in growth regulation. Tannins that are known to form complexes with metal ions and be involved in oxidative reactions that can cause degradation of cellulosic materials, known as ink corrosion (Arpino et al., 1977; Rouchon- Quillet et al., 2004; Rouchon et al., 201), constitute 5-10% of oak wood. The sensory impact of oak tannins is widely assumed to have an impact on astringency and mouth feel of a wine. In oak wood, hydrolysable tannins consist mainly of gallotannins and ellagitannins where the galloyl- and hexahydroxydiphenic acid (HHDP) moieties, respectively, are esterified to a core molecule, generally glucose (Du Penhoat et al., 1991; Mämmelä et al., 2000; Scalbert et al., 1988).

The aim of this work was the gravimetric determination of hydrophilic extractives content into Turkey oak (*Quercus cerris* L.), as well as for Hungarian oak, since in now days, they are used in many wine barrels in Albania. In general, the wood does not contain many organic compounds soluble in water, although higher amounts of tannin and arabino-galactane are presented in some species. The main components of wood parts, soluble in cold water, consist of carbohydrates, proteins, tannins, gums, coloring matter and inorganic salts. Water moves inorganic compounds from wood with the extraction step.

MATERIAL AND METHODS

The study was focus in the species Turkey oak (*Quercus cerris* L.) and the Hungarian oak (*Quercus frainetto* Ten.), that together are the most common types of oak in Albania. They are found in the same habitat forming mixed forests with other species.

Research Locations

The study was carried out in six sites along longitudinal gradient (Tab 1). In the northeast part our sampling sites were Kukes (KU) and Diber (DI), in the northern central part of Albania we chose Ulza (UL) and Rreshen (RR), while from southern-central Albania we took samples from Graceni (EL) and Belsh (BE). All studied sites represent the natural habitats of mixed forest stands of Turkish oak (*Q. cerris* L.) and Hungarian oak (*Q.frainetto* Ten.) managed intensively as coppice for a long time. The core sampling at first five sites was carried out during period October - November 2012, while the samples from Belshi area were taken on February 2014. The study sites are located in different exposition and in a broad altitudinal range from 240 m until 692 m a.s.l (Table 1). Natural understory vegetation consists of common hornbeam (*Carpinus betulus* L.), common juniper (*Juniperus communis* L.), common hawthorn (*Crataegus monogyna* Jacq.) and herbaceous vegetation. These forest stands are grown on brown soils formed on limestone bedrock. Such soils have medium thickness with a sub-clay structure.

Table 1. Sampling sites location

Nr	Sampling sites	Sampling sites location		
		Longitude	**Latitude**	**Altitude (m. a.s.l)**
1	Kukes (KU)	20° 23' 35" E	42° 05' 01" N	365
2	Diber (DI)	20° 23' 46" E	41° 45' 07 " N	616
3	Rreshen (RR)	19° 53' 10" E	41° 48' 09 " N	240
4	Ulez (UL)	19° 54' 07" E	41° 39' 28 " N	241
5	Elbasan (EL)	19° 57' 51" E	41° 08' 58 " N	692
6	Belsh (BE)	19° 56' 47" E	40° 54' 09 " N	136

Samples preparation

Three stem discs from the bole R1, middle R2 and top R3 of the stem were taken from each tree. The stem discs were air dried and sanded until the tree-ring patterns were perfectly visible. Tree-ring width (TRW) was measured to the nearest 0.001 mm using a linear table, LINTAB (Frank Rinn S.A, Heidelberg, Germany) and the TSAP-Win program. For each sampled tree, height (H) was measured with Vertex, while diameter at breast height (DBH) with caliper.

Discs are cleaned from the bark and any possible knock. Using a chainsaw, do some cutting, collecting necessary sawdust. In each case, we took care to remain unchanged correct proportion of sapwood and heartwood. Sawdust is riddled collected and stored fraction that runs the sieve 40mesh (0.400mm) and remains 60mesh sieve (0.250mm). When was needed was grinding in a hand- driven grinding mill, (TAPPI T 257 2002).
For each sample previously determined the moisture according TAPPI T 264 (TAPPI T 264 2007).

Cold- water Solubility

We weighted about 2grams (with an accuracy of 0.0001g) of wood meal, we placed sample in a plastic bottle of 500 ml and added slowly 300 ml of distilled water, so that the wood meal got all wet, to avoid the tendency to float. Extraction conducted at 23 ± 2 ° C with constant

mixing for 48 hours. (Samples were placed in an electric shaker which makes continuous and uniform mixing) (TAPPI 207).

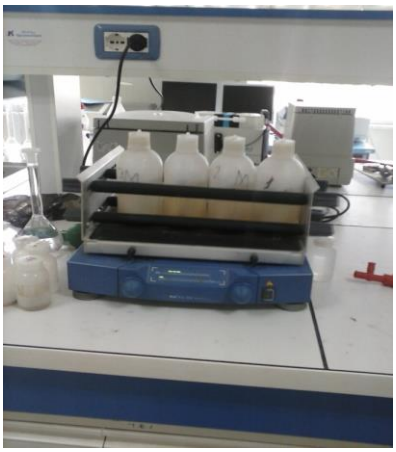


Figure1. The extraction proces

After extracting we filtered the extract, we used it in a vacuum filtration system, transferring the material to a glass medium tare filter which was previously dried to constant weight at 105 ± 3ºC. We rinsed samples with 200ml cold distilled water and filter together with the extracted wood meal and we dried it to constant weight at 105 ± 3ºC temperature. After cooling in desiccators, we weight the filter and residues accurately to 0.0001g.

For to calculation the wood solubility in cold water we used the following equation [1].
SCW% = [(C-D)*100]/C (1)

Where: SCW = Solubility %, C = first weight of oven dry tested specimen (grams). D= weight of oven dry specimen, after extraction (grams).

RESULTS AND DISCUSSION

The amount of extractives that is dissolved in cold water is also known as the solubility of wood in cold water SCW (%). For both types we have analyzed the solubility of extractives cold water mainly for basal discs (R1), and in fewer cases those of middle R2 and peak R3.

From the measurements carried out in the laboratory it was found that for Turkey oak (*Q. cerris*) the average value of solubility for the basin type disc was 7.03 ± 1.83%. The maximum solubility value was reached in the wood sample No. 8 at the Ulza station (10.1%), while the minimum solubility value was found in the tree no.4 of the Belsh station (3.9%).

For Hungarian oak type (*Q. frainetto*), the average solubility of SCW for the base disc was 8.96 ± 2.68%, while the maximum solubility in cold water was reached at tree No. 5 of Elbasan Station (12.00%), while the minimum value of cold water solubility was reached on the tree no.3 of the Belsh station (3.55%).

As seen in both types, the lowest values of cold water solubility have been reached on the samples taken at Belsh station.

Figure 2 presents the boxplots of cold water solubility values for the R1 disc of the two species studied. As can be seen from the graphical presentation below, the cold water solubility of the Hungarian oak species is higher (it has a higher average value), while the amplitude of the distribution of the solubility values of the samples around the average is greater in Turkey oak. This shows that Hungarian oak samples have a higher solubility in cold water according to stations than Turkey oak samples.

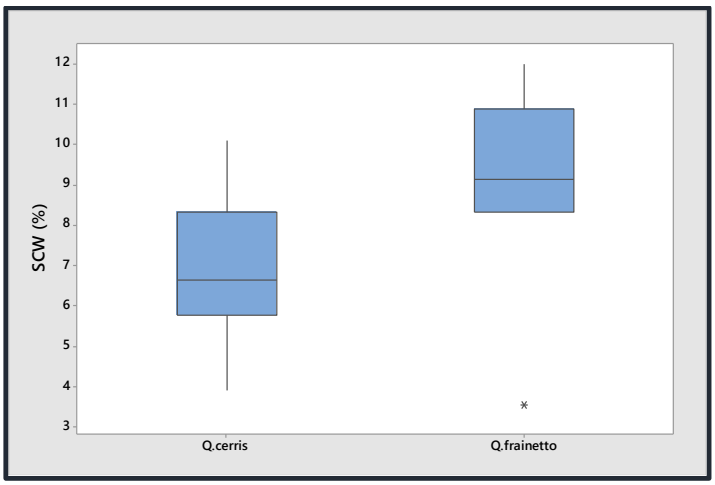


Figure 2. Box plots of SCW values (%) for samples of both species

From the correlation analysis between the cold water solubility SCW (%) and variables such as: sample diameters, age of trees and annual width of the two species, no significant correlation was observed ($p < 0.05$).

To identify the change in the solubility ratio of the extractives according to the samples taken according to the length of the stem for both species, we analyzed the solubility of the extractives in the cold water of the R1 disc (obtained at the base of the tree) and the R2 disc obtained in the middle. As can be seen from Figure 3 we saw that in the case of Tukey oak type the solubility of the extractives was higher in the base disc.

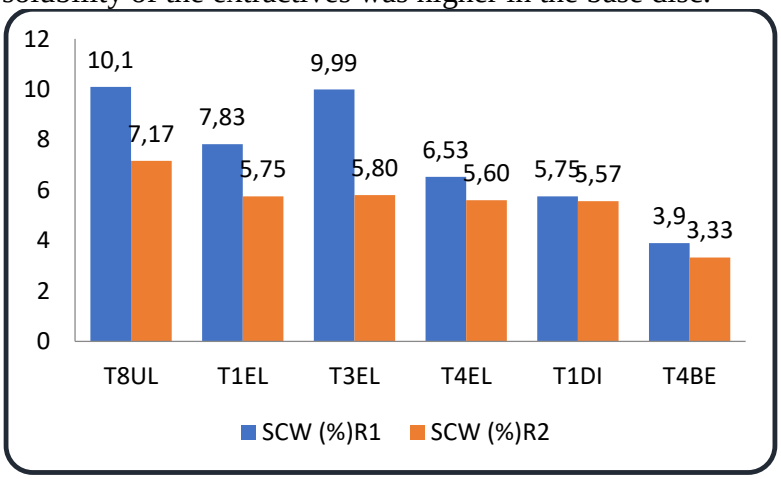


Figure 3. Solubility variability in cold water SCW (%) between base and middle disc (R1 and R2) for Turkey oak

The same conclusion was also reached for Hungarian oak samples. The sample solubility percentage was higher in the base samples and lower in the middle ones (Figure 4).

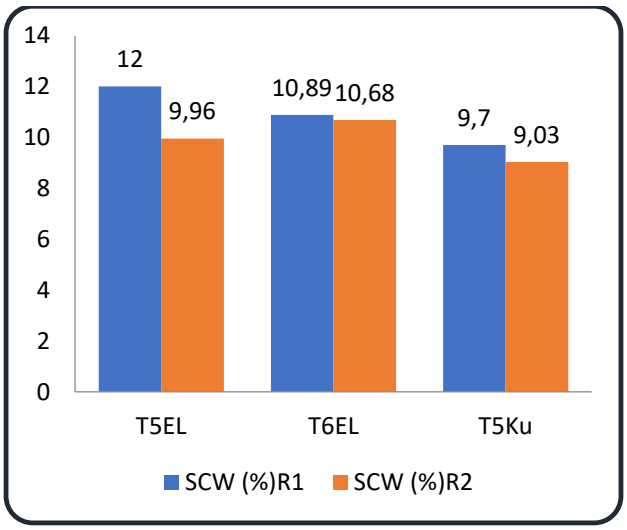


Figure 4. Solubility variability in cold water SCW (%) between the base and middle disc (R1 and R2) for the Hungarian oak type wood

Cold water solubility for the 3 discs (R1; R2; R3) was analyzed only on trees 4 (Turkey oak), 5 (Hungarian oak) and 6 (Hungarian oak) taken at Elbasan station. As can be seen from the figure 5, the solubility values in cold water SCW (%) fall from the base to the peak of the tree. From the graphic presentation it turns out that the solubility values of Turkey oak species in cold water are much smaller than for the Hungarian oak species.

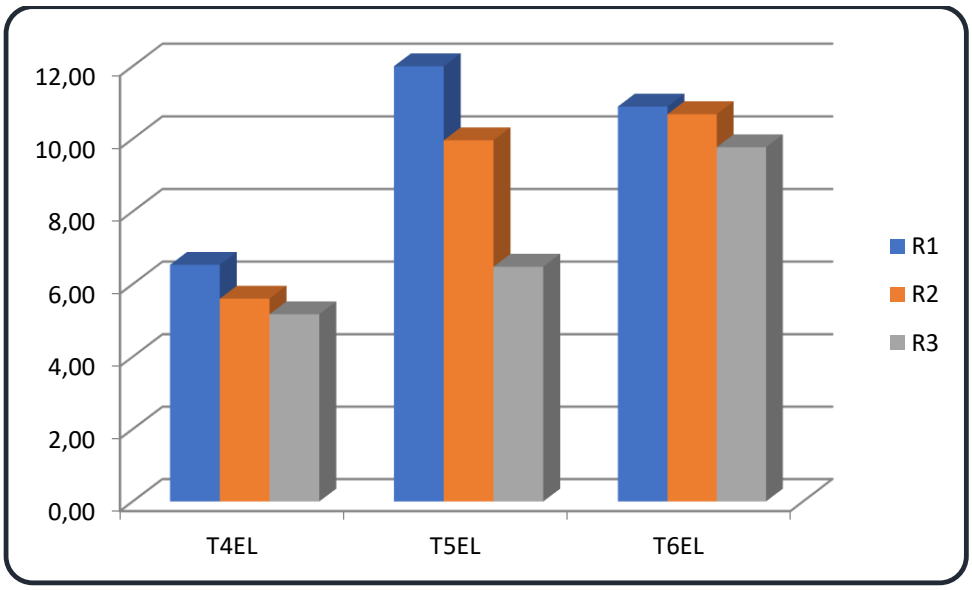


Figure 5. The variability of SCW (%) in relation to the vertical position of the wood sample

For wood samples T5R2EL, T6R1EL and T12R1RR, we determined the solubility in cold water SCW (%) for the whole section of the disk (without bark) and then for the heartwood (after we first split it from the sapwood) The analysis showed that the solubility values in cold water were much higher for the heartwood than for the whole disk (without bark) (Figure 6).

Hillis (1987) has shown that wood extractives tend to be higher in heartwood. The size of heartwood decreases starting from crown starting level. Consequently, the tree's peak contains less heartwood and less extractive than the rest of the trunk. However, the level of reduction in the heartwood area from the base to the top depends on the species.

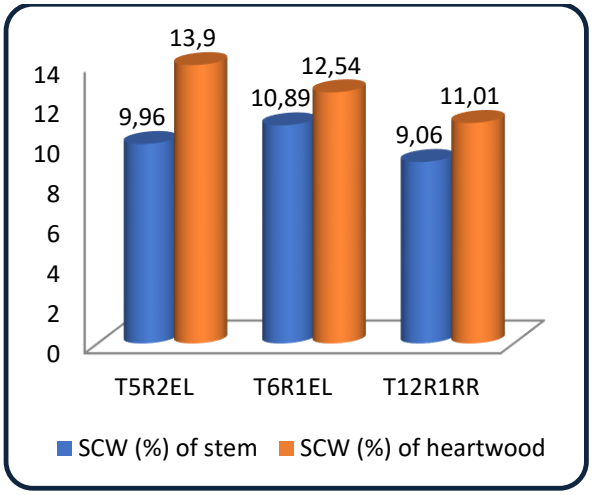


Figure 6. Solubility in cold water SCW (%) on the stem (sapwood and heartwood) and on the separated heartwood

For wood no. 6 at Elbasan Station (T6R2EL), the solubility in cold water SCW (%) was also determined for the bark. The results of cold water solubility for the bark were much higher than those in the whole transversal section of the tree trunk without bark (figure 7).

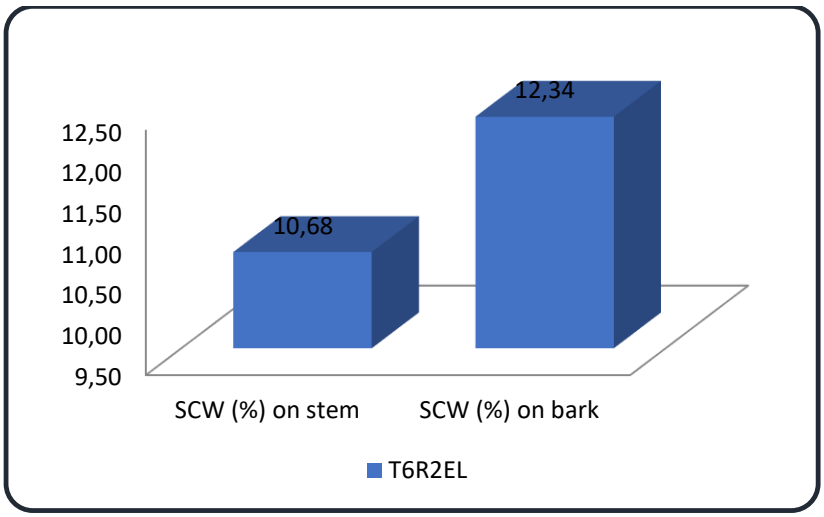
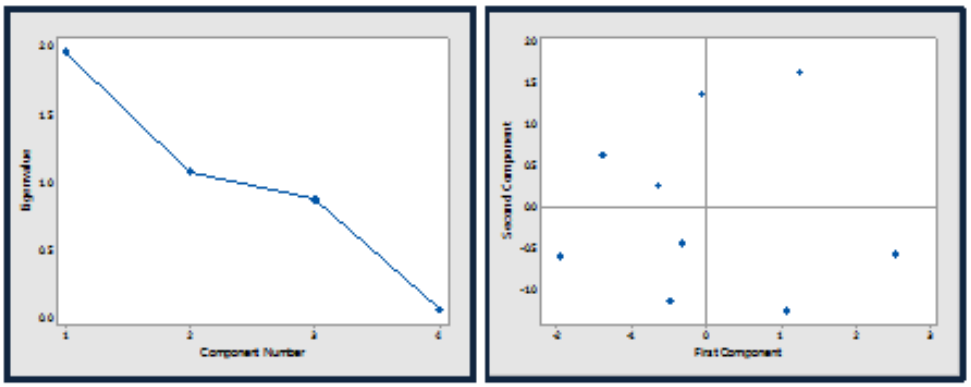


Figure 7. Solubility in cold water SCW (%) on the trunk and in the cortex

For sample basal disks (R1) multivariable statistical analysis were performed for the variables: tree age, cutting diameter, annual average ring width and cold water solubility SCW.

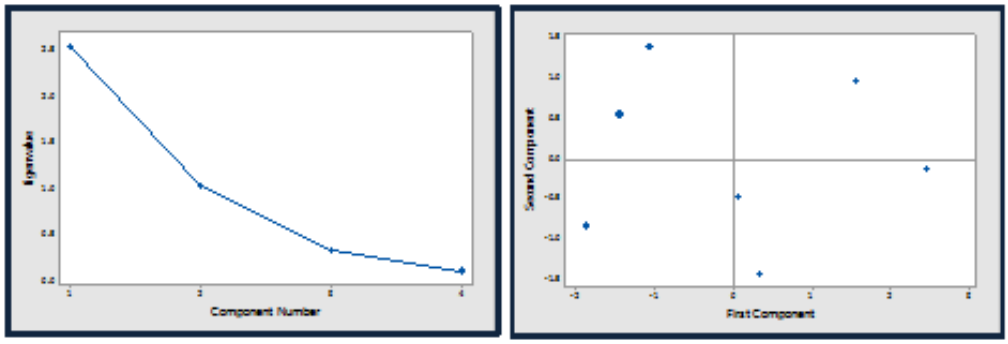
For Turkey oak (*Q. cerris*), the first principal component PC1 that causes variation is the diameter of the tree samples which causes about 49.1% of the total variance, while the second PC2 component that causes the variation is the average width of annual rings that causes a variance of 27.1%, and both together account for about 76.2% of all variance (figure 8).



a) b)

Figure 8. Analysis of the principal components PCA for variables: wood age, cutting diameter, TRW and solubility in cold water SCW (%) for Q.cerris on R1 basal disc samples in all areas studied; screen plot a) and score plot b).

For *Q. frainetto* the principal component analysis showed that the first principal component (PC1) that causes the largest variation is the sample diameter (with eigenvalue 2.5333) that causes 63.3% of the total variance, while the second principal component (PC2) that causes variation is the age of trees which contributes with 25.9% of total variance and both together account for about 89.3% of all variance (Figure 9).



a) b)

Figure 9. Analysis of principal components PCA for variables: wood age, cutting diameter, TRW and cold water solubility SCW (%) for Q. *frainetto* on R1 basal disc of samples in all areas studied. screen plot a) and score plot b).

To see how the samples are grouped according to their similarity to cold solubility, we used the Euclidean distance method. For Turkey oak specie, the samples were grouped in 2 clusters. In the first cluster the samples were grouped: T8R1UL (1), T11R1RR (2), T4R1BE (3), T3R1EL (5), T1R1DI (7), T4R1KU (9) and T7R1DI (8), while in the second cluster samples T1R1EL (4) and T4R1EL (6) of the Elbasan station (Figure 10).

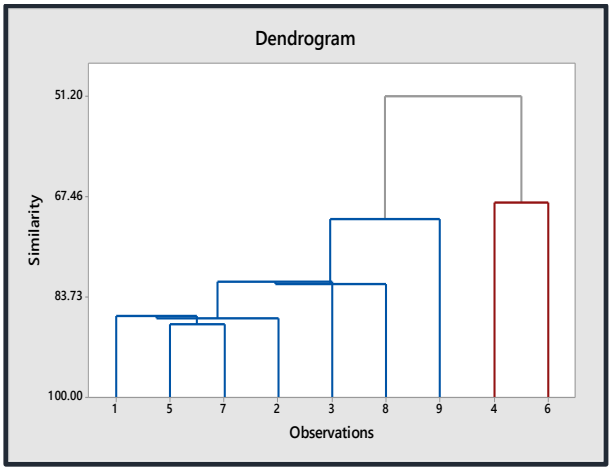


Figure 10. Dendrogram created by HFC for cold water solubility SCW (%) for Q.cerris R1 discs

Even in the case of the Hungarian oak specie it resulted that the samples according to the cold water solubility were grouped in 2 clusters. The T2R1UL (1), T12R1RR (2), T3R1BE (3), T5R1KU (6) and T3R1DI (7) samples are included in the first cluster, while the T5R1EL (4) and T6R1EL (5) in the second cluster of Elbasan Station (Figure 11).

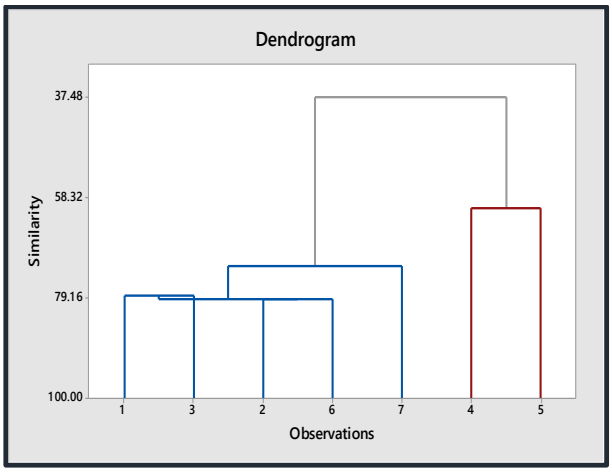


Figure 11. Dendrogram created by HFC for Cold Water Solubility CSW (%) for *Q. frainetto*

The separation of the samples from the Elbasan area can be influenced by two factors; first the samples ages from this area as a consequence and the diameters were larger as well as from other areas; secondly this area is managed not as coppice.

CONCLUSIONS

From the results of our work it is evident that these two species (Turkey oak and Hungarian oak) vary widely according to their content, in extractives components, extracted in cold water.

There was no correlation found between solubility in cold water SCW (%) and variables such as the cutting diameter, age and annual rings width TRW, for samples of both species. Hungarian oak samples have a higher solubility in cold water according to stations. The solubility values in cold water SCW (%) fall from the base to the peak of the tree. Results for the bark and the heartwood solubility were much higher than those in the entire cross section of the trunk wood without bark.

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ESTIMATION OF ANTI-HEMOLYTIC AND ANTI-INFLAMMATORY EFFECTS OF *DAUCUS GRACILIS* STEM. METHANOLIC EXTRACT FROM EASTERN ALGERIA

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ABSTRACT

This study aims to exploit the biological virtues of the local plants studied for the first time. The methanolic extract (ME) of *Daucus gracilis* was tested for possible antimicrobial, anti-hemolytic and anti-inflammatory properties. The ME was prepared by hydro-alcoholic maceration in a water/methanol mixture, and then the total polyphenols were assayed by the Folin-Ciocalteu method. The antimicrobial activity was carried out by the agar diffusion method using disks containing 70 mg/ml. The anti-hemolytic test is carried out by the stabilizing power of human red blood cell (HRBC) and that of the anti-inflammatory activity by the measurement of protein denaturation. The extract showed moderate activity on 28% of the tested strains; *B. cereus* (9.5 mm), *L. monocytogenes* (8mm), *P. Aeruginosa* (8mm) and *C. freundii* (7mm). The anti-hemolytic activity was dose-dependent and interesting but far from being compared to the Diclofenac Sodium ($P<0.05$). For the anti-inflammatory test, the Diclofenac (IC_{50}: 218.67 µg/ml) was slightly more active than the extract (IC_{50}: 554.07 µg/ml). In conclusion, we can say that our extract is moderately endowed with interesting biological activities that can be exploited later.

Keywords: *Daucus gracilis, Apiaceae, Antimicrobial, Anti-hemolytic, Anti-inflammatory*

INTRODUCTION

Historically, natural products have proved to be the most prolific and diverse source of antibiotics (Nguta et al., 2016). According to World Health Organization (WHO), about threequarters of the world population depends on traditional medicines (mainly herbs) for their healthcare (Shaikh et al., 2016). After great advances in modern medicine, problems of resistance, drug insecurity and side effects have appeared in recent years. Among these problems, the antibiotic resistance which has become a serious and widespread problem in developing countries, both in hospitals and the community, causing high mortality each year (Wikaningtyas and Sukandar, 2016), the problems of inflammation and those related to the fluidity of blood. *D.gracilis* is an Apiaceous species which has not been studied before. It is an annual plant with stems up to 40 cm, long pedunculate umbels, yellowish fruit, and 3.5 x 2 mm bristling with whitish short hairs. (Quezel et Santa, 1963; Sáenz Laín, 1981). It belongs to the class of Magnoliopsida, order of Apiales. In order to limit these health problems, we studied the antimicrobial, anti-inflammatory and anti-hemolytic properties of the methanolic extract of this species.

MATERIALS AND METHODS

Plant material

The plant was harvested at Djebel Felfla (Skikda) at an altitude of 550 m. Then it was freed from impurities and dried in the shade at an ambient temperature. The species was identified

by Professor Laouer Hocine, Professor at the laboratory of valorization of natural biological substances.

Methanolic extract

The aerial parts are crushed and reduced to powder, and then 20 g are macerated in 100 ml of a water-alcoholic mixture of methanol (1-10 V/V) at ambient temperature for 72 hours. After filtration with filter paper several times, the methanol is removed from the filtrate by evaporation under reduced pressure in a rotary evaporator (BÜCHI), to obtain the methanolic extract (**ME**).

Total phenolic contents (TPC)

The TPC in methanolic extracts was determined by spectrometry using ‘‘Folin-Ciocalteu’’ reagent assay (Li et al., 2007). A volume of 1 ml of Folin-Ciocalteu reagent diluted 10 times with water was added to 200 µl of ME with suitable dilutions. After 4 min, 800 µl of a sodium carbonate solution (75 mg/ml) were added to the reaction medium. After 2 h of incubation at room temperature, the absorbance was measured at 765 nm using a UV-vis spectrophotometer.

Anti-Hemolytic Activity

The principle involved here is stabilization of human red blood cell (HRBC) membrane by hypotonicity induced membrane lysis. The assay mixture contains 1ml phosphate buffer [pH 7.4, 0.15 M], 2 ml hypo saline [0.36 %], 0.5 ml HRBC suspension (10 % v/v) with 0.5 ml of plant extracts and standard drug Diclofenac Sodium of various concentrations (50, 100, 250, 500, 1000, 2000 µg/ml) and control (distilled water instead of hypo saline to produce 100 % hemolysis) were incubated at 37oC for 30 min and centrifuged respectively. The hemoglobin content in the suspension was estimated using spectrophotometer at 560 nm (Chippada et al., 2011). The percentage of hemolysis membrane can be calculated as follows:

% hemolysis = ($OD_{\text{Test sample}}$ / OD_{Control}) X 100

Anti-inflammatory activity in vitro

It was evaluated by the protein denaturation method (Alhakmani et al., 2013). Diclofenac Sodium, a potent anti-inflammatory drug was used as a control. The reaction mixture consists of 2 ml of the various dilutions of the ME or control and 2.8 ml of phosphate buffer saline (pH 6.4) mixed with 2 ml of egg albumen (fresh), then the mixture is incubated at 27 ° C for 15 minutes. The denaturation is induced in a water bath at 70 ° C for 10 minutes. After cooling, the absorbance was measured at 660 nm. The inhibition percentage of protein denaturation is calculated as follows:

% Inhibition = (A_{test}-$A_{control}$) x 100 / $A_{control}$.

Statistical analysis

The results of the various tests are analyzed by the univariate ANOVA followed by the Dunnett and Tukey test for multiple comparisons and determination of significance rates. The statistics are made by Graphpad Prism 5.

RESULTS AND DISCUSSION

TPC

The ME containing the total biomolecules has a brown color; it is soluble in Dimethyl sulfoxide (DMSO) and in methanol. The yield of extraction is 3.74%.

Gallic acid was used as a standard for the calibration curve (0-160 µg/ml) (Figure 1) and was expressed as micrograms of Gallic acid equivalents per milligram of extract (µg GAE/mg ME).

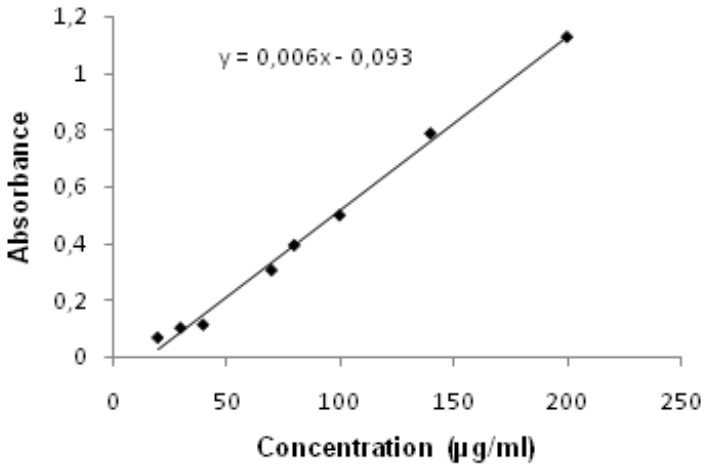

Figure 1. Calibration curve of Gallic acid

The ME of *D. gracilis* is rich in polyphenols 149.05 ± 3.33 µg EAG/mg. This content is significant compared to the majority of species of the same genera. These quantities are close to that found by (Bendiabdellah et al., 2013) in the extract of *D. crinitus*; 130.19 µgmg. Previous studies have shown that the amount of polyphenols in plants depends on the biological (genotype, organs and ontogenesis), edaphics, and on environmental conditions (temperature, salinity, water stress and light intensity) (Ksouri et al., 2008).

Anti-hemolytic activity

This approach allows us to know the positive or negative effect of our extracts on the anti-free radical system of red cells. The anti-hemolytic activity of the extract is tested on human erythrocytes and then compared with that of Diclofenac Sodium, which is known to be a good anti-hemolytic agent at non-toxic concentrations. The ME has an important membrane-stabilizing activity close to that of Diclofenac Sodium and its efficacy is dose-dependent (Figure 2), it showed maximum inhibition of 78.7% at the concentration of 1000 µg / ml and Diclofenac showed the maximum inhibition of 98.66% at the same concentration. Erythrocytes are considered the major target for free radicals due to the presence of both high membrane concentrations of polyunsaturated fatty acids and the transport of oxygen associated with hemoglobin molecules which are potent promoters of reactive species to oxygen. It appears that the high total phenol content in the extract results in potent anti-hemolytic activity.

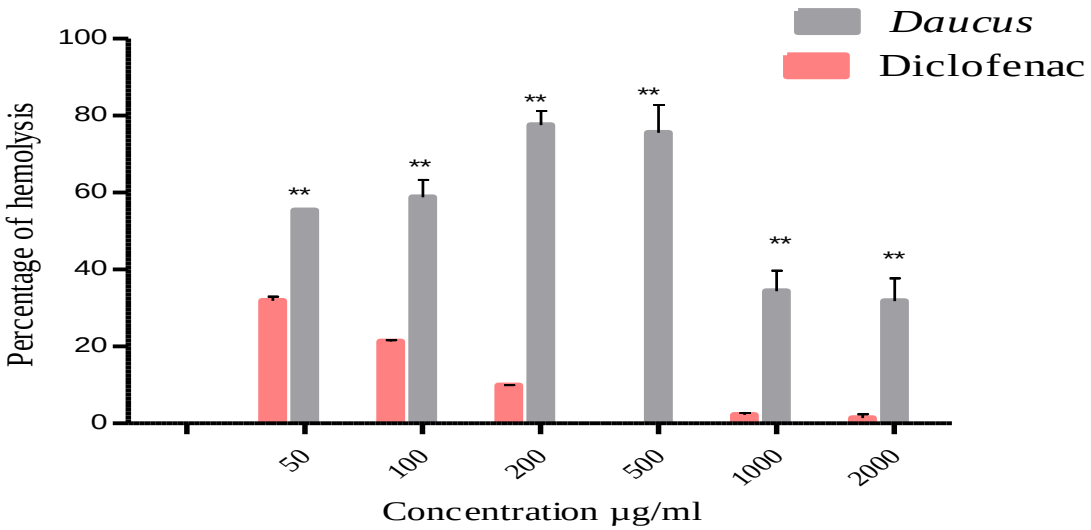

Figure 2. Anti-hemolytic activity of the ME (n = 3 ± SEM). ***: p ≤ 0.01

Anti-inflammatory activity

In vitro anti-inflammatory activity may be related to denaturation of proteins. The percentage of denaturation inhibition is compared with that of Diclofenac (Figure 3). The inhibition of denaturation is directly related to the concentration of the extract. Diclofenac is slightly more active with an IC_{50} of 218.67 µg / ml followed by *Daucus* with an IC_{50} of 554.07 µg / ml. Comparison of the extracts with Diclofenac shows that the difference between the activities is not significant at 95%.

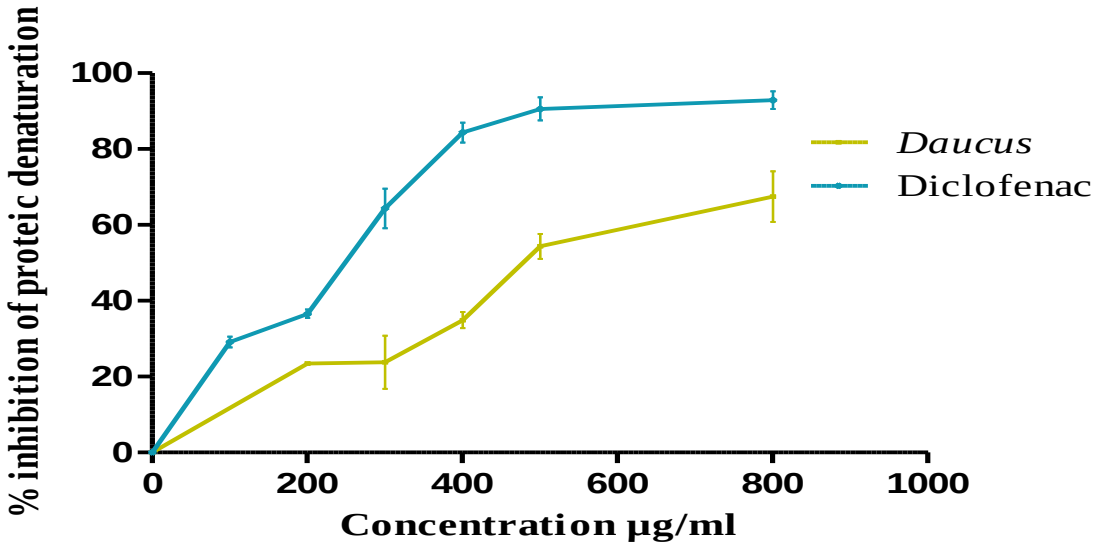

Figure 3. Anti-inflammatory activity of the ME (n = 3) ± SEM

Extracts from other *Daucus* species have also been shown to be anti-inflammatory; polyphenols appear to be responsible for this activity; By studying the anti-inflammatory effect of propolis, Valenzuela-Barra et al. (2015) attributed this effect to the presence of polyphenols in considerable percentages. Bacterial infections cause an increase in the number of neutrophils, which produces an oxidative effect at the site of the microbial invasion. The erythrocytes were used as a model for studying drug-cellular membrane interactions. Anti-inflammatory effects stabilize erythrocytes against hemolytic stress induced by hypo-tonicity which prevents the release of hemoglobin. This membrane stabilization activity of erythrocytes is used in vitro to evaluate the anti-inflammatory activity of various compounds. The erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract can also stabilize the lysosomal membrane which is important in limiting the inflammatory response by preventing the release of the lysosomal constituents of activated neutrophils such as bacterial proteases and Which also cause inflammation of tissues and lesions (Kumar et al., 2013).

CONCLUSION

The results of this study can be considered the first information on anti-hemolytic and anti-inflammatory properties of the methanolic extract of *D. gracilis*. This extract had a remarkable anti-hemolytic and anti-inflammatory activities which can be exploited after clinical confirmation and pharmacological standardization. Likewise, further studies are being made to test this extract in vivo to evaluate its cyto-toxicity.

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PHYSIOLOGICAL STUDY OF CULTIVARS CARRYING THE 1BL.1RS WHEAT-RYE CHROMOSOMAL TRANSLOCATION IN BREAD WHEAT

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ABSTRACT

In order to study the effect of the 1BL.1RS wheat-rye chromosome translocation, on yield and other agronomic traits, three Hellenic spring wheat varieties with (cvs. Acheron, Elissavet and Orfeas) and six cultivars without the translocation (cvrs. Apollonia, Acheloos, Vergina, Doirani, Nestos and Strymonas) were used. The Russian cultivar KVZ/Cgn, one of the donators of the aforementioned translocation to modern wheat cultivars, was used as check. The complete randomized block design was used with four replications and the experiments were established in the main farm of the Western Macedonia University of Applied Sciences in Florina for two successive years. The following physiological traits were measured: total chlorophyll content, chlorophyll fluorescence, CO_2 assimilation rate, stomatal conductance, intercellular CO_2 concentration and transpiration rate. Significant differences were recorded in yield and in three traits, i.e. total chlorophyll content, CO_2 assimilation rate and transpiration rate. Regarding yield, despite the existing variability between cultivars with and without the translocation, no effect of the translocation was noticed. The two cultivars with (Elissavet and Acheron) performed equally with three of the top yielding varieties without translocation (Doerani, Apollonia and Achellos). Also, the same three wheat cultivars without the translocation were ranked in top three places according to the total chlorophyll content but they did not differentiated from cv. Elissavet which is one of the translocation carriers. In CO_2 assimilation rate, no significant effect of the translocation was observed and a similar situation was noticed in the transpiration rate. In order to find out the effect of the physiological traits on yield potential, the above results were compared to the yield performance of the corresponding cultivars. This comparison led to the conclusion that the 1BL.1RS chromosome translocation does not give any significant advantage regarding the physiological traits studied. Further research is needed to confirm the results of the present study.

Keywords: *Yield potential, Drought resistance, Chlorophyll, Assimilation rate.*

INTRODUCTION

One of the main problems in releasing new cultivars is that this new germplasm will probably be grown on marginal environments due to the irrational waste of territorial resources

noticed in previous years. The drought conditions prevailing in spring especially in the last decades, is the main obstacle of agricultural production in the southern regions of Europe (Yau and Saxena, 1997) complicating the problem of food sufficiency. Thus, one of the most decisive factors in all breeding programs is the identification and integration of genes into cultivated varieties that confer resistance or tolerance to drought (Blum, 1988) and avoid mistakes of the past (Acevedo and Fereres, 1994). This led breeders and especially those working on wheat, to look for new gene pools to face the problem (Fehr, 1987). According to various reports bread wheat *(Triticum aestivum* L em Thell) cultivars carrying the 1BL.1RS wheat-rye chromosome translocation are characterized among other by high yield potential (Kim et al., 2004; Xynias et al., 2007) and resistance to drought (Hoffmann, 2008). The 1BL.RS translocation is originated from cv. Kavkaz/Cgn (Schlegel and Meinel, 1994) and it's unique traits are attributed to genes located on the short arm of the first chromosome of rye (Schlegel and Meinel, 1994; Xynias et al., 2007). According to Acevedo and Fereres (1994) one crucial mistake in developing new germplasm was the non-sufficient exploitation of certain physiological traits, rendering cultivated plants more competitive to weeds. Common physiological traits used to recognize increased stress tolerance are was exchange parameters such as assimilation rate (A), stomatal conductance (g_s), transpiration rate (E) and intercellular CO_2 concentration (c_i), chlorophyll content and chlorophyll fluorescence (Hura et al., 2007; Živčák et al., 2008). The above physiological traits have exhibited a good correlation with tolerance to stresses and yield parameters with a high heritability and repeatability (Fotovat et al., 2007; Sayar et al., 2008).

In a preliminary report Pankou et al. (2017) stated that no evidence was obtained confirming the positive effect of the translocation on relevant physiological traits. They suggested that research had to be continued and this was the aim of the present study: was to investigate the effect of the 1BL.1RS wheat-rye chromosomal translocation on certain physiological traits and elucidate how they affect yield and drought resistance.

MATERIALS AND METHODS

For the purpose of the study, nine Hellenic bread wheat cultivars (eg. Acheron, Elissavet, Orfeas, Apolonia, Acheloos, Vergina, Doirani, Nestos and Strymonas) that were developed at the Cereal Institute of Thessaloniki (Anonymous 1985) were used. The Russian cultivar Kavkaz/Cgn, one of the donors of the 1BL.1RS wheat-rye chromosome translocation (Xynias et al., 2006; Weng, 2007) was used as check. Three of the Hellenic cultivars were found to carry the 1BL.1RS wheat-rye chromosome translocation (cvs. Acheron, Elissavet and Orfeas) whereas the other six cultivars, were lacking the specific translocation (Xynias et al., 2006; Peros et al., 2015).

Method

Experimenting lasted for two successive years. The examined cultivars and the control were sown in early November 2015 and at the same time in November 2016, in a field of the University of Applied Sciences of W. Macedonia Farm in Florina Greece (40°46′ N, 21°22′E, 707 m asl), in a sandy loam soil with pH 6.3, organic matter content14.0 g/kg, N-NO_3 100 mg/kg, P (Olsen) 50.3 mg/kg and K 308 mg/kg and water holding capacity 21.8% (0 to 30 cm depth). Seedbed preparation included mouldboard plough, disc harrow and cultivator. Nitrogen and P_20_5 at 80 and 40 kg ha^{-1}, respectively, were incorporated into the soil as diammonium phosphate (20-10-0) before sowing. The crop was kept free of weeds by hand hoeing when necessary. The Randomized Complete Blocks (RCB) experimental design (fixed model) was applied, with four replications (Steel and Torrie, 1960). The plots were consisted of five rows (plot area $3m^2$) of which the three inner were threshed (harvest area $1.8m^2$).

The mechanically harvested grain was weighed and a sample of grain was dried in an oven at 105° C for 24 h to determine grain moisture content. Grain yield was referred to 12% grain moisture. Chlorophyll content readings were taken with a hand-held dual-wavelength meter (SPAD 502, Chlorophyll meter, Minolta Camera Co., Ltd., Japan). For each plot six fully expanded flag leaves per plot were used when the plants were at physiological maturity with six measurements per leaf, a total of 36 readings per plot. A portable photosynthesis system (LI-6400 XT, Li-Cor, Lincoln, Nebraska, USA) equipped with a 2X6 (12 cm^2) open top narrow leaf chamber was used for determinations of CO_2 assimilation rate (A), transpiration rate (E), stomatal conductance to water vapour (g_s), and intercellular CO_2 concentration (C_i) during grain filling period. Leaf gas exchange was measured on the fully expanded flag leaf during the grain-filling period on six plants from each plot from 09:00-12:00 in the morning to avoid high vapor-pressure deficit and photoinhibition at midday. The quantum photosynthetic yield of photosystem (PS) II or Y was measured with the portable OS5p Chlorophyll Fluorometer (Opti-Sciences Inc. Hudson, NH, USA).

The means were compared according to the L.S.D. method. The data obtained were analyzed statistically with Mstat-C (Freed and Eisensmith, 1986).

RESULTS AND DISCUSSION

Significant differences were recorded between the examined cultivars in yield (significant differences at p = 1%, Table 1). Regarding of the physiological traits studied, revealed significant differences in total chlorophyll content, CO_2 assimilation rate and transpiration rate. No differences were observed in the other physiological traits between the examined cultivars regardless of the presence of the translocation (Table 1).

Table 1. Analysis of variance of bread wheat with and without the 1BL.1RS wheat-rye chromosomal translocation regarding yield and six physiological traits.

Source	df	Yield	Total chlorophyll content	Chlorophyll fluorescence	CO_2 assimilation rate	Stomatal conductance	Intercellular CO_2 concentration	Transpiration rate
Years (Y)	1	ns	**	**	ns	ns	**	ns
Factor (A)	9	**	**	ns	**	ns	ns	**
(Y) x (A)	9	**	* *	*	**	ns	ns	**
Error	54							
CV		18.57	8.13	16.38	13.22	40.97	6.08	13.69

The cultivars were classified according to their yield performance into four groups (Table 2). The first one included the top yielding varieties, (marked with A) cultivars Doerani, Apollonia, Elissavet*, Acheloos and Acheron* (cultivars marked with a * are those carrying the 1BK.1RS translocation). The second group included cultivars Apollonia, Elissavet*, Acheloos, Acheron* and KVZ/Cgn* (marked with B). The third group was consisted on the cultivars Acheloos, Acheron*, KVZ/Cgn* and Strymonas (marked with C). Finally, the fourth group marked with D, was consisted of the lower yielding cultivars Nestos, Orfeas* and Yecora. It is obvious from the above results that there was no particular yield differentiation of varieties studied. For this, it could be stated that no particular effect of the translocation exists and this is in disagreement with the conclusion of Kim et al. (2004), who reported a positive effect of the translocation on

yield performance. Sufficient yield can be produced by elite cultivars despite the effect of the translocation. The insufficient yield performance of cv. Yecora could be attributed to the very low temperatures prevailing in Florina during winter. However, there is no excuse for the inferior performance of cvrs. Orfeas* and Nestos, which are supposed to be low-temperature-resistant.

Table 2. Ranking of the bread wheat cultivars according to chlorophyll content.

Cultivar	Yield	Total chlorophyll content	CO_2 assimilation rate	Transpiration rate
Acherontas*	ABC	BC	AB	AB
Elissavet*	AB	AB	DE	CD
KVZ/Cgn*	BC	CD	CDE	AB
Orfeas*	D	CD	ABC	BCD
Apollonia	AB	AB	A	A
Acheloos	ABC	A	ABC	ABC
Yecora	D	D	E	D
Doerani	A	A	ABC	AB
Nestos	D	BC	BCDE	ABCD
Strymonas	C	AB	BCD	ABC
LSD	102.1	3.327	1.968	0.6056

*Cultivars carrying the 1BL.1RS wheat-rye chromosomal translocation

The same view existed and in the ranking of the physiological traits (Table 2). The cultivars were classified in four groups in total chlorophyll content and transpiration rate and in five groups in CO_2 assimilation rate. For total chlorophyll content, cultivars Acheloos and Doerani were ranked in the first two places, they differed from three of the cultivars with the translocation (Acheron*, KVZ/Cgn*, and Orfeas*) but did not differ from the fourth cultivar with the translocation (Elissavet*). A similar view was found in assimilation and transpiration rates, where Apollonia was ranked first. The physiological traits studied in the present work (assimilation rate, stomatal conductance, transpiration rate and intercellular CO_2 concentration, chlorophyll content and chlorophyll fluorescence) are commonly used to recognize increased stress tolerance (Hura et al., 2007; Živčák et al., 2008), correlated with yield parameters (Fotovat et al., 2007; Sayar et al., 2008). However, yield performance was not affected by the aforementioned physiological traits and by the presence of the translocation. Only one cultivar with the translocation performed well (cvr Elissavet*). This questionable effect of the translocation was also reported in other studies. Lazaridou et al. (2017) suggested that any positive effect of the translocation on the host cultivar is determined by the genetic background of the host cultivar itself. The best yielding cultivars did not carry the translocation and

exhibited the highest values in all physiological traits studied. The aforementioned results support the view of Acevedo and Fereres (1994) who stated that exploitation of certain physiological traits could be beneficial in producing high yield varieties. Cvs. Apollonia and Doerani which were ranked in first places not only in yield but also in all physiological traits studied are good examples of the former statement.

CONCLUSIONS

The comparison performed in the present study, supported previous statements that the 1BL.1RS chromosome translocation does not give any significant advantage regarding the physiological traits studied (assimilation rate, stomatal conductance, transpiration rate and intercellular CO_2 concentration, chlorophyll content and chlorophyll fluorescence). Its effect is probably influenced by the genetic background of the host cultivar. However, further research is needed to confirm the results of the present study.

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DRYING OF KIWI FRUIT SLICES BY APPLYING VACUUM IMPREGRATION

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ABSTRACT

Kiwifruit is native to north-central and eastern China and has been cultivated in our country for about 30 years. Kiwifruit is quite rich in vitamins, minerals, antioxidants, phytochemicals and fiber content. The kiwi plantation area in our country is showing a rapid increase and parallel to this, production is increasing significantly. Therefore, kiwifruit produced in our country is becoming an important industrial product with minimal loss of quality. In this project, Actinidia deliciosa cv. Hayward was used as material. Kiwifruits were sliced to a thickness of 9 mm and dried in a hot air dryer at 65 ° C. Vacuum impregnation and osmotic dehydration technique which saves energy in drying technology were used and also 30%, 40% and 60% brix sucrose concentrations used as pre-treatments. The drying process continued until the water activities (aw) of the products were 0.60-0.65. As a control, the fruits were dried without any treatment in the hot air dryer. In this study, effect of vacuum impregnation method of kiwifruit on osmotic dehydration mechanism and the effects of different osmotic solution concentrations on the dried quality qualities of this mechanism have been examined. For this purpose, total dry matter, aw (water activity), L*a*b*- chroma- hue color values and sensory evaluation were performed and the best solution concentration was recommended according to these analyses. 30% brix concentration sensually recommended since samples with a solution concentration of 30 brix was best preserved in terms of colour that provides the best sour sweet balance.

Keywords: *Actinidia deliciosa, Dried fruit, Quality Analyses, Vacuum İmpegration*

INTRODUCTION

Kiwifruit is a kind of fruit that is attracted by consumers due to its taste and high nutritional qualities. In parallel with the increase in the world, the production of kiwi in Turkey is increasing every year. It is reported that the production of kiwifruit, which was 1,400 tons in 2000, rose to 43,000 tons nowadays (TUIK, 2017).

Due to the short shelf life of the kiwifruit it is necessary to increase the product range by using different processing methods to increase the commercial life. For this purpose, one of the methods applied to maintain the quality characteristics of the fruits for a long time is dried fruit. The osmotic dehydration technique, which is widely used in the world together with the drying method, has gained an increasing interest in recent years due to the advantages such as the reduction of the energy input required by the drying technology and the desired product quality. Osmotic dehydration is not a drying method alone and is considered as a pre-treatment before the actual drying process. Osmotic dehydration can be used alone, or it can be used with techniques such as vacuum impregnation and air drying.

The vacuum impregnation prevents oxidative browning and fading of the fruit pieces by expelling the gas in the pores. Several studies have been carried out on the drying of kiwi slices

(Tylewicz et al., 2011; Nowacka et al., 2014, Allaeddini et al., 2004), and it has been reported that the kiwi can be successfully used in industrial applications. For kiwi and other sliced fruits, it has also been reported that osmotic pre-treatment may be recommended to prevent loss of aroma. It has been recommended to obtain better quality dried products, which would be beneficial to investigate the drying of the kiwifruit by different drying methods (Orikasa et al., 2014).

In this study, the effects of the quality of the kiwifruit slices of the hot air-dried dryer were investigated. Vacuum impregnation-osmotic dehydration was used as pre-treatment to determine the best application by analyzing the dried kiwi quality properties (moisture content, water activity, and surface colour of kiwi samples). In our country, dried and processed kiwi sliced is very limited. This work will create an opportunity area for the production of dried kiwi. At the same time, this new product will increase consumers' chances of being easy to eat, rich in nutritional value, and superior in terms of sensory properties such as colour, appearance, taste, smell and aroma.

MATERIAL AND METHODS

Plant Materials

A. deliciosa cv. Hayward was used as a material. Fruits were obtained from the kiwi garden of Yalova Atatürk Horticultural Central Research Institute (Viticulture Department).

Methods

1. Harvest and storage fruits: Kiwifruits were harvested when reached 7 Brix. Fruits were kept in the cold weather storage area of the post-harvest section and when reached to Brix value of 11, kiwi-fruits were processed.

2. Preparation of kiwi slices:

The kiwi fruit was peeled and then sliced circularly with a slitter thickness of 9 mm.

3. Preparation of osmotic solution and pre-drying in osmotic solution:

As a pre-treatment in the osmotic drying process, a "vacuum impregnation" process was carried out using three osmotic solutions. For that, sucrose solutions were prepared with water-soluble dry matter content (Brix) of 30%, 40% and 60%.

Brix values of the sucrose solutions and of fruits (fresh and dried) were determined with an Atago brand refractometer (PAL-3 model, Tokyo, Japan). The saccharose solution prepared for the osmotic dehydration is used at a temperature of 30 °C. The kiwi slices and the osmotic dehydration solution in a ratio of 1/4, were placed in a container (Fig. 1) in which a vacuum pressure was applied. The vaccum pressure was maintained at 78 kPa for 30 min (Tylewicz et al., 2011; Nowacka et al., 2014, Allaeddini and Emam-Djomeh, 2004; Karacaoğlu ve ark, 2016).

4. Drying of the fruit: Kiwi samples were dried in a drying oven at a temperature of 65 ° C and a relative humidity of 1.0% at relative humidity 10%.

5. Tests and analyses made: Water activity analysis was performed with a Novasina water activity device and sorbent isotherms were determined (when the aw value is between 0 and 1, the measurement accuracy is ± 0.001).

Colour analysis. Colour analysis on fresh and dried fruits L * a * b *, chroma and hue values were determined with the Konica-Minolta CR 400 instrument. H = tan-1 (b / a) C = (a2 + b2) ½. (L is a brightness value, 0 is black, 100 is white, a is red, -a is green, b is yellow and -b is blue)

Total Dry Matter Detection. Fresh, osmotic and dry kiwi samples (between 2-6 g) were dried to constant weight under vacuum at 65 °C 100 mmHg (13,3 kPa) and the results were calculated as % (AOAC, 1990). Amount of water soluble in dry matter; The amount of water-soluble dry matter measured at room temperature (20 °C) with the "Atago" brand hand refractometer (Atago S-28, Japan), with 3 replicate fruit juice removed from fresh kiwi samples. The results are expressed in %.

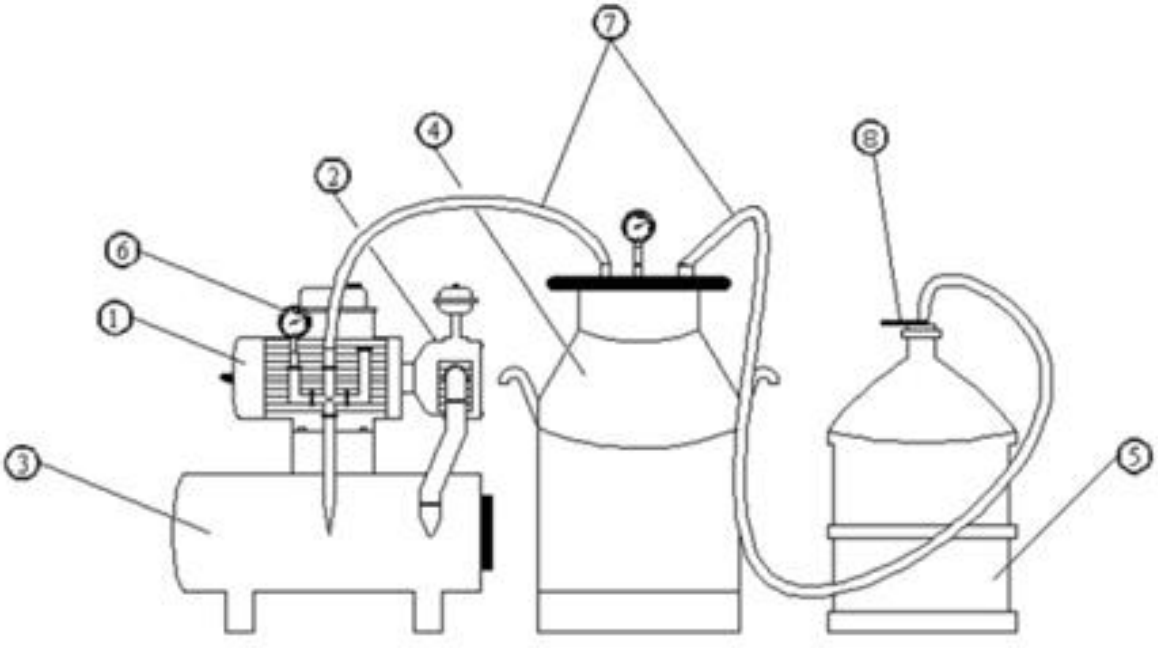

Figure 1. General view of the vacuum impregnation system (1.Single-phase electric motor 2. Vacuum pump 3. Vacuum tank 4. Sample area 5. Solution area 6. Manometer 7. Transparent plastic hose 8. Globe valve

RESULTS AND DISCUSSION

Vacuum impregnation was applied to the solutions prepared at different concentrations in the study and the results of the kiwifruit analyses are given below.

Flesh colour: In colour analysis of kiwi slices fresh and dried in three different osmotic solutions, L * value changed between 45.13-37.37, a colour value was between -6.76 and 0.6, b * colour value was 17.35-22.91, Chroma 18.72-22.91, H ° -0.65 and 0, respectively. As the concentration of osmotic solution increased, the overall brightness decreased, therefore the L* value, which was 45.13, decreased to 37.37 at the highest sugar concentration. The a* value increased, the dark green colour changed from green, this is from value of -6.76 to 0.6 as the sugar concentration increased. The b* value was also found to change from yellow to dark yellow. Instrumental colour measurements correspond visually to high-quality food products. The preservation of natural colour in processed and stored food is very difficult.The green colour found in the fruit is determined by chlorophyll pigments, which degrade during heat treatments and eventually change the green colour.

In many studies on green colour changes due to time and temperature applications, it has been determined that the green colour is decreased and the change to brown colour (Monslaves et al., 1998). As the concentration of osmotic dehydration increased, Chroma rose slightly while it decreased in applications with less concentrated solutions. Also in the brightness, it was obtained the similar results. Colour changes varied depending on drying time and concentration

of sucrose. While the 60 Brix concentrated kiwi slices are dried for 6 hours, the drying time of the lower concentration fruit samples were prolonged.

The results obtained in the study were found to be consistent with the results obtained in other kiwifruit drying studies (Talens et al. 2003; Allaeddini and Emam-Djomeh, 2004; Mohammadi et al., 2008; Orikasa et al., 2014).

Moisture Ratio and Water Activity: Moisture content is an important factor in preserving the quality of food. In addition to affecting microbial quality, the increase in moisture content has negative effects such as oxidation, hydrolysis and nonenzymatic browning, and increased enzymatic activity in the structure of food. In ripe kiwifruit, it was determined that Brix value was 15.1, the dry matter amount was in the range of 23.76-26.89, and the moisture % was between 73.11% and 76.24%. Also it was determined that the kiwi slices dried by applying three different osmotic concentrations at 30, 40, and 60% changed dry matter contents from 88.35 to 89.14% and moisture content from 10.86% to 12.62%. In similar studies, it was determined that the total dry matter content varied between 86.98% and 89.64%.

Water activity is different from aw humidity in food quality; it is determine the physical, chemical and microbiological stability. Water activity is known to be moderate to severe food products of 0.60-0.90 and includes a large group of foodstuffs that are spontaneously stable, directly consumable foods (Aguilera et al., 1992). No microbiological activity is observed when the water activity value falls below 0.60. In general, dry fruits have a water activity value of 0.60 to 0.75. The values of aw were determined within the scope of this study, considering the importance of preserving the quality in food and aw importance in drying process. The water activity of the freshly untreated kiwi was determined to be 0.95. Talens et al. (2003) have found water activity value of 0.97 in their study.

In addition, they reported that it would be beneficial to apply osmotic dehydration in a short period of time to prevent damage to the fruit tissues or cells. In our study, vacuum impregnation has been shown to be beneficial in shortening the duration of dehydration. Similar comments have also been made by other researchers (Nowacka et al, 2014; Talens et al. 2003). Even though the immersion method, which is an osmotic dehydration method, slows down in terms of mass transit, it is a process that supports the improvement of the quality of the final product and energy saving. The most important advantage is to prevent microbial degradation of foodstuffs by reducing water activity prior to drying (Mujica-Paz et al., 2006).

CONCLUSIONS

In this study, it was determined that the water activity of dried kiwi slices by applying 30, 40 and 60 Brix osmotic dehydration solution is between 0,594 and 0,630. It has been observed that the water activity results of dried fruits are in the range of food safety and can be consumed for a long time, being preserved microbiologically.

The osmotic dehydrated dried fruits by increasing sucrose concentrations were found to be partially reduced in water loss and increased dry matter concentration. As the sucrose concentration rises, it is thought to form a layer on the fruit surface that prevents mass transfer. Dried kiwi can be used both in the mixed fruit cocktail as dry kiwi and in various products (fruit cake, etc.) to replace fresh fruit after being rehydrated. Sensory evaluation of colour, texture and taste has been performed to select the most appropriate solution. The sucrose solution concentrated at 30% was sensory recommended since samples pre-drying with this osmotic solution were best preserved in terms of colour and provides the best sour/sweet balance.

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EFFECT OF GROWTH PROMOTERS ON GROWTH PERFORMANCE, CARCASS CHARACTERISTICS, HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS OF IRAQI SHARABI CALVES FATTENED UNDER NINEVEH PROVINCE ENVIRONMENT, IRAQ

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ABSTRACT

The experiment was carried out at the calves farm of Al-Rasheida Animal station, Mosul, Iraq. To investigate the effect of using growth promoters on growth and some carcass traits, hematological and biochemical traits of Iraqi sharabi calves, 16 sharabi local calves were used (140-165kg) live body weight and 8-10 months old. Divided into 4 main groups (4calves/group),each main group was divided into 2 subgroup (2 calves /group) according to their live body weight. 1st group was fed on experimental ration only, while 2nd, 3rd and 4th groups were fed on experimental ration and supplemented with 200 g of Biolaczym, or 200 g of Sorbotiol or 25 gm. of Stymulan /100kg experimental ration, respectively, for 120 days. The rations and wheat straw were given on the rate of 2.5% and 1% of B.W., for all the groups respectively. At the end of feeding trails, blood samples were withdrawal from jugular vein from each calf and all calves were slaughtered. The results indicated that adding growth promoter type of stymulant to the ration of 4th calves group has significantly ($p \leq 0.05$) increased the daily gain, total gain ,final weight, somebody dimensions, hot and cold carcass weights , loin eye area ,dressing and main cuts percentages, hemoglobin, red & white blood cell counts, packed cell volume ,total protein and globulin, while cholesterol, triglycerides and urea were decreased significantly ($p \leq 0.05$) as compared with other calves groups 1st ,2nd and 3rd respectively. It was concluded that using Stymulan as growth promoter in fattening Sharabi calves had improved some production performance, carcass traits, hematological and biochemical parameters.

Keywords: *Growth promoters, Some growth and carcass traits, Hematological and biochemical traits,Iraqi sharabi calves.*

INTRODUCTION

Growth promoters are defined as alternative sources of antibiotics used in animal meat diets, as a partial or total replacement of these substances (El-Ashry et al., 2006). In the last decade,WHO has promoted the use of growth promoters as alternative sources of antibiotics in meat-animal diets .These Growth promoters are added to cattle, buffalo, sheep and goats rations in order to increase growth speed, body weight and improve some carcass characteristics may be due to its ability to improve digestion in the stomach and improve the feed efficiency by increasing the ability of bacteria to benefit from food compounds and identify the growth and activity of micro-organisms harmful and a positive reflection on some of the qualities of production of animals (Ahmed et al., 2009). Different types of growth

promoters are available in the market, such as enzymes (Zeid et al., 2008), juices of certain fruits and vegetables (Ahmed, et al., 2009), herbs and spices (Frankic et al., 2009), the oils of some medicinal plants (Khattab et al.,2010) and some medicinal plants seeds (Abdullah et al., 2012).It was noted that the addition of the growth promoter of the type of Megavit- DB to the fattening of the Red Chittagonger and Holstein crossbreed bull calves caused a significant increase in the rate in daily gain, total body gain (Sarker et al., 2010), in addition that adding growth promoter type of stymulan to the fattening ration of sharabi calves have significantly improved the rate of daily gain, total body gain as compared to control (Nasser et al. 2012). Bakr et al. (2009) reported that the addition of the growth promoter type of Biovet to buffalo calves diets significantly increased the number of red blood cells and the percentage of packed cell, also Shams al-dain et al. (2014c) reported that the addition of the growth promoter type of Stymulan to local calf significantly increased total protein and globulin concentration in local calf serum as compared to control group. Because of the availability of many growth promoters in the local markets of Mosul. This study was designed to use three types of growth promoters, Biolaczym, Sorbotiol and Stymulan to adding to the local fattening calves rations to observe their effect on productive efficiency, some characteristics of the calve carcasses, some hematological and biochemical parameters.

MATERIALS AND METHODS

This work was carried out at cattle farm of Al-Rashidiya station, Department of Agricultural Research in Mosul, State of Board of Agricultural Research, Ministry of Agriculture. Sixteen local sharabi calves aged 8-10 months and weights 140-165 kg calves were assigned randomly into 4 main groups(4 calves s/group) according to their live weight ,each main group was divided into 2 subgroup (2 calves/group), and housed in 8 semi-shaded well ventilated pen (10 x 5 m) containing a stage for calves movement and exposure to sunlight .All groups of calves were fed on the experimental ration (Table 1) prior to formulating the experimental ration, the main feed ingredients were analyzed according to AOAC (2007),which supply from feed factory at Al-Rashidiya station from the Feedstuffs which available at the station, to cover the need for the growing calves of protein and energy represented by the American Research Council (NRC.,1994) (table 1) the experimental ration was provided at rate 2% of the live body weight of the calves group with wheat straw fed *ad libitum* for fifteen days as a preliminary period, at the end of the preliminary period all calves reweighted again in the morning and before the introduction of experimental ration .These weights were considered the initial weights for experimental for the four main calves groups.

The growth promoters were obtained from the local markets of Mosul, North part of Iraq. growth promoter type of Biolaczym, containing *Bacillus subtililis*, *Lactobacillus acidophilus*, *Saccharomyces cerevisiae* and *Excipients*, while the growth promoter type of Sorbotiol, containing Sorbotiol, L-lysin, DL-methionine, and B12 ,the products of Biolaczym and Sorbotiol, are produced by Vietnamese company Minh Dung ,and growth promoter type of Stymulan contains some medicinal plants (cumin and fennel) and oils of some active compounds (cinnamon oil, clove oil, peppermint oil) and some extracts such as peppermint, carotene and some vitamins A, D3, E, K, C and beta and some mineral salts such as magnesium, Cobalt, manganese and sodium chloride and product by Polish Biopoint Company.

Table 1. Feed stuffs (gm/kg feed) and chemical analysis (%) of experimental ration

Feed stuffs		Chemical analysis determined (DM% basis)*		
Ingredients	g/kg feed	items	ration	straw
Barley grain	**400**	Dry matter(%) *	**92.28**	93.67
Wheat bran	420	Crude protein(%)*	**15.80**	**2.94**
Soy bean meal	**90**	Ether extract(%)*	**2.80**	**0.64**
Yellow corn	**80**	**(%) ****Crude fiber	**6.34**	**38.38**
Nacl) **(**Salt	**5**	Ash (%)*	**5.48**	**9.9**
Limestone(Caco3)	**5**	ME. (Kcal/kg) **	**2744**	**41.81**

*Determined on dry matter base according to AOAC (2007).
*Calculated from chemical analysis tables for Iraqi feed stuffs (Al-khawaja et al. 1978).
*

The 1st group calves was fed on the first ration (experimental ration only), while the 2nd, 3rd and 4th calves groups were fed on the second ration (experimental ration +200g of Biolaczym/100kg ration),third ration (experimental ration+100g of Sorbiol/100kg.ration), and fourth ration (experimental ration +25 g of Stymulan/100 kg ration), respectively. The four experimental rations were provided at two meals at 8:00 am and 5:00 pm. The rations and the wheat straw were provided with 2.5% and 1% of the live body weight of the calves, respectively, for 120 days. The remaining ration was reweighted at morning before the morning meal to calculate the quantities of feed intake for each group of calves. Calves were weight every fifteen days before the presentation of the morning meal, on this basis the amounts of experimental ration and the wheat straw were adjusted to each calves group.Fresh water and minerals blocks were freely available at all times in front of calves, and all calves were subjected to veterinary health care throughout the period of the experiment.

Measurements of the body dimensions (body length, chest girth, abdomen girth, front height, and wither height,) were taken at the end of the fattening period (120 days) before the animals were slaughtered using a measuring tape and numbered ruler according to the method mentioned by Shams al-dain et al.(2014b). Blood samples were individually collected early in the morning after fasting overnight from all calves groups, at the end of the experimental periods (120 days) before slaughter calves via jugular vein using a 10 ml plastic disposal syringe. About 10 ml of blood were obtained from each calf by using two vacationer tubes. The first 5 ml of the blood was put in vacationer tubes containing ethylene- demine tetra-acetic acid (EDTA), the tubes were inverted several times to ensure adequate mixing of the blood with anticoagulant and transported to the laboratory for hematological analysis ,the samples were analyzed within two hours after collection, the hematological analysis included ,total of erythrocyte (RBC's $\times 10^{6}$ cells/ul) and leukocyte (WBC's $\times 10^{3}$ cells/ul) counts were determined manually by using the hemocytometer as described by Schalm (1977), hemoglobin concentration (Hb, g/dl) was determined according to Schalm (1977), packed cell volume (PCV%) was estimated by the use of the microhematocrit method according to Cole (1987) and expressed as a percentage. The second 5 ml of the blood but in non heparin zed glass tubes, blood samples were centrifugation at 4000 rpm /15 minutes and stored at $-20C^{0}$ until biochemical analysis , the biochemical analysis of blood serum was used to determined the total protein (T.P), albumin (AL), globulin (GL), glycerol (G), triglycerides (TG) ,urea blood (BU) and blood glucose (BG) in serum by using commercial kits (Biolabo Meriex, France) according to the procedure outlined by the manufacturer and by automatic spectrophotometer. At the end of the fattening period (120 days), after fasting overnight (12 hours)**,** prior to slaughter, all the calves were weighted (final weight) before slaughter. The slaughter was carried out at slaughter house at Al-Rashidiya station, after the slaughter, carcasses were washed, identified and

weighted before being taken to the cooling chamber in order to obtain the hot carcass weight. The weight of the edible parts (testes, liver, heart, spleen and kidneys) and non edible parts (trachea, lungs, digestive tract, genital tract, head, foot and skin), subcutaneous fat weights around the internal intestines and kidneys was recorde

After the cooling period for 24 hours at a temperature of -2°C , the carcasses were reweighted to obtain the cold carcass weight and and losses due to cooling. A horizontal cut was taken between the 12^{th} and 13^{th} rib in order to expose the Longissimus dorsi area (loin eye area), and fat thickness were measured between the 12^{th} and 13^{th} ribs by using a plastic grid and vernier caliper, the fat thickness was obtained by the average of three observations in the same place .The degree of fullness of the carcass was measured by using the European grading system, which was calculated according to the method described by Commission of the European Communities. (1982),five degrees of acceptance, 1 = incomplete , 2 = medium full, 3 = well filled, 4 = very well filled and 5 = very full), also to measure the degree of carcass fat and the degree of lipid coating of the kidneys(kidneys fat degree), the degree of carcass fat packaging was measured by visual assay of the external fat on the carcass,(Z = lean, C = weak muscle and fat, 1 = Lipid, 2 = very low fat, L3 = very low fat, H3 = very high fat, 4 = high fat and 5 = hyper fat. The lipid level of the kidneys was also measured by the visual estimate of the fat coating of the kidneys 1 = Very fat, 2 = low fat, 3 = good fat, 4 = high fat, 5 = excessive fat which was calculated according to the method described by Commission of the European Communities (1982). The three (11, 12 and 13) ribs regions were taken from the left side of all the calves carcasses, weighted and then put in polyethylene bags and placed in frozen until the physical section. Physical section was carried out after removing the freezing state by placing them in the refrigerator for 24 hours, the ribs were then physically separated into their components by bone, muscle and fat tissues by using scalpels and sharper knives, inside the room to avoid refrigerant evaporation as much as possible, and then recorded weights of the above components and the percentages of the rib components were calculated according to the method of Rouse et al. (1970). After completing the physical section of the ribs (11, 12 and 13), the muscle and fat tissues of all three ribs of the calve carcasses were cuts with sharp knives into small pieces and mix them well, the mixture was then mixed with an electric machine, the resulting meat was then mixed thoroughly with an electric mixer for the purpose of homogenizing. Three samples were taken from each of the three rib (11, 12 and 13) of all calves' carcasses. Chemical analysis was carried out according to the methods described by A.O.A.C. (2007) to estimate the crude fat, protein and ash. As for moisture it was calculated by taking a weight of 3-4 g of meat sample in a moisture tray after mixing it with 5 g Dry sand and dried at 50 ° C for 24 hours.

The experiment was designed by the complete randomized design (CRD). Data generated from the experiment were statically analyzed by analysis of variance was carried out on all data according to SAS (2004). Then means were separated by Duncan's multiple range tests to determine the significant at 0.05 % level of probability (Duncan, 1955).

RESULTS AND DISCUSSION

Productive performance of calves

The statistical results of the productive performance were presented in the Table 2, showed that there were a significant ($P \leq 0.05$) differences between the four calves groups in the daily and total gain and the final weight. The 4^{th} group of calves that fed the fourth ration (experimental ration + Stymulan) was significantly ($p \leq 0.05$) higher on those traits as compared with the other groups of calves that fed the first ration(experimental ration only) and the second ration (experimental ration + Biolaczym) and the third ration (experimental ration + Sorbotiol) respectively,the daily weight gain was increased and a cumulative which caused a positive

reflect to increased the total weight, causing its superiority significantly ($p \leq 0.05$) in the final weight, possibly due to that growth promoter type of Stymulan containment some medicinal plants such as cumin, fennel and the oils of some effective compounds, such as cinnamon oil, clove oil, peppermint oil and some extracts of substances such as mint, carotene and antioxidants vitamin C and E, which has a positive effect on improving the immune status of animals(Safaa,2005),or perhaps that growth promoter reduce the acid function (pH) of the intestines, which increases the absorption of digested food compounds (Allam et al., 1999).These results were consistent with the results of Nasser et al.(2012) which indicated that the addition of growth promote type of Stymulan to fattening local calves rations at rate of 25 and 50 gm/100 kg ration had a significant improvement in the rate of daily weight gain and final weight as compared with those calves group that took control.The results in Table 2 showed a mathematically reduction in the daily and total feed intake by the calves groups in 2nd ,3rd and 4th groups , respectively, that fed the second ration (experimental ration +Biolaczym),third ration(experimental ration + Sorbotiol) and fourth ration (experimental ration + Stymulan), respectively as compared to those in 1st(experimental ration) ,this may be due to that rations containing growth promoters as food additives, which improved the conditions of the rumen by determining the growth and activity of microorganisms and improving the efficiency of feed utilization by increasing the ability of bacteria to benefit from the compounds food (Ahmed et al., 2009), which improved the dry matter digestion factor as a result of increased feed utilization (El-Ashryet al.,2006). These results were consistent with the results of Sarker and Yang (2010),which indicated that no significant effect for the addition of growth promoter type of propolis or illite in the daily and total feed intake by Korean calves Han woo as compared to those calves that took control, but pointed to a reduction in the amount of daily feed intake of the animals containing growth promoter as compared to those control group. The decrease in daily and total feed intake was reflected by the 4th calves group (Table 2) which resulted in improved the feed efficiency as compared with the other groups of calves. These results were consistent with the results of Shams al-dain,et.al. (2014a), which indicating that there was no significant effect on the use of of growth promote type of Biolaczym in feed efficiency.

The Cost for 1 kg consumed feed for all calves groups, 550, 560,560 and 575 I.D/1kg /feed, respectively, were almost same, but the cost of feeding to produce one kg of weight gain in the fourth group 4801 I.D., was more efficient than the other groups, and were 5770 , 5676 and 5422 I.D., respectively.

The results presented in table (3) showed a significant ($p \leq 0.05$) effect of the addition of growth promoters in the rations in some body measurements. The 4th group of calves was significantly exceeded ($p \leq 0.05$) in the body length, chest and abdomen girth as compared with other calves groups ,1st ,2nd and 3rd,respectively, this may be attributed to the presence of a significant differences ($p \leq 0.05$) in the final weights of calves groups(Table 2), which resulted in a significant ($p \leq 0.05$) differences in the body length, chest and abdomen girth of the calves fed experiential ration + Stymulan, with positive and significant correlation coefficient between live weight and body length, chest and abdomen girth (Shamsal-dain et al.,2014b). These results were consistent with the results of Nasser et al. (2012),which indicated a significant effect for the addition of growth promoter type of Stymulan (0.25 and 0.50 kg/100 ration) to local Sharabi calves in body length, chest and abdomen girth .While there was no significant effect on the use of growth promoter in the front and wither height, These results were consistent with the results of Shamsal-dain et al. (2014 a) which indicating that there was no significant effect for the addition of growth promoter type of Biolaczym (0 and 200g /100kg ration) to local calves in front and wither height.

Table 2. Effect of growth promoters in some productive performance of calves (Mean±S.E.)

Treatments / Studied traits	T1 ER	T2 ER +Bi	T3 ER +So	T4 ER +St
Initial weight, kg	144 a± 25	143 a± 26	145 a± 25	142 a± 27
Final weight, kg	205.25 b±62.0	202.33 b± 60.5	207.50 b± 53	219.25 a ±69
Average Daily gain,g	510.42 b±111.18	494.42 b± 100.23	520.83 b±123.10	644.38 a± 141.80
Total gain, g	61.25 b± 16.25	59.33 b ± 17.55	62.50 b± 5.66	77.25 a±22.18
Experimental ration consumption (kg/calf/day)	3.99 a±0.71	3.79 a0.75±	3.93 a0.72±	3.96 a0.73±
Straw consumption (kg/calf /day)	1.40 a 0.42 ±	1.29 a0.42±	1.38 a0.31±	1.39 a± 0.35
Total feed consumption (kg/calf /day)	5.39 a± 0.91	5.08 a±0.91	5.31 a0.80±	5.35 a0.83±
Feed conversion efficiency (g feed/g gain)	10.57 b± 4.4	10.28 b± 4.4	10.19 b± 5.1	8.31 a± 3.5
Cost for 1 kg consumed feed (Iq.dinar/ calf /day)	550	556	556	575
Cost for 1 kg weight gain (Iq.dinar/ calf /day)	5770	5676	5422	4801

* ER= experiential ration, ER +Bi= experiential ration + Biolaczym, ER + So= experiential ration + Sorbotiol, ER + St= experiential ration + Stymulan)

**Mean with different letters within the same line are significantly different (p≤0.05)

Table 3. Effect of growth promoter in different body measurements and dimensions (cm) (Mean ±S.E.)

Treatments / Studied traits	T1 ER	T2 ER +Bi	T3 ER +So	T4 ER +St
Body length	149.50 b± 18.1	148.90 b±18.9	150.60 b± 17.3	169.70 a±13.8
Chest girth	138.30 b± 15.4	137.80 b± 16.3	138.60 b± 14.1	149.50 a±11.3
Abdomen girth	142.60 b± 9.87	141.90 b± 9.87	141.20 b± 5.5	154.40 a± 3.2
Front height	104.10 a± 8,8	103.60 a 8.8 ±	103.20 a± 8.3	109.20 a± 4.7
Wither height	116.2 a ± 9.4	115.80 a± 9.4	116.50 a±9.2	120.7 a± 6.1

ER= experiential ration, ER +Bi= experiential ration + Biolaczym, ER + So= experiential ration + Sorbotiol,*ER + St= experiential ration + Stymulan)

** Means with different letters within the same line are significantly different (p≤0.05).

Characteristics of calve carcasses

The statistical results of the data on the carcass characteristics (Table 4) showed a significant superiority ($p \leq 0.05$) for the 4thcalf groups (the experiential ration +Stymulan) in the hot and cold weight, dressing percentage and loin eye area as compared with other calves groups, 1st group (experiential ration only), 2nd group (experiential ration+Biolaczym) and 3rd group(experiential ration+Sorbiol),respectively, and the results in table 4 showed a significant ($p \leq 0.05$) increased in fat thickness (mm), kidney and pelvic fat percentage(%) and main cuts percentage(%), while the secondary cut percentage (%) was a significantly ($p \leq 0.05$) decreased in the 4th calf group as compared to those 1st group , this may be attributed to a positive relationship between fat thickness and kidney and pelvic fat percentage and the amount of cholesterol and triglycerides (both high and low-density lipoproteins) in the blood of buffalo calves fed to the lactobacillus +saccharamyces (Bakr et al., 2009), or perhaps the enhancers have improved the conditions of the rumen, which led to increased energy digestibility, especially the representation of energy in the showed there was no significant effect on the use of any type of the growth promoter in the percentages of the edible and non-edible parts.

Table 4. Effect of growth promoter in some carcass traits of calves (Mean±S.E.)

Treatments / Studied traits	T1 ER	T2 ER +Bi	T3 ER +So	T4 ER +St
Slaughter weight (kg)	205.25 b±62.0	202.33 b± 60.5	207.50 b± 53	219.25 a ±69
Hot carcass weight (kg)	106.59 b±25.1	102.80 b±28.3	106.27 b± 27.1	118.65 a± 32.6
Cold carcass weight (kg)	104.40 b± 24.2	101.10 b± 26.3	104.90 b± 25.8	116.30 a±19.9
Dressing percentage(%)	50.86 b± 2.11	49.96 b± 2.51	50.54 b± 2.36	52.09 a±2.78
Loin eye area (cm)	51.12 b± 8.89	51.93 b± 9.23	52.01 b± 9.23	56.25 a± 5.21
Fat thickness (mm)	5.83 b± 2.1	5.94 ab± 2.5	6.10 ab± 2.5	6.33 a± 2.9
Edible parts percentages(%)	9.34 a± 1.2	9.27 a±0.86	9.21 a± 0.93	10.85 a± 0.88
Non-edible parts percentages(%)	17.92 a± 1.88	17.11 a± 2.11	16.28 a± 2.11	15.25 a±1.94
Kidney and pelvic fat percentage(%)	2.14 b± 0.56	2.68 ab± 0.64	3.39 a± 0.82	3.51 a±0.89
Main cuts percentage(%)	67.28 b± 2.31	68.56 ab± 1.98	69.12 a± 1.83	70.76 a± 1.55
Secondary cuts percentage(%)	32.72 a± 2.31	31.44 ab± 1.98	30.88 a± 1.83	23.24 b± 1.55

ER= experiential ration, ER+Bi= experiential ration+Biolaczym, ER+So=experiential ration+Sorbotiol, * ER+St=experiential ration+Stymulan) ** Means with different letters within the same line are significantly different ($p \leq 0.05$)

The obtained results are in accordance with those reported by Adnan et al. (2012), they found that supplementation with growth promoter type of Stymulan to ration of local sharabi calves was increased significantly ($p \leq 0.05$)the hot and cold weight and dressing percentage as compared to those in control group, also the results were consistent with the results of Titi et al. (2008), which indicated that there was a significant effect to add growth promoter type of yeast in the diets of fattening male cows in kidney and pelvic fat percentage and accordance with those reported by Shams al-dain et al. (2014a), they found no significant effect that supplementation with growth promoter type of Biolaczym to ration of local Sharabi calf in the percentages of the edible and non-edible parts as compared to those in control group.

Table 5. Effect of growth promoter in physical section of three ribs region (Mean ± S.E.)

Treatments / Studied traits	T1 ER	T2 ER +Bi	T3 ER +So	T4 ER +St
three ribs region weight, gm.	1185 ±240	1152 ±240	1177 ±240	1224 ±210
Muscle tissue weight, gm.	653 ±110	643 105±	667 110±	691 98±
fat tissue weight,gm.	186 28±	184 27±	196 28±	202 31±
Bone tissue weight,gm.	346 54±	325 48±	314 54±	331 55±
Percentage of Muscle tissue(%)	55.11 9.2±	55.82 9.7±	56.67 9.9±	56.45 9.6±
Percentage of Fat tissue(%)	15.69 2.5±	15.97 2.9±	16.65 3.7±	16.50 3.8±
Percentage of Bone tissue(%)	29.20 9.2±	28.21 9.7±	26.68 9.9±	27.05 9.6±
Percentage of Muscle/fat tissue	3.51 0.93±	3.49 0.91±	3.40 1.06±	3.42 1.02±
Percentage of Muscle/bone tissue	1.89 0.21±	1.98 0.28±	2.12 0.23±	2.09 0.35±
Percentage of Fat /bone tissue	1.86 0.21±	1.77 0.16±	1.60 0.16±	1.59 0.13±
Percentages of Muscle +fat tissues	70.80 ±4.8	71.19 4.9±	73.32 5.2±	72.95 ±5.4

ER= experiential ration, ER +Bi= experiential ration + Biolaczym, ER + So= experiential ration + Sorbotiol, * ER + St= experiential ration + Stymulan)

The results presented in table 5 showed that there was no significant effect on the use of any type of growth promoter in the weights and percentages of bone, meat and fat tissues (physical section) of the three ribs (11,12 and 13) region. The percentages of the muscle and fat tissues were increased arithmetically, while the percentage of bone tissue was decreased arithmetically of the three rib region of the 3rd and 4th calves groups carcasses as compared with those on 1st calves group .These results were consistent with the results of Mahyuddin and Widiawati (2010) which indicated that there was no significant effect to add the growth promoter type of yeast and medicinal herbs in the fattening calves of the buffalo calves in the percentage of muscle tissue of the region of the three ribs, and in accordance with the results of Nasser et al. (2012), which indicated that there was no significant effect to add the growth promoter type of stymulan in the fattening of local calves in the percentage of bone tissue of the region of the three ribs. The results of the statistical analysis presented in Table 7 showed

no significant effect on the use of any type of growth promoter in the chemical analysis of the three rib regions. The dry matter and protein percentages were increased arithmetically while the moisture and fat percentages were decreased arithmetically of the three ribs region of the 2nd ,3rd and 4th calves groups as compared with those in 1stcalves groups.

Table 6. Effect of growth promoter in chemical analysis of three ribs region (Mean±S.E.)

Treatments / Studied traits	T1 ER	T2 ER +Bi	T3 ER +So	T4 ER +St
Moisture percentage	58.67 0.48±	58.47 ±0.44	58.33 ±0.39	58.24 ±0.36
Dry matter percentage	41.33 ±0.48	41.53 ±0.48	41.67 ±0.48	41.76 ±0.48
Protein percentage	±0.16 99.15	16.21 ±0.19	16.45 ±0.34	16.54 ±0.27
Fat percentage	24.08 ±0.19	24.15 ±0.28	24.21 ±0.19	24.36 ±0.32
Ash percentage	1.18 ±0.06	1.17 ±0.06	1.14 ±0.06	1.01 ±0.06

ER= experiential ration, ER +Bi= experiential ration + Biolaczym, ER + So= experiential ration + Sorbotiol, * ER + St= experiential ration + Stymulan).

The results presented in Table 7 showed no significant effect on the use of any type of growth promoter in carcass grade, carcass fat grade and kidneys fat grade respectively. The values of the carcass grade, carcass fat grade and kidneys fat grades in the 4th were increased arithmetically as compared with those in 1st ,2nd and 3rd calves group respectively.

Table 7. Effect of growth promoter in carcass, carcass fat and kidneys fat grades (Mean±S.E.)

Treatments / studied traits	T1 ER	T2 ER +Bi	T3 ER +So	T4 ER +St
Carcass Grade	3.10 0.15±	3.15 ±0.25	3.20 0.25±	3.40 ±0.20
carcass fat grade	3.00 0.20±	3.20 ±0.15	3.25 ±0.15	3.45 ±0.10
kidneys fat grade	3.10 0.25±	3.20 ±0.20	3.30 0.15±	3.50 0.15±

ER =experiential ration, ER +Bi= experiential ration + Biolaczym, ER + So= experiential ration + Sorbotiol, * ER + St= experiential ration + Stymulan)

Hematological and biochemical parameters

Data in table(8) clearly indicated that supplementation with growth promoter type of Stymulan was significantly($p \leq 0.05$) affected the total of erythrocyte count (RBC), total of leukocyte count (WBC) ,hemoglobin concentration (Hb)and packed cell volume (PCV).Supplementation of growth promoter type of Stymulan to 4th groups was significantly ($p \leq 0.05$) the RBC, WBC ,Hb and PCV%, as compared to those in other groups.The significant increase in the total of RBC, Hb and PCV% in 4td group may be due to that growth promoter Stymulan contains some active compound.

Table 8. Effect of growth promoter in some hematological traits (Mean ±S.E.)

Traits / Factors	RBC ($\times10^6$µl)	WBC ($\times10^3$µl)	Platelets ($\times10^3$µl)	Hb. (g/dl)	PCV **(%)**
T4 ER +St	11.72 b ±1.34	12.01 b±0.95	4.33 a±0.29	11.97 b±1.34	59.32 3.12b±
T4 ER +St	99.11 b±1.44	12.24 b±1.11	4.47 a±0.30	14.12 b±1.44	32.87 b ±3.19
T4 ER +St	12.24 b±1.11	12.27 b±1.09	4.51 a±0.31	12.26 b±1.11	06.33 b±2.67
T4 ER +St	12.58 a±1.41	12.77 a±0.95	4.62 a±0.29	12.69 a±1.41	34.89 a±3.12

*RC=control, CR +Bi=control+ Biolaczym, CR + So=control+ Sorbotiol, CR + St= control+ Stymulan) ** Means with different letters within the same column are significantly different ($p\leq0.05$)

like medicinal plants (cumin and fennel) and oils of some active compounds (cinnamon oil, clove oil, peppermint oil) and some extracts such as peppermint and carotene. These results were consistent with the results of Ahmed et al. (2009) which indicated that there was a significant effect of growth promoter (0, 2.5%, 5% and 7.5% of vegetable and fruit juices)on RBC and Hb in the blood of buffalo calves, and also accordance with those reported by Shams al-dain et al.(2014a),they found a significant effect that supplementation growth promoter type of Biolaczym (200 g/100kg feed) to ration of local sharabi calf in the RBC, Hb and PCV% as compared to those in other groups. The results in Table 9 indicate that there is a significant effect ($p\leq0.05$) for the growth promoter in most of the studied biochemical traits. The total protein was significantly increased ($p\leq0.05$) in the 4th calves group, this may be due to the fact that the total protein status of the serum reflects the animal's feeding status and that there is a positive relationship with the food protein (Taha and Shamsal-dain, 1998), this was reflected by a significant increase ($p\leq0.05$) in the level of globulin in the blood of calves fed on the 4th ration with the addition of the growth promoter stymulan. This may be due to increased immunity of the body through the increase of globulin, due to the presence of some extracts of medicinal plants in the growth promoter Stymulan, that growth promoter are used to reduce the incidence of inflammation and stimulate the immune system of the animals receiving the growth promoter (Avita et al., 1995), or as a result of the effect of positive food enhancers on the balance of intestinal bacteria (Fuller, 1989). These results were consistent with the results of Bakr et al. (2009), who indicated that there was a significant effect of growth promoter Biovet (0,15 and 25 g/calf/day) on the total protein concentration in the buffalo calf serum and also accordance with those reported by Shamsal-dain et al. (2014a), they found a significant effect that supplementation growth promoter Biolaczym (200 g/100kg feed) to ration of local Sharabi calf in the total protein and globulin as compared to those in other groups.

While the results in Table 9 indicated that adding the growth promoter type of stymulan to 4th calves group caused a significant decrease ($p\leq0.05$) in the amounts of cholesterol and triglycerides in the serum, which may be due to the fact that the growth promoter stymulan may caused an increased in the growth of muscle and fat composition of the body of animals (Table5), or perhaps because growth promoter stymulan may prevent the synthesis of cholesterol directly (Taranto et al., 1998).The results were consistent with the results of Vasiijevic and Shah (2008), which showed a significant decrease ($p\leq0.05$) in the blood cholesterol of milk cows fed on diets containing the growth promoter, also the results were consistent with the results of Bakr et al.(2009), which showed a significant decrease ($p\leq0.05$)

in the blood cholesterol of buffalo calf serum fed on diets containing the growth promoter Biovet (0.15 and 25 g/calf/day), also the results were consistent with the results of Shamsaldain et al. (2014c), which showed a significant decrease ($p \leq 0.05$) in the blood cholesterol and triglycerides in the local calf serum fed rations containing the growth promoter type of Stymulan (0.15 and 30 g/100kg feed). While the results in Table 9 showed a significant decrease ($p \leq 0.05$) in blood urea in the 4th calves group as compared to those other groups. These results were consistent with the results of Zeid et al. (2008), which indicated a significant ($p \leq 0.05$) reduction in blood urea in Friesian dairy cows fed on diets containing growth promoter (10 g xylanase, 10 g yeast, 5 g xylanase + 5 g yeast). But the results in Table 9 indicate that there was no significant effect of the growth promoter in blood glucose. These results are consistent with the results of Shams-al-dain et al. (2014c), who indicated that there is no effect of growth promoter type Stymulan in serum glucose of local calves.

Table 9. Effect of growth promoter in some biochemical parameters(Mean ±S.E.)

Traits / Factors	T/protein (g/dL)	Globulin (g/dL)	Albumin (mg/dL)	Cholesterol (mg/dL)	Triglyceride (mg/dL)	Glucose (mg/dL)	Urea (mg/dL)
T1 CR	7.06 0.24b±	3.38 0.16b±	3.68 0.09a±	135.89 3.54a±	42.26 a ±1.14	70.38 2.82± a	58.83 a2.24 ±
T2 CR +Bi	7.45 0,35b±	3.66 0.11b±	3.79 0.06a±	134.17 12a 4±	41.99 a±0.76	69.94 a±1.54	58.71 a±1.87
T3 CR + So	7.44 0.42b±	3.68 0.09b±	3.76 0.08a±	134.12 3.75a±	41.97 a±0.48	69.97 a±21.1	58.68 a±1.92
T4 CR + St	7.98 0.38a±	4.41 0.08a±	3.37 0.06a±	131.99 b±6.54	36.74 b±0.71	69.89 a±1.67	52.91 b±2.27

RC=control, CR +Bi=control+ Biolaczym, CR + So=control+ Sorbotiol, CR + St= control+Stymulan)
mean with different letters within the same line are significantly different ($p \leq 0.05$)**

It can be concluded that using growth promoter Stymulan in ration of Iraqi Sharabi calves had improved the studied production performance of calves, characteristics of calves carcasses, hematological and biochemical parameters without adverse effect on the animal health's as compared with others growth promoter types Biolaczym and Sorbotiol. However further studies are needed in this aspect.

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ALTERNATING ELECTRIC CURRENT AFFECTS ADVENTITIOUS ROOTING OF 140RU GRAPEVINE ROOTSTOCK

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ABSTRACT

Rooting capability of hardwood cuttings varies depending on grapevine rootstock used in viticulture. In present research, it was utilized from alternating electric current applications (AC) at different electrical potentials, including AC-0, AC-110, AC-220 and AC-380 V and application durations, including 1, 2 and 3 hour to enhance adventitious rooting potentials of hardwood cuttings from 140Ru grapevine rootstock. Study results revealed that although increasing electrical potential from AC-0 to AC-220 V were improving rooting characteristics, reducing application durations from 3 to 1 hour positively affected rooting characteristics of hardwood cuttings. In terms of adventitious rooting rates, the highest adventitious rooting rate was 87.50% for AC-220 V for 1 hour application compared to AC-0 V for 3 hour application (37.50%). Similarly, AC-220 V for 1 hour application (13.87 no) led to the highest adventitious root number than AC-0 V for 3 hour application (6.08 no). Consequently, AC-220 V for 1 hour application caused the best rooting characteristics of hardwood cuttings from 140Ru grapevine rootstock.

Keywords: *Grapevine rootstock, Hardwood cutting, Adventitious root, Difficult-to-root, Alternating electric current*

INTRODUCTION

Grapevine (*Vitis vinifera* L.) belongs to the family Vitaceae and there are more than 10.000 grape cultivars under the genus Vitis (Galet, 2000 a, b).

Due to various abiotic and biotic stress factors, the vegetative propagation of grapevine by grafting became a part of the modern viticulture activity (Spoerr, 1902). For this aim, grape growers are grafted scions of susceptible *Vitis vinifera* L. cultivars onto the cuttings of tolerant grapevine rootstocks bred from different North American species and after that these omega grafted cuttings were rooted under the suitable conditions for obtaining healthy grafted-rooted grapevine saplings (Kok and Bahar, 2017a; b; Kok, 2018b). Rooting ability of grapevine rootstocks depends on genetic characteristics, environmental factors and endogenous and exogenous supply of biochemical constituents (Somkuwar et al., 2011). Some of the hardwood cuttings from grapevine rootstocks, including 41B, 140Ru, 420A, Dod Ridge and Ramsey may occasionally exhibit rooting problems during their rooting processes (Çelik, 2011; Kök, 2017; Kok, 2018a).

In hardwood cuttings of difficult-to-root grapevine rootstocks, it may be taken some measures to increase rooting rates such as *I*) application of hardwood cuttings with different bacteria types, II) immersion of hardwood cuttings into hot water for a long time, III) immersion of the hardwood cuttings into lime containing water, IV) immersion of hardwood cuttings into 2-4% sucrose solution for 24 hour, V) immersion of hardwood cuttings into 0.5-1.0% hydrogen peroxide solution for 24 hour, VI) application of short-term high-voltage alternating current (1100 V) to hardwood cuttings (Çelik, 2011; Ağaoğlu, 2002).

There are two different types of electrical currents such as direct electric current (DC) and alternating electric current (AC) in nature. Alternating current is an electric current, which periodically reverses direction in contrast to direct current, which flows only in one direction (Herman, 2011).

It is well known fact that external electric current applications may affect polarization of dipoles in living cells (Moon and Chung, 2000). There are various studies on effects of electric current applications on plants, including root elongation and root number of Rhizophora mucronata seedlings (Kathiresan and Rajendran, 2000), chilling effects in vernalization of winter wheat (Filek et al., 2002), breaking bud dormancy of grape (Kurooka et al., 1990) and germination of tomato seeds (Moon and Chung, 2000).

The latest studies on rooting of plants have reported on influences of electric current on different physiological and biochemical responses of various plant species (Mishra et al., 2001; Filek et al., 2002; Filek et al., 2003; Wawrecki and Zagorska-Marek, 2007; Köse, 2007).

Köse (2007) declare in a study that direct electric current (DC) application with 60 V for 3 hour results in the highest increases in rooting characteristics of hardwood cuttings from grapevine rootstock Ramsey compared with Control application, which can be clarified by effects of direct electric current on plant metabolism like hormonal and enzyme activities and on movements of endogenous solutes, especially carbohydrates, plant growth regulators and enzymes.

The purpose of present study was to determine the effects of various electrical potentials of alternating electric current applied at different application durations on the rooting of hardwood cuttings from 140Ru grapevine rootstock.

MATERIAL AND METHODS

Research site

This study was carried out under the laboratory conditions of electrical department at Tekirdağ Technical and Industrial High School, Tekirdağ, Turkey in 2014 year.

Plant material and preparing hardwood cuttings

In the study, it was benefited from 140Ru grapevine rootstock, which is bred from hybrid of Berlandieri Resseguier No. 2 x Rupestris du Lot (St. George) 140 Ruggeri. It is well known fact that hardwood cuttings of 140Ru grapevine rootstock have low rooting potential.

Harwood cuttings from the previous season of 8-10 mm diameter and having 3-4 buds were taken from the mother grapevine rootstocks grown in Tekirdağ Viticulture Research Institute, Turkey during the dormant period of 2014 year.

Applications of alternating electric current and rooting procedure of hardwood cuttings

In order to apply various electrical potentials of alternating electric current to hardwood cuttings, it was utilized from electrical test set and electrical potential values of alternating electric current applications were adjusted by a rectifier shown in Figure 1.

Figure 1. Electrical test set and rectifier

During the alternating electric current applications, a procedure declared by Köse (2007) was followed. Prior to alternating electric current applications, all prepared hardwood cuttings were kept in water to obtain good soaking for 24 hours. In the research, a special electric current application system was designed for applying of alternating electric current to hardwood cuttings and well-wetted hardwood cuttings were placed on this electric current application system (Figure 2).

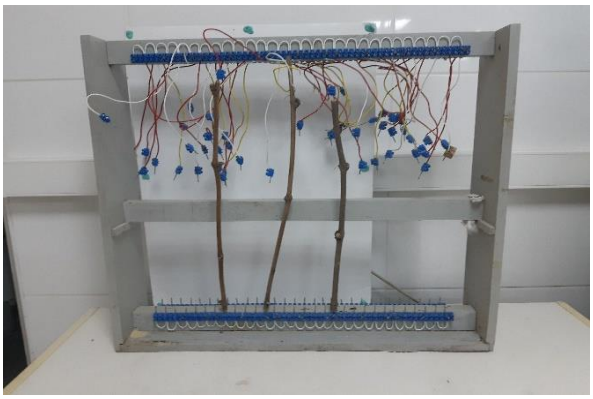

Figure 2. Electric current application system

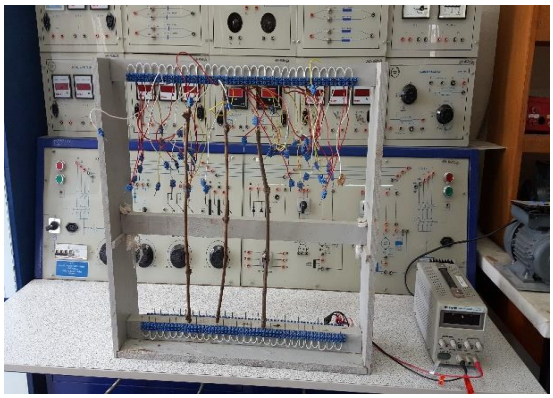

Figure 3. Applications of alternating electric current

In this system displayed in Figure 2 and 3, a stainless-steel electrode wires (0.69 mm diameter) were inserted into the pith of hardwood cuttings to a depth of 10 mm. Because negatively charged ions of indole-3-acetic acid (IAA), which encourage adventitious root formation in plants, could move to the positively charged cutting base, anode was set to basipetal end and cathode was set to acropetal end of hardwood cuttings during the electric current applications (Jacobs, 1979; Moore, 1989; Köse, 2007). Subsequently, alternating electric current was applied to hardwood cuttings at certain electrical potential values and application durations (Figure 3) and all hardwood cuttings were wrapped with stretch film to keep the humidity and were moved into growth bed containing sand-perlite for rooting process. In the course of rooting period, related cultural practices, including irrigation and shading were performed. After 75 days, the study was ended and all hardwood cuttings were planted out from the rooting medium and certain calculations and measurements were respectively carried out for hardwood cuttings (Kök, 2012; 2017).

In this research, it was respectively utilized from three application durations and four electrical potentials of alternating electric current (Table 1).

Table 1. Different application durations and electrical potentials of alternating electric current

Application duration (hour)	Electrical potential (volt)
	AC-0 V
	AC-110 V
1, 2, 3	AC-220 V
	AC-380V

AC-0 V : 0 volt alternating current AC-110 V: 110 volt alternating current
AC-220 V: 220 volt alternating current AC-380 V: 380 volt alternating current

Calculations and measurements

After the study was completed, some calculations and measurements, consisting of bud bursting rate (%), shoot length (cm), shoot weight (mg), adventitious rooting rate (%), adventitious root number (no), adventitious root length (cm) and adventitious root weight (g) were performed for omega grafted cuttings in the research.

Statistical analysis

The study was planned according to randomized complete parcels design as 2 factors with 4 replicates and each replicate consisted of 15 hardwood cuttings. Statistical analysis was performed by statistical software of TARIST and differences among the means were compared by Fisher's Least Significant Difference (LSD) test at 5% level.

RESULTS AND DISCUSSION

Table 2, 3 and 4 present interaction effects of application duration and electrical potential, main effect of application duration and main effect of electrical potential.

As shown in Table 2, there are no significant differences in terms of interaction effects of application duration and electrical potential of alternating electric current on characteristics of shoot and root ($p<0.05$). Based on bud bursting rates in Table 2, rising electrical potential values of alternating electric current applications up to AC-220 V for 1 h application duration (75.00%) increased bud bursting rates than AC-0 V for 3 h application duration (51.87%). Concerning shoot length in Table 2, the highest shoot length mean was recorded for AC-220 V for 1 h application duration (19.75 cm) when the compared with AC-0 V for 3 h application duration (10.34 cm). With respect to shoot weight in Table 2, AC-220 V for 1 h application duration (4.83 g) resulted in the highest shoot weight mean than AC-0 V for 3 h application duration (2.40 g). Regarding adventitious rooting rates represented in Table 2, the highest

Table 2. Interaction effects of application duration and electrical potential of alternating electric current on characteristics of shoot and root

Application duration (hour)	**Electrical potential (volt)**	Bud bursting rate (%)	Shoot length (cm)	Shoot weight (g)	Adventitious rooting rate (%)	Adventitious root number (no)	Adventitious root length (cm)	Adventitious root weight (g)
	AC-0 V	56.25	11.23	2.73	40.62	6.16	5.22	3.44
1 h	AC-110 V	71.87	16.92	3.97	78.13	12.96	9.93	4.22
	AC-220 V	75.00	19.75	4.83	87.50	13.87	11.08	4.82
	AC-380 V	62.50	13.00	3.22	62.50	7.12	6.25	3.77
	AC-0 V	55.62	10.51	2.70	37.50	6.12	5.15	3.30
2 h	AC-110 V	65.62	15.12	3.75	76.25	12.03	9.86	4.11
	AC-220 V	71.87	17.53	4.29	84.37	13.33	10.72	4.45
	AC-380 V	59.37	12.75	2.93	59.37	6.75	5.83	3.59
	AC-0 V	51.87	10.34	2.40	37.50	6.08	5.10	3.22
3 h	AC-110 V	59.37	14.19	3.09	67.50	11.84	9.27	4.00
	AC-220 V	68.75	17.19	4.01	81.25	13.00	10.08	4.27
	AC-380 V	59.37	12.02	2.83	56.25	6.50	5.50	3.53
	$LSD_{5\%}$	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

Different letters within the same columns denotes significant differences ($p<0.05$) N.S.: Not significant

AC-0 V : 0 volt alternating current AC-110 V: 110 volt alternating current AC-220 V: 220 volt alternating current
AC-380 V: 380 volt alternating current

adventitious rooting rate was obtained from AC-220 V for 1 h application duration (87.50%) compared to AC-0 V for 3 h application duration (37.50%). In point of adventitious root number demonstrated in Table 2, adventitious root number ranged from 6.08 (AC-0 V for 3 h) to 13.87 no. (AC-220 V for 1 h). Concerning adventitious root length in Table 2, the highest root length mean was recorded for AC-220 V for 1 h application duration (11.08 cm) when the compared with AC-0 V for 3 h application duration (5.10 cm). As far as adventitious root weight was concerned in Table 2, the lowest adventitious root weight mean was obtained from AC-0 V for 3 h application duration (3.22 g) whereas the highest mean was 4.82 g in AC-220 V for 1 h application duration.

Table 3 show that main effects of application duration of alternating electric current have no significant effects on characteristics of shoot and root ($p<0.05$).

Table 3. Main effects of application duration of alternating electric current on characteristics of shoot and root

	Application duration (hour)			
Characteristics	**1 h**	**2 h**	**3 h**	**$LSD_{5\%}$**
Bud bursting rate (%)	66.40	63.12	59.84	N.S.
Shoot length (cm)	15.22	13.98	13.43	N.S.
Shoot weight (g)	3.69	3.42	3.08	N.S.
Adventitious rooting rate (%)	67.18	64.37	60.62	N.S.
Adventitious root number (no)	10.03	9.56	9.35	N.S.
Adventitious root length (cm)	8.12	7.89	7.49	N.S.
Adventitious root weight (g)	4.06	3.86	3.75	N.S.

Different letters within the same columns denotes significant differences ($p<0.05$) N.S.: Not significant

Among the application durations, the best results were respectively obtained from 1, 2 and 3 h application duration for all characteristics of shoot and root (Table 3). Regarding bud bursting rates, the highest means were respectively recorded for 1, 2 and 3 h application durations (66.40, 63.12 and 59.84%). In terms of shoot length in Table 3, 1 h application duration (15.22 cm) caused the highest shoot length mean compared to 2 and 3 h application durations (13.98 and 13.43 cm). Based on shoot weight in Table 3, 1 h application duration (3.69 g) led to the highest shoot weight mean than 2 and 3 h application durations (3.42 and 3.08 g). In terms of adventitious rooting rates presented in Table 3, 1 h application duration (67.18%) had remarkable effect on adventitious rooting rate when the compared with 2 and 3 application durations (64.37 and 60.62%). With respect to adventitious root number, the highest adventitious root number mean was recorded for 1 h application duration (10.03 no) than 2 and 3 h application durations (9.56 and 9.35 no). As displayed in Table 3, it was observed that 1 h application duration (8.12 cm) led to the highest increase in adventitious root length mean compared to 2 and 3 h application durations (7.89 and 7.49 cm). As far as adventitious root weight was concerned in Table 3, the highest adventitious root weight means were respectively obtained from 1, 2 and 3 h application durations (4.06, 3.86 and 3.75 g).

Table 4 exhibit that main effects of electrical potential of alternating electric current have significant roles on characteristics of shoot and root except for bud bursting rate and adventitious root weight ($p<0.05$).

Table 4. Main effects of electrical potential of alternating electric current on characteristics of shoot and root

	Electrical potential (volt)				
Characteristics	**AC-0 V**	**AC-110 V**	**AC-220 V**	**AC-380 V**	**LSD5%**
Bud bursting rate (%)	54.58	65.62	71.87	60.41	N.S.
Shoot length (cm)	10.69 c	15.41 ab	18.16 a	12.59 bc	3.69
Shoot weight (g)	2.61 c	3.61 ab	4.38 a	2.99 bc	0.95
Adventitious rooting rate (%)	38.54 c	73.96 ab	84.37 a	59.37 b	17.33
Adventitious root number (no)	6.12 b	12.28 a	13.40 a	6.79 b	2.34
Adventitious root length (cm)	5.15 b	9.68 a	10.63 a	5.86 b	1.80
Adventitious root weight (g)	3.32	4.11	4.52	3.63	N.S.

Different letters within the same columns denotes significant differences ($p<0.05$)
N.S.: Not significant AC-0 V : 0 volt alternating current AC-110 V: 110 volt alternating current AC-220 V: 220 volt alternating current
AC-380 V: 380 volt alternating current

As examined in Table 4, it can be seen a linear relationship among the electrical potential values of alternating electric current from AC-0 V to AC-220 V application except for AC-380 V application. Concerning bud bursting rates in Table 4, it can be seen gradually increases among the electrical potential values from AC-0 V to AC-220 V application (54.58, 65.62 and 71.87%). Concerning shoot length in Table 4, the highest shoot length mean was recorded for AC-220 V application (18.16 cm) than AC-0 V application (10.69 cm). Regarding shoot weight in Table 4, AC-220 V application (4.38 g) caused the highest shoot weight mean, while the lowest mean was obtained from AC-0 V application (2.61 g). It was figured out from Table 4 that the AC-220 V application (84.37%) resulted in highest adventitious rooting rate than AC-0 V application (38.54%). While the applications of AC-110 V and AC-220 V (12.28 and 13.40 no) were causing the highest adventitious root number means, the lowest means were obtained from applications of AC-0 V (6.12 no) and AC-380 V (6.79 no) (Table 4). As far as adventitious root length was concerned in Table 4, the applications of AC-110 V and AC-220 V (9.68 and 10.63 cm) led to the highest adventitious root length means when the compared with AC-0 V application (5.15 cm). In respect of adventitious root weight in Table 4, AC-220 V application (4.52 g) caused the highest adventitious root weight mean than AC-0 V application (3.32 g).

CONCLUSIONS

Some grapevine rootstocks used in viticulture can have difficulties in rooting of their hardwood cuttings. In this case, it can be taken different measures to overcome this difficulty.

The results of present study, in which alternating electric current applications were used to increase the rooting rates of hardwood cuttings, revealed that decreasing application durations such as 3, 2 and 1 hour had crucial roles on rooting characteristics of hardwood cuttings. Likewise, increasing electrical potential values from AC-0 to AC-220 V application also caused significant improvements in rooting characteristics of hardwood cuttings.

As a result, the best rooting characteristics of hardwood cuttings from 140 Ru grapevine rootstock were especially obtained from AC-220 V for 1 hour application.

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RESPONSE OF CALLUSING ATTRIBUTES OF CABERNET SAUVIGNON/5BB GRAFTING COMBINATION TO APPLICATIONS OF DIFFERENT PHENOLIC COMPOUNDS

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ABSTRACT

Phenolic compounds have remarkable roles in regulation of plant development and growth and these compounds enhance plant vigor under the biotic and abiotic stress conditions. This research was conducted to find out influences various doses of different phenolic compound applications on Cabernet Sauvignon/5BB grafting combination. In order to improve grafting characteristics, it was benefited from three doses (Control, 100 and 200 ppm) of three different phenolic compounds (benzoic acid, citric acid and oxalic acid). In the research, after omega grafting procedure was completed, these phenolic compounds at varying doses were applied onto grafting points of the grafted cuttings. Despite the fact that various doses of different phenolic compound applications had changing effects on characteristics of shoot and grafting, the favorable results were respectively recorded for increasing doses of oxalic acid (OA), benzoic acid (BA) and citric acid (CA) for grafting characteristics. Consequently, the best results for grafting characteristics were especially obtained from applications of OA 100 ppm and OA 200 ppm for Cabernet Sauvignon/5BB grafting combination.

Keywords: *Grapevine rootstock, Omega grafting, Callus promoting chemicals, Benzoic acid, Oxalic acid, Citric acid*

INTRODUCTION

Grafting, consisting of cutting and scion so that both these plant parts grow together as one, is broadly used in horticulture as a method to improve disease resistance, tolerance to abiotic stress, fruit quality and plant size (Mudge et al., 2009).

Since grapevine rootstocks increase tolerance to various biotic and abiotic stresses agents, grafting is a viticultural technique frequently used in world viticulture (Cookson et al., 2013). For this reason, the grafting procedure is very important and includes complex biochemical and structural changes in the course of the adhesion of two grafted partners, followed by callus formation and the establishment of a functional vascular system (Hartmann et al., 1990; Cookson et al., 2013).

When the grape growers are grafted sensitive *Vitis vinifera* cultivars onto grapevine rootstocks by omega grafting method, they can encounter incompatibility problem in some scion/rootstock combinations (Çelik, 2011; Kok and Bahar, 2017a, b; Kok, 2018).

Successful grafting is associated with callus production, which is essential for graft union formation (Hartmann et al., 1990). Therefore, it should be taken some precautions such using of various plant growth regulators, plant growth enhancing bacteria and chemical compounds for improving callus formation at graft union (Kok and Bahar, 2017a, b; Kök, 2017; Köse, 2005; 2006).

Benzoic acid is naturally synthesized by plants and classified in the group of carboxylic acids. It seems to work like a stress signaling compound and is potentially known to provide abiotic stress tolerance (Shater Cabd Allah et al., 2015).

Citric acid is antioxidant and anti-stress agent and also act as a signaling molecule in some plant physiological processes and defense mechanisms (El-Kobisy et al., 2005). Because citric acid has stimulatory effects on growth and productivity of most plants, it is considered to have auxinic actions (Ragab, 2002).

Oxalic acid (OA) is a phenolic acid ubiquitously present in living organism that plays a considerable role in different metabolic process in plants (Shimada et al., 1997).

The purpose of present study was to find out influences of various doses of different phenolic compound applications on shoot and callusing attributes of Cabernet Sauvignon/5BB grafting combination under the callusing room conditions.

MATERIAL AND METHODS

Study site

The current research was performed at Tekirdağ Namık Kemal University, Agriculture Faculty in grafting unit of Horticulture Department in the course of 2014 year.

Plant materials

In the study, it was utilized from scions of Cabernet Sauvignon wine grape cultivar and one-year-old cuttings of 5BB (Berlandieri x Riparia Teleki 8B, Selection Kober 5BB) as plant materials.

Callus-enhancing chemicals used in the research

In order to improve callus attributes in Cabernet Sauvignon/5BB grafting combination, benzoic acid ($C_7H_6O_2$, ≥99.5%, Sigma-Aldrich), citric acid ($C_6H_8O_7$, ≥99.5%, Sigma-Aldrich) and oxalic acid ($C_2H_2O_4$, ≥99%, Sigma-Aldrich) from phenolic compounds were used and preferred doses of these phenolic compounds were respectively Control, 100 and 200 ppm (Table 1).

Table 1. Various doses of different phenolic compound applications used in the study

C	
BA 100 ppm	CA 200 ppm
BA 200 ppm	OA 100 ppm
CA 100 ppm	OA 200 ppm

C : Control BA 100 ppm : Benzoic acid 100 ppm BA 200 ppm : Benzoic acid 200 ppm CA 100 ppm : Citric acid 100 ppm CA 200 ppm : Citric acid 200 ppm OA 100 ppm : Oxalic acid 100 ppm OA 200 ppm : Oxalic acid 200 ppm

Providing of scions and cuttings and their storage up to the time of omega grafting

During the dormant period, scions and cuttings were successively taken from a commercial vineyard and mother grapevines of rootstock grown in Tekirdağ Viticultural Research Institute in Tekirdağ, Turkey. Afterward, these mentioned materials were stored at cold storage with 1-4 °C temperature and 85-90% relative humid until the time of omega grafting (Çelik, 2011).

Omega grafting procedure and applications of callus-enhancing chemicals

Prior to omega grafting procedure, cuttings were prepared to be 30-35 cm long and scions with single winter bud were prepared to be 4-6 cm long. Omega grafting operations were

performed at first week of April 2014. In the course of the omega grafting procedure, care should be paid to the fact that diameters of scion and cutting to be used in omega grafting were close to each other. After all omega grafting applications were completed, grafting point of grafted cuttings were dipped into solutions of various doses of different phenolic compounds applications for 1 hour (Table 1). Thereafter, all grafted cuttings were dried for 5 minutes and paraffinized. Later, grafted cuttings in plastic boxes with the water at the bottom were transferred to callusing room and were kept in here under the conditions at 28-30 °C temperature and 85-90% relative humid for 21 days. During this period, related operations were respectively carried out in callusing room (Korkutal et al., 2011).

Calculations and measurements

After grafted cuttings were taken out from the callusing room, some calculations and measurements, including bud viability rate (%), bud bursting rate (%), shoot length (cm), shoot weight (mg) as shoot characteristics and callus growth rate at grafting point (%), callus weight of scion (mg) and callus weight of cutting (mg) as grafting characteristics were respectively conducted.

Statistical analysis

The study was laid out in accordance with completely randomized blocks with 4 replicates and each replicate consisted of 15 omega grafted cuttings. Statistical analyzes were conducted by statistical software of TARIST. Differences among the means were compared by Fisher's Least Significant Difference (LSD) test at 5% level.

RESULTS AND DISCUSSION

Shoot characteristics

Table 2 presents the influences of various doses of different phenolic compound applications on bud viability rate, bud bursting rate, shoot length and shoot weight and bud bursting rates were statistically affected by various doses of different phenolic compound applications ($P<0.05$).

Table 2. Influences of various doses of different phenolic compound applications on shoot characteristics

Applications	Bud viability rate (%)	Bud bursting rate (%)	Shoot length (cm)	Shoot weight (mg)
C	70.00	50.00c	4.29	363.75
BA 100 ppm	70.00	50.00c	5.15	390.00
BA 200 ppm	70.00	55.00bc	5.77	412.50
CA 100 ppm	80.00	80.00a	7.25	567.50
CA 200 ppm	85.00	85.00a	7.55	612.50
OA 100 ppm	75.00	70.00abc	6.13	462.50
OA 200 ppm	78.75	75.00ab	6.41	501.25
$LSD_{5\%}$	N.S.	22.46	N.S.	N.S.

Different letters within the same columns denotes significant differences ($p<0.05$) N.S.: Not significant C : Control BA 100 ppm : Benzoic acid 100 ppm BA 200 ppm : Benzoic acid 200 ppm CA 100 ppm : Citric acid 100 ppm CA 200 ppm : Citric acid 200 ppm OA 100 ppm : Oxalic acid 100 ppm OA 200 ppm : Oxalic acid 200 ppm

Concerning bud viability rates displayed in Table 2, although there were no significances difference among the various doses of different phenolic compound applications (P<0.05), CA 200 ppm application caused the highest bud viability rate (85.00%) than C application (70.00%).

With respect to bud bursting rates given in Table 2, it was observed that various doses of different phenolic compound applications led to significant differences in bud bursting rates (P<0.05). The study findings revealed that the highest bud bursting rates were respectively recorded for applications of CA 100 ppm (80.00%) and CA 200 ppm (85.00%) than C application (50.00%).

As far as shoot length was concerned in Table 2, various doses of different phenolic compound applications had no significant effects on shoot length (P<0.05). While the lowest shoot length mean was 4.29 cm for C application, the highest mean was obtained from CA 200 ppm application (7.55 cm).

Shoot weight means in Table 2 were not significantly affected by various doses of different phenolic compound applications (P<0.05) and CA 200 ppm application resulted in the highest shoot weight mean (612.50 mg) when the compared with C application (363.75 mg).

Grafting characteristics

It was evidently observed in Figure 1 that various doses of different phenolic compound applications significantly affected callus growth rate at grafting point (P<0.05) and

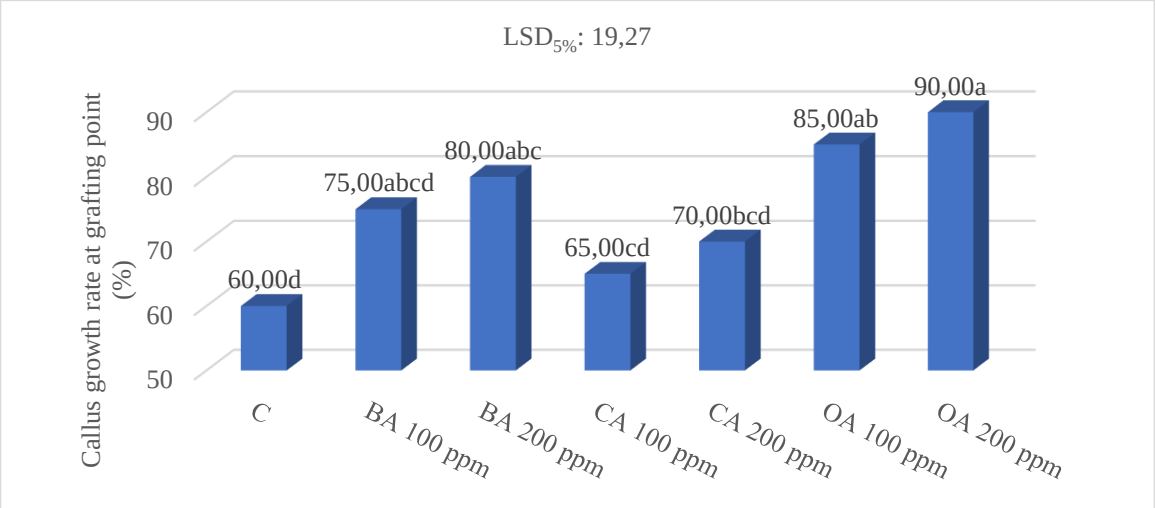

Figure 1. Influences of various doses of different phenolic compound applications on callus growth rate at grafting point

callus growth rates at grafting point ranged from 60.00 (C application) to 90.00% (OA 200 ppm application).

As indicated in Figure 2, callus weight means of scion were not significantly affected by various doses of different phenolic compound applications (P<0.05). The highest callus weight mean of scion was obtained from OA 200 ppm application (239.74 mg) whereas the lowest callus weight mean of scion was 67.76 mg for C application.

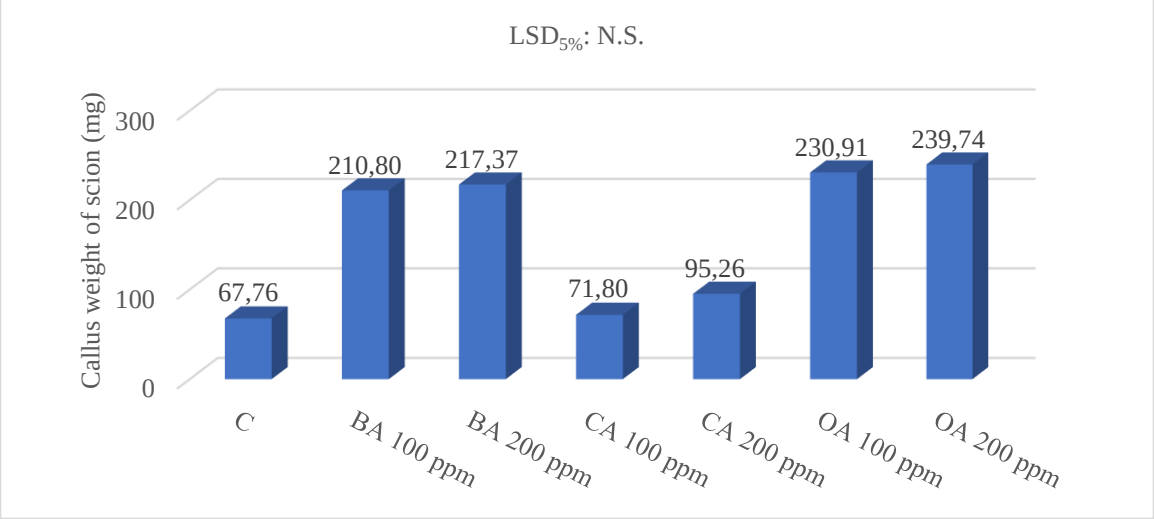

Figure 2. Influences of various doses of different phenolic compound applications on callus weight of scion

Regarding callus weight means of cutting displayed in Figure 3, there were significant differences among the various doses of different phenolic compound applications (P<0.05) and the highest callus weight of cutting was obtained from OA 200 ppm application (721.80 mg) whereas the lowest callus weight of cutting was 282.36 mg for C application.

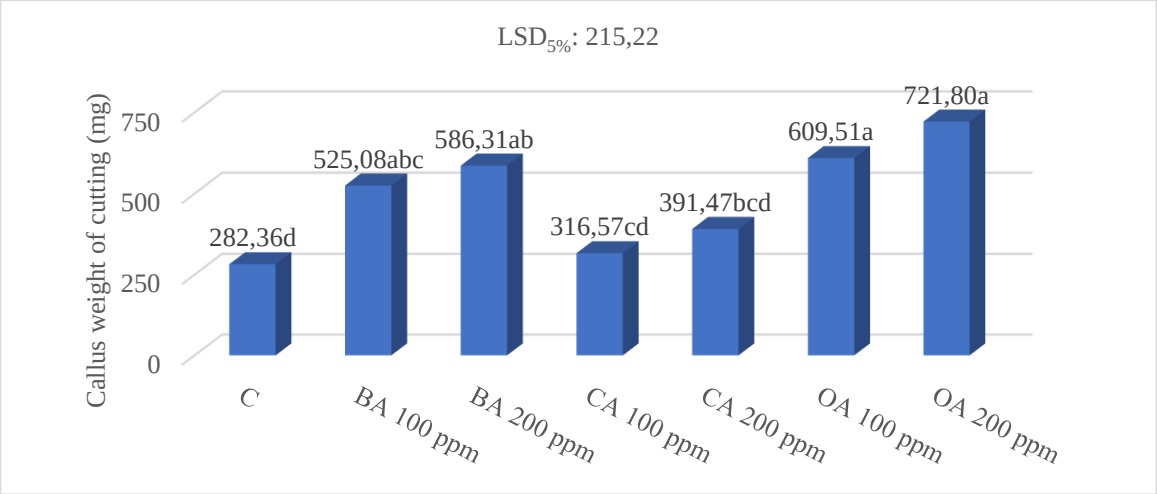

Figure 3. Influences of various doses of different phenolic compound applications on callus weight of cutting

CONCLUSIONS

Good callus formation between scion and rootstock is a desirable situation for grapevine sapling production. Despite the fact that various doses of different phenolic compound applications had varying effects on characteristics of shoot and grafting, it was especially observed that oxalic acid applications had advantageous effects on characteristics of omega grafting. As a result, applications of OA 100 ppm and OA 200 ppm particularly caused the best results for callusing attributes, contributing to improvements in success rate of omega grafting in Cabernet Sauvignon/5BB grafting combination.

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DETERMINATION OF FATTY ACID COMPOSITION OF SESAME (*SESAMUM INDICUM* L.) GROWN IN ÇUKUROVA

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ABSTRACT

With its oil content and quality sesame (*Sesamum indicum* L.) is one of the oldest and the most important oil plant cultured in the world. The most important feature of sesame oil is its resistance to oxidative deterioration. In this study, fatty acid compositions of some local sesame seeds cultivated in Çukurova were determined. As a result of this study, it was determined that the composition of fatty acids is made up of palmitic acid (9.21-9.78 %), stearic acid (4.64-5.08 %), oleic acid (37.50-40.13 %), linoleic acid (42.64-44.56 %) arachidic acid (0.56-0.60 %) and linolenic acid (0.34-0.40 %). Palmitic acid content in saturated fatty acid was the highest in the Cumhuriyet 99 (9.78 %) while the oleic acid value of the unsaturated fatty acid was found to be the highest in Muganlı 57 (40.13 %). The location affected the rates of palmitic acid, stearic acid and arachidic acid, while planting condition affected linoleic acid and palmitic acid. From the results of this study, it can be concluded that the fatty acid profile of the sesame oil was significantly influenced by genotypes.

Keywords: *Sesame, Sesame oil, Fatty acid composition, Palmitic acid, Oleic acid, Çukurova*

INTRODUCTION

Sesame (*Sesamum indicum* L.) is one of the oldest oil crops in the world that has been cultivated for about 4000 years (Morris, 2002). Today it is mainly produced in Asian countries like India, China, Afghanistan, Pakistan, Bangladesh, Indonesia and Sri Lanka. Sesame ranks 5th in oilseeds produced in Turkey. Sesame is widely used as vegetable oil (77.6%) and the other part is consumed in pastry (20.1%) and seed (2.3%) (Tan and Kaya, 2001). In addition to being an important oil seed, it has also found a wide range of applications in the pharmaceutical and cosmetic industries due to the antioxidant compounds it contains. Though sesame oil is edible oil, its use as vegetable oil in our country is limited because it is not economical to use. Sesame seeds, on the other hand, constitute raw material in the production of tahini and tahini halva in our country. It is also used in spices and pastry products. Sesame varieties have four different colours, white, yellow, brown and black (Dashak and Fali, 1993). Varieties contain high fat, protein and essential amino acids. Sesame seeds are especially rich in amino acids such as lysine, methionine and cysteine. Sesame seeds contain 40-60% fat. The most common fatty acids in sesame oil are; oleic acid (35.9-42.3%), linoleic acid (41.5-47.9%), palmitic acid (7.9-10.2%), stearic acid (4.8-6.1%), low linolenic acid (0.3-0.4%) and low arachidic acid (0.3% -0.6%) (TFCC, 2012).

Sesame oil is widely used in the manufacture of medicines, paints, margarines, soaps, cosmetics, and insecticides, as well as foodstuffs. After removing the oil from the sesame seeds, 43% of crude protein is present in the remaining seeds. For this reason, it has an important place in animal feeding. Sesame seeds are also considered as human food by adding bread in some countries (Ilısulu, 1973). Due to the shortening of the growing period, low production inputs and the rotation with many plants, sesame is considered to be important crop plants

(Atakisi, 1985). Due to the fact that the growing period is short, it is possible to alternate with all kinds of cultivated plants.

In the world sesame cultivation area is 7.7 million hectares and production is 4 million tons. In Turkey, sesame cultivation area is 28017 hectares, while production is 21036 tonnes (FAO, 2009). Turkey has 0.69% of world sesame production (FAO, 2009). The climate conditions of the Aegean, Mediterranean and South-eastern Anatolian regions of our country are suitable for sesame cultivation, the short growing time of sesame, low production cost and possibility of growing as second crop increase the importance of sesame furtherly.

Sesame is grown as a second crop after harvest in the southern and south-eastern provinces of Turkey after wheat and barley farming, and makes a substantial contribution to the national economy. The fact that the harvest is done by hand provides an important workforce in the regions where it is grown (Arioglu, 2007). Therefore, sesame is one of the most important crops in Turkey. It is grown in the South-eastern Anatolia Region of Turkey mostly.

In this study, some local sesame seeds cultivated in Çukurova were investigated for fatty acid composition in seed oil.

MATERIAL AND METHODS

In this research, 8 different local sesame seeds were used. Three of these varieties (Muganlı-57, Özberk -82, Baydar-2001) belong to the Western Mediterranean Agricultural Research Institute and five of these varieties (Kepsut-99, Cumhuriyet-99, Osmanlı-99, Tan-99, Orhangazi-99) were registered by the Aegean Agricultural Research Institute. These Sesame varieties were planted as the main crop and second crop in the trial area of Eastern Mediterranean Agricultural Research Institute and in the trial area of Tarsus Soil and Water Resources campus.

Extraction of sesame seed oil

10 g of sesame seeds from each variety were milled in a laboratory mixer and transferred to Soxhlet cartridges. The cartridges were placed in hangers in the extraction beaker and 150 ml of petroleum ether (boiling point of 40-60 ºC) was added. After 4 hours at 160 ºC, the cartridges were removed from the extraction beakers and the extraction beakers were dried at 103 ºC for 1 hour and cooled in a desiccator. As a result of the weighing, the oil of the samples was calculated as percentage (AOAC, 1990).

Fatty Acid Analysis by Gas Chromatography

The extracted sesame oils were analysed by gas chromatography (GC) after the esterification. GC equipment featured a capillary column (Fused silica, 100 m × 0.25 mm × 0.2 μm) and a flame ionization detector (FID), (Agilent 7890A, Agilent Technologies, USA). The GC conditions used to determine fatty acid methyl ester (FAME) were as follows: an injection volume of 1μL; a temperature program of 175°C for 10 min, 5°C/min to 210°C, 5°C/min to 230 °C and a final temperature of 230°C for 15 minutes; a detector temperature of 260°C; an injector temperature of 250°C; a gas carrier flow of N_2, 1mL min−1; a 1:20 split and a total run time of 58.5 minutes (TFCC, 2010)

Statistical Evaluations

In this study, the fatty acid results of sesame varieties were analysed according to split parcel trial design divided by random blocks using JMP 7.0. statistical package program. The data obtained from the study were subjected to analysis of variance. The Least Significant

Difference (LSD) test was used to compare the statistical significance of the differences between the varieties.

RESULTS AND DISCUSSION

Palmitic Acid

As shown in Table 1, palmitic acid were affected from location ($P < 0.05$), growing season ($P < 0.01$) and variety ($P < 0.01$). Looking at the interactions between the parameters, It was determined that there was a statistically significant effect on the palmitic acid ratio of location *variety ($P < 0.05$), growing season*variety ($P < 0.05$) and location*growing season ($P < 0.01$) interactions. However, the interaction between location*growing season*variety was not statistically significant.

According to the results of multiple comparisons, it was determined that the rates of palmitic acid were higher in Adana location (9.55%) and main crop conditions (9.56%), while the highest rate of palmitic acid in varieties was found in Cumhuriyet 99 (9.78%). The lowest palmitic acid level was found in Tarsus location (9.41%) and in secondary crop conditions (9.40%). Also, it was observed that the lowest palmitic acid level belongs to Muganlı 57 variety (9.21%) among the varieties. The results of this study are in correlation with the study of Baydar (2005) and Bahkali et al. (1998).

Stearic Acid

As shown in Table 1, location ($P < 0.05$) and variety ($P < 0.01$) were statistically significant effects on stearic acid according to the variance analysis. However, it was determined that the growing season had no significant effect on the stearic acid ratios. When the relationship between the parameters was considered, the interaction of location*growing season ($P < 0.05$) was found to have a statistically significant effect on the stearic acid ratios but the interaction of location*variety, growing season*variety and location*growing season*variety was not important as the statistically.

According to the results of multiple comparisons, it was determined that the stearic acid ratio (4.87%) was higher in the Adana location, while the highest stearic acid ratio in the varieties was found to be in the Osmanlı 99 (5.08%). It was seen that the lowest stearic acid level was in the Tarsus location (4.75%) and Orhangazi 99 variety (4.64%). Stearic acid rates were primarily affected by variety and location, but not by growing season. The results of this research are in harmony with the study of Baydar (2005) and Bahkali et al. (1998).

Oleic Acid

As shown in Table 1, according to the variance analysis performed, variety ($P < 0.01$) has a statistically significant effect on oleic acid. However, it had been determined that there was no significant effect on the oleic acid ratios of the location and growing season. It was determined that there was a statistically significant effect on the oleic acid ratios of the interaction between location*growing season ($P < 0.01$) and location*growing season*variety ($P < 0.05$). But the interaction of location*variety and growing season*variety was not important, statistically.

Table 1. Summary of the variation analysis from fatty acid composition

Parameters	Palmitic Acid (%)	Stearic Acid (%)	Oleic Acid (%)	Linoleic Acid (%)	Arachidic Acid (%)	Linolenic Acid (%)
Locations						
Adana	9.55a	4.87a	38.83	43.50	0.57a	0.37
Tarsus	9.41b	4.75b	38.45	43.88	0.56b	0.38
LSD $_{0.05}$	0.076	0.084	-	-	0.0058	-
Growing Season						
Main Crop	9.56a	4.78	38.97	43.17b	0.57	0.38
Second Crop	9.40b	4.85	38.30	44.20a	0.57	0.37
LSD $_{0.05}$	0.048	-	-	0.394	-	-
Varieties						
Muganlı 57	9.21d	4.89b	40.13a	42.64c	0.57b	0.34b
Baydar 2001	9.38c	4.83bc	39.63a	42.99c	0.56b	0.37ab
Özberk 82	9.55b	4.80b-d	39.46a	43.11c	0.56b	0.37ab
Kepsut 99	9.40c	4.71de	37.88b	43.50bc	0.57b	0.37a
Cumhuriyet 99	9.78a	4.81b-d	38.08b	44.19ab	0.56b	0.39a
Tan 99	9.59b	4.72c-e	38.26b	44.09ab	0.57b	0.37ab
Osmanlı 99	9.66ab	5.08a	37.50b	44.44ab	0.60a	0.40a
Orhangazi 99	9.28cd	4.64e	38.14b	44.56a	0.56b	0.40a
LSD $_{0.05}$	0.145	0.113	0.782	0.847	0.0172	0.031
CV(%)	2.57	2.65	2.15	2.59	3.3	10.81
Variance Analysis P Values						
Location	0.0118*	0.0209*	0.0744 NS	0.3091 NS	0.0101*	0.1172NS
Growing Season	0.0002**	0.1764 NS	0.0552 NS	0.0007**	0.5082NS	0.2150NS
Variety	<.0001**	<.0001**	<.0001**	<.0001**	0.0013**	0.0152*
Location*Variety	0.0163*	0.7247 NS	0.0679 NS	0.0535 NS	0.6634NS	0.2027NS
Location*Growing Season	0.0033**	0.0305*	0.0063**	0.0001**	0.2612NS	0.0452*
Growing Season*Variety	0.0338*	0.1140 NS	0.1258 NS	0.2200 NS	0.0455*	0.2182NS
Location*Growing Season*Variety	0.1121 NS	0.1789 NS	0.0123*	0.2570 NS	0.2607NS	0.1818NS
* p< 0.05; ** p< 0.01 important within error boundaries, NS: Not Significant						

According to the results of multiple comparisons, the highest oleic acid ratio was found in Muganlı 57 variety (40.13%) and the lowest oleic acid ratio was found in Osmanlı 99 variety (37.50%) in the studied varieties. Oleic acid ratios were affected by variety but not by location and growing season. Baydar (2005) and Bahkali et al. (1998) had similar results to the results of this research.

Linoleic Acid

As shown in Table 1, according to the analysis of variance, growing season ($P < 0.01$) and variety ($P < 0.01$) were statistically significant effects on linoleic acid. Also, it was determined that there was no statistical effect of the location on linoleic acid. The interaction of location*growing season ($P < 0.01$) had a statistically significant effect on linoleic acid ratios. However, it was seen that the interaction between the location*variety, growing season*variety and the location*growing season*variety was not significant effect statistically.

According to the results of multiple comparisons, linoleic acid ratio (44.20%) was found to be higher in the second crop conditions, while the highest linoleic acid ratio was found in Orhangazi 99 variety (44.56%) among the all varieties. The lowest linoleic acid ratios were found in the main crop condition (43.17%) and belong to Muganlı 57 variety (42.64%). Linoleic acid ratios were primarily affected by variety and growing season, and were not affected by location. Baydar (2005) and Bahkali et al. (1998) had similar results to the results of this research.

Arachidic Acid

Arachidic acid rates were high in Adana location (0.57%) and low in Tarsus location (0.56%). Among the varieties, the highest ratio of arachidic acid was found in Osmanlı 99 variety (0.60%). It was determined that arachidic acid ratios were primarily affected by variety and location, respectively, but not by growing season.

Linolenic Acid

The highest linolenic acid ratio was found to be in the Osmanlı 99 and Orhangazi 99 varieties (0.40%) and the lowest linolenic acid ratio belonged to Muganlı 57 variety (0.34%) among the varieties. Also, It was determined that the linolenic acid ratios were affected by variety, and were not affected by location and growing season.

CONCLUSIONS

In recent years, sesame seeds, which can be replaced with all kinds of cultivated plants due to the shortness of growing period, have been planting widely in the Aegean, Mediterranean and South-eastern Anatolia regions as the second crop after the cereals planting. The increase in sesame production will be possible in the different regions of our country, with the its suitability as main and second crops for sesame cultivation, with varieties have to be high yield potency in dry and watery conditions. As a result of this study, it was determined that palmitic acid ratios, stearic acid ratios, oleic acid ratios, linoleic acid ratios, arachidic acid ratios and linolenic acid ratios was in the range of 9.21-9.78%, 4.64-5.08, 37.50-40.13, 42.64-44.56, 0.56-0.60 0.34-0.40%, respectively. While the location has effect on palmitic acid, stearic acid and arachidic acid, growing season affects linoleic acid and palmitic acid. The varieties were statistically effective on all given fatty acids. The relationship between location and growing season was also found to be significant in all fatty acids except arachidic acid. The Çukurova region has suitable climate and favourable soil structure for sesame growing. It is thought that sesame, which can be grown both as a main crop and a second crop, will make a significant contribution to the country's economy. In this study, it was shown that the fatty

acid compositions of local sesame varieties differ in Çukurova conditions and the numerical values of these differences were determined and contributed to the literature.

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COMPARISON OF THE CHEMICAL COMPOSITION AND STEVIOL GLYCOSIDE PROFILES OF *STEVIA REBAUDIANA* (BERTONI) VARIETIES CULTIVATED IN SOUTH OF TURKEY

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ABSTRACT

This study was carried out to determine the chemical composition and total steviol glycoside (TSG) content of some stevia varieties cultivated in Adana. For this reason, Criolla and Morita II leaves were used. Additionally, the results were compared with each other in terms of commercial production. Some physical properties (harvesting period, height of plant, leaf width and length, total carbohydrate and dietary fiber contents and crude protein content) of the samples were determined. Steviol glycoside analysis was performed using HPLC device. As a result, harvesting period of Morita II was shorter than Criolla and the TSG content of Morita II was 17.58%, while the ratio of Reb A and Stevioside were 13.37% and 3.10%, respectively. It was determined that the content of TSG and Reb A of Morita II was higher than that of Criolla, but the content of stevioside was lower. These results indicated that the Morita II variety has a better quality than Criolla for commercial production.

Keywords: *Stevia, Criolla, Morita II, Steviol glycosides, Chemical composition.*

INTRODUCTION

Stevia (*Stevia rebaudiana* Bertoni) is a wild, small, bush-like plant of the *Asteraceae* family, and its homeland is the Amambi mountains on the border of Paraguay and Brazil. It is known that it has been extensively used by the indigenous tribe Guarani who lived in South America for 1500 years. Stevia is named with different names such as "sweet herb" and "honey leaf" by the Paraguayan Indians. Stevia is a sweet plant of semi-humid subtropical regions and its length is 60-90 cm. It can grow at about 25˚C and some species can grow at 2300-2900 meters altitude (İnanç and Çınar, 2009; Yapar, 2004; Pala, 2010; Karaca, 2010; Kola, 2017). Stevia can be grown between 45th northern latitudes and 35th southern latitudes. Stevia is processed in 7 countries including Turkey. Stevia production is mostly done in China followed by Paraguay, Brazil and India. The highest consumption is in USA followed by Japan, China and France. In 2017, stevia was grown in Turkey about 1260 decares (about 500-600 tons of dry leaves) (Kola, 2015; Kola, 2017).

In 1897, the famous Swiss botanist Moisés Santiago Bertoni made a detailed explanations about stevia, which is a new sweet sugar source known by indigenous people living in Central and South America for a long time, and its sweetening properties as a result of a study in the eastern parts of Paraguay. In 1931, two French chemists (M. Briedel and R. Lavieille) isolated glycosides, which gave Stevia's unique sweetening properties. These compounds have been identified as Stevioside and Rebaudioside and have been found to be 250-300 (~520) times more sweet, non-fermented sweetener

with high temperature and pH stability compared to sucrose (Wallin, 2007; Kola et al., 2011; Kola, 2013; Kola et al., 2013; Kola, 2015; Kola, 2017). Stevia is a plant with high adaptability, high yield and high degree of sweetness. Main composition of dry stevia leaf is given in Table 1.

Table 1. Main composition of dry stevia leaf (Karaca, 2010)

Component	Amount
Moisture (g)	7
Energy (kcal)	270
Protein (g)	9.8
Fat (g)	2.5
Carbohydrate (g)	52
Ash (g)	10.5
Crude fiber	18.5
Minerals	
- Calcium (mg)	464.4
- Phosphorus (mg)	11.4
- Iron (mg)	55.3
- Sodium (mg)	190
- Potassium (mg)	1800
Antinutritional factors	
- Oxalic acid	2295
- Tannins	0.01

Steviol glycosides are non-toxic substances which are known as healthy, low calorie (the calorie value of sugar obtained from sugar cane is 4 Kcal / g 16.7 KJ / g and the steviol glycoside have only 0.003% of the calorie of sugar obtained from sugar cane), high density sweeteners (250-520 times sweeter than the sugar obtained from sugar cane) with their own unique taste (Savita, 2004; Kola and Kenar, 2014). It is stable at high temperature values and wide pH ranges. When it is added to a foodstuff, the foodstuff is not denaturated and easily spoilt. They are resistant to acid and alkaline conditions. The foodstuffs which are added steviol glycoside can be preserved for a long time without drying or mold (Kola, 2015; Kola, 2017).

Steviol glycosides can be substituted for sugar in obesity, diabetes, hyperlipidemia, coronary diseases and phenylpropanoid ketone patients (PKU). At the same time, it can also serve as a supporter for the cure. It helps preventing tooth decay and enteric diseases. Steviol glycosides are not digestible. They do not cause an increase in blood sugar and insulin concentration after taking in the body. They are suitable for diabetes patients. According to clinical studies, stevia is particularly effective in improving insulin sensitivity in Type 2 diabetes. Steviol glycosides can help growing of bifidobacteria and lactobacilli. These bacteria produce acetic acid, propionic acid, butyric acid and lactic acid, which inhibit the growth of pathogens such as *Escherichia coli*, *Clostridium* and *Salmonella* sp. by lowering the intestinal pH by fermenting the carbohydrates in the leaves. Steviol glycosides reduce the formation of deterioration and helping to relieve intestinal peristalsis and constipation. The molecular structure of steviol glycoside determines its lipid adsorption ability. Steviol glycoside helps lowering the concentration of cholesterol and triglycerides and reducing the Low-Density Lipoprotein (LDL) concentration in the human body. HDL / LDL ratio increases because it is not effective on the High

Density Lipoprotein (HDL) concentration. In a study of the function and tolerance of steviol glycosides when taken orally, steviol glycoside was found to decrease the systolic and diastolic blood pressure ($P < 0.05$) and no obvious side effects were observed. The Stevia fermentation extract has a marked function on histamine antagonist (antihistamine), has allergic rhinitis, hives (urticaria) and asthma relieving effects (Kola, 2015; Kola, 2017). When Stevia sweeteners are classified, they are in the class of natural, calorie-free and high-density sweeteners as seen on Figure 1.

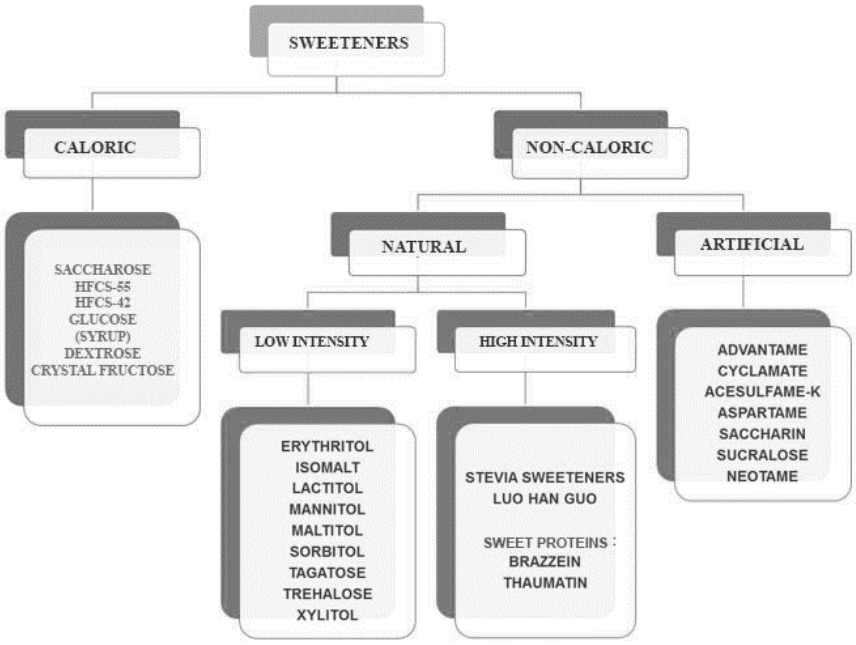

Figure 1. Classification of sweeteners

TSG (Total Steviol Glycoside) is produced after extraction and purification processes from dry stevia leaves (Figure 2). Rebaudioside A and other steviol glycosides are obtained by TSG separation techniques, purification or enzymatic processes (Karaca, 2010; Kola, 2017).

Stevia-derived sweeteners are approved by the FDA, EFSA and JECFA. These regulations are given in Table 2 (Kola, 2017).

There are several studies on stevia varieties grown in our country (especially *Stevia rebaudiana* Bertoni). However, there is no study comparing the characteristics of the wild variety Criolla and the industrial variety Morita II. Many of these studies have been done by Kola et al. (Kola et al., 2011; 2013; 2016a; 2016b; Kola, 2013; Kola and Kenar, 2014; Kola, 2015; Kola, 2017). This study was carried out to determine the chemical composition and total steviol glycoside (TSG) content of some stevia varieties cultivated in Adana. For this reason, Criolla and Morita II leaves were used. Additionally, the results were compared with each other in terms of commercial production.

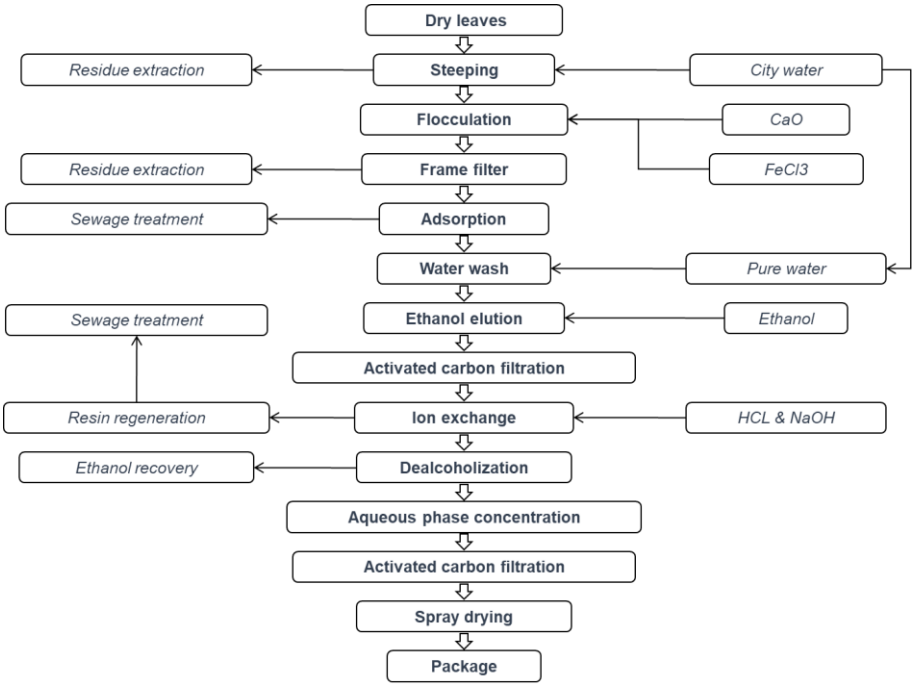

Figure 2. Extraction process of TSG (total steviol glycoside) and Reb A (Rebaudioside A) from dry stevia leaf (Kola, 2015; Kola, 2017)

Table 2. Some regulations about safety of stevia-derived sweeteners

Regulation number	Regulation
07 – 2004	The Codex Alimentarius Commission, which acknowledges that Stevia is safe on the human body, has reached an agreement with JECFA.
12 – 2008	FDA accepted RebA %95 as GRAS. After a while, Australia, New Zealand, Switzerland, Taiwan and France accepted RebA %95 as GRAS.
03 – 2010	FDA accepted RebA %60, %80 and TSG%90 as GRAS.
04 – 2010	EFSA put some restrictions on the safety of stevia glycoside on human body. (ADI: 4 mg/kg).
07 – 2011	EU published the G/SPS/N/EEC/409 report. In the Commission's draft regulation, the regulation of the food additive in the second part of EC regulation 1333/2008 was revised and the authority of the use of stevia glycosides was referred. According to the information, long-term use of stevia is good for patients with cardiovascular, hypertension and diabetes.
06 – 2013	According to Turkish Food Codex Regulation on Food Additives, steviol glycosides (E 960) is allowed to use as sweetener in Turkey.

MATERIALS AND METHODS

Materials

The dry leaves of Criolla and Morita II varieties were obtained directly from the producers in Adana. The chemicals used in the analyzes and preparation of standard solutions were obtained from "Sigma-Aldrich" and "Merck". Stevia active substances to be taken for this purpose; Stevioside (50956), Stevioside hydrate (S3572), Steviol (19345), Steviol hydrate (H8664), Steviolbioside

(59754), Rebaudioside A, B, C and D (01432, 49747, 30987, 19819), Dulcoside A (90387) standards were obtained from Sigma-Aldrich.

Steviol Glycoside Analysis

Steviol glycosides were analyzed according to the method of "FAO JECFA Monographs 10" by HPLC (FAO, 2010). The injection volume was 5 µL and the mobile phase was 32:68 acetonitrile and 10 mmol/L: sodium phosphate buffer (pH 2.6). Capcell pak C18 MG II column and UV-210 nm detector was used. The analysis was performed at a flow rate of 1.0 mL/min for 30 min and the column temperature was 40°C. Identify the peaks from the sample solution by comparing the retention time with the peaks from the mixture of steviol glycosides standard solution (see under figure). Measure the peak areas for the steviol glycosides from the sample solution. Measure the peak area for stevioside and rebaudioside A from their standard solutions.

Calculate the percentage of each of the eight steviol glycosides neexcept rebaudioside A in the sample from the formula (FAO, 2010):

$$\%X = [W_S/W] \times [f_X A_X/A_S] \times 100$$

Calculate the percentage of rebaudioside A in the sample from the formula (FAO, 2010):

$$\%\text{Rebaudioside A} = [W_R/W] \times [A_X/A_R] \times 100$$

Where:

X is each steviol glycoside;

W_S is the amount (mg) calculated on the dried basis of stevioside in the standard solution;

W_R is the amount (mg) calculated on the dried basis of rebaudioside A in the standard solution;

W is the amount (mg) calculated on the dried basis of sample in the sample solution;

A_S is the peak area for stevioside from the standard solution;

A_R is the peak area for rebaudioside from the standard solution;

A_X is the peak area of X for the sample solution; and

f_X is the ratio of the formula weight of X to the formula weight of stevioside: 1.00 (stevioside), 1.20 (rebaudioside A), 1.00 (rebaudioside B), 1.18 (rebaudioside C), 1.40 (rebaudioside D), 1.16 (rebaudioside F), 0.98 (dulcoside A), 0.80 (rubusoside) and 0.80 (steviolbioside).

Calculate the percentage of total steviol glycosides. In addition to the general analyzes (total dry matter, humidity, etc.), the following measurements and analyzes were performed on the obtained samples. These were harvesting period (day), height of plant (cm), leaf width - length (cm), leaf production yield (kg/da), total carbohydrate analysis (%), total dietary fiber content (g/100 g) and crude protein content (%).

RESULTS

Comparison of the Some Properties of Criolla and Morita II Varieties

Some characteristics of the Criolla and Morita II varieties of stevia are given in Table 3. It was determined that harvesting period of Morita II (70-80 days) was shorter than Criolla (110-120 days). Dry leaf yield is higher in Criolla variety than in Morita II variety. Some other parameters are also given in Table 3.

Table 3. Comparison of some parameters belong to Criolla variety and Morita II variety

Parameters	Criolla	Morita II
Harvesting Period (day)	110-120	70-80
Height of Plant (cm)	95-110	65-75
Leaf Width - Length (cm)	1.79-2.21	2.56-3.12
Leaf production yield (kg/da)	550-630	450-500
Total Carbohydrate Analysis (%)	62	71
Total Dietary Fiber Content (g/100 g)	31.50	30.60
Crude Protein Content (%)	14	11

Steviol Glycoside Content of Criolla and Morita II Varieties

Total steviol glycosides (TSG) and steviol glycoside profiles were analysed and retention times (RT) were determined in this study. These were Stevioside (RT: 6.840), Rebaudioside A (RT: 6.432), Rebaudioside B (RT: 17.097), Rebaudioside C (RT: 8.747), Rebaudioside D (RT: 3.155), Rebaudioside F (RT: 7.579), Dulcoside A (RT: 9.576), Steviolbioside (RT: 18.539) and Rubusoside (RT: 12.656).

Steviol glycoside content is given in Table 4 and Figure 4. According to the Table 4 and Figure 4, The TSG content of Morita II variety (17.58%) was higher than Criolla variety (12.41%) and Rebaudioside A content of Morita II variety (13.37%) was found to be higher than Criolla variety (4.90). However, the rate of stevioside is higher in the Criolla variety (6.74%).

Tablo 4. Steviol glycoside content of Criolla and Morita II varieties (%)

Steviol Glycosides	Criolla	Morita II
Stevioside	6.74	3.10
Rebaudioside A	4.90	13.37
Rebaudioside B	0.02	0.09
Rebaudioside C	0.36	0.64
Rebaudioside D	0.00	0.19
Rebaudioside E	0.00	0.01
Rebaudioside F	0.22	0.14
Dulcoside A	0.17	0.02
Steviolbioside	0.00	0.01
Steviol	0.00	0.00
Rubusoside	0.00	0.01
Total Steviol Glycosides (TSG)	**12.41**	**17.58**

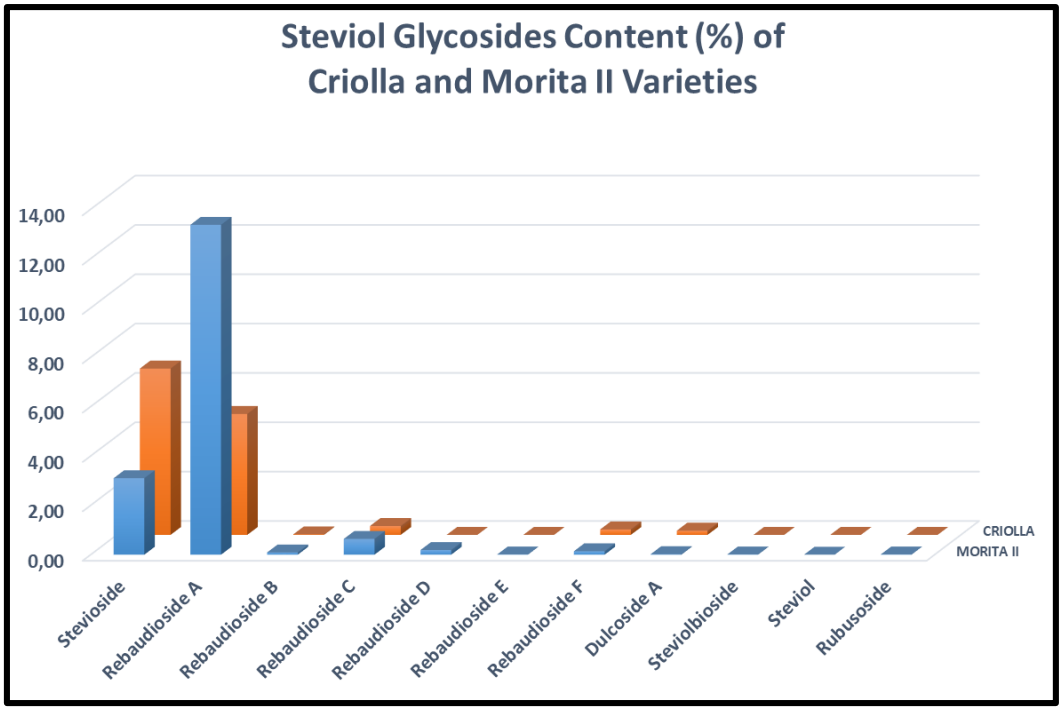

Figure 4. Steviol glycoside content of Criolla variety and Morita II variety

TSG, Stevioside and Rebaudioside A content of Criolla and Morita II are seen in Figure 5. According to these results, the TSG content of Morita II was 17.58%, while the ratio of Reb A and Stevioside were 13.37% and 3.10%, respectively. It was determined that the content of TSG and Reb A of Morita II was higher than that of Criolla, but the content of stevioside was lower.

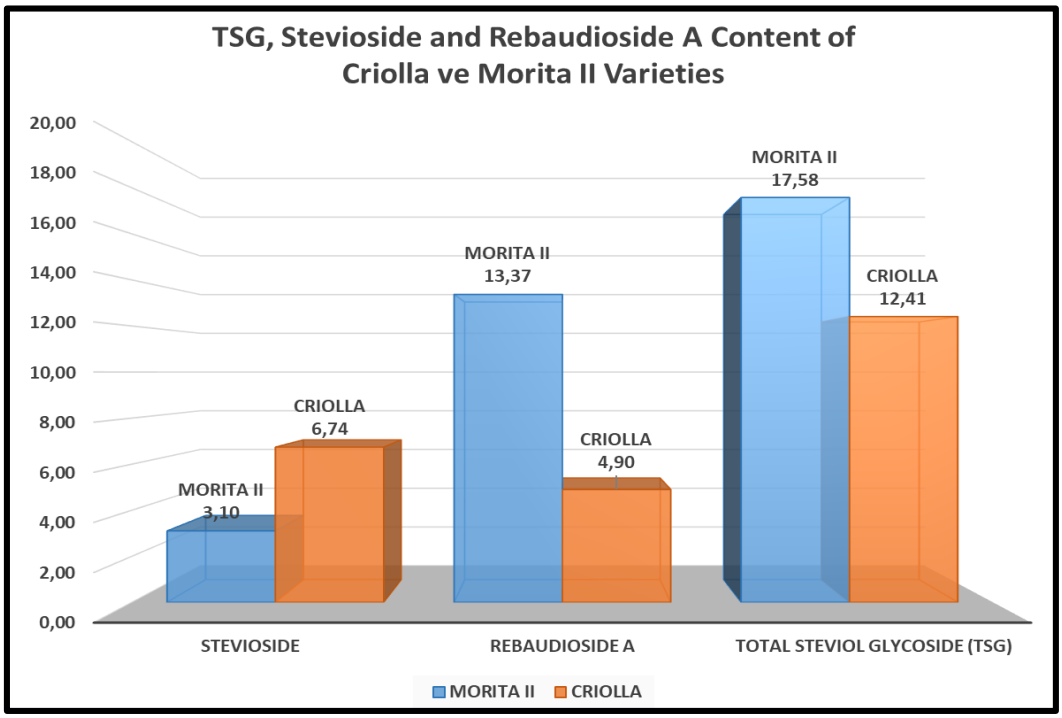

Figure 5. TSG, Stevioside and Rebaudioside A Content of Criolla variety and Morita II variety

Approximately 76% of the steviol glycoside content of the Morita II variety is composed of Reb A as seen in Figure 6. For this reason, it is a more valuable variety in terms of commercial production.

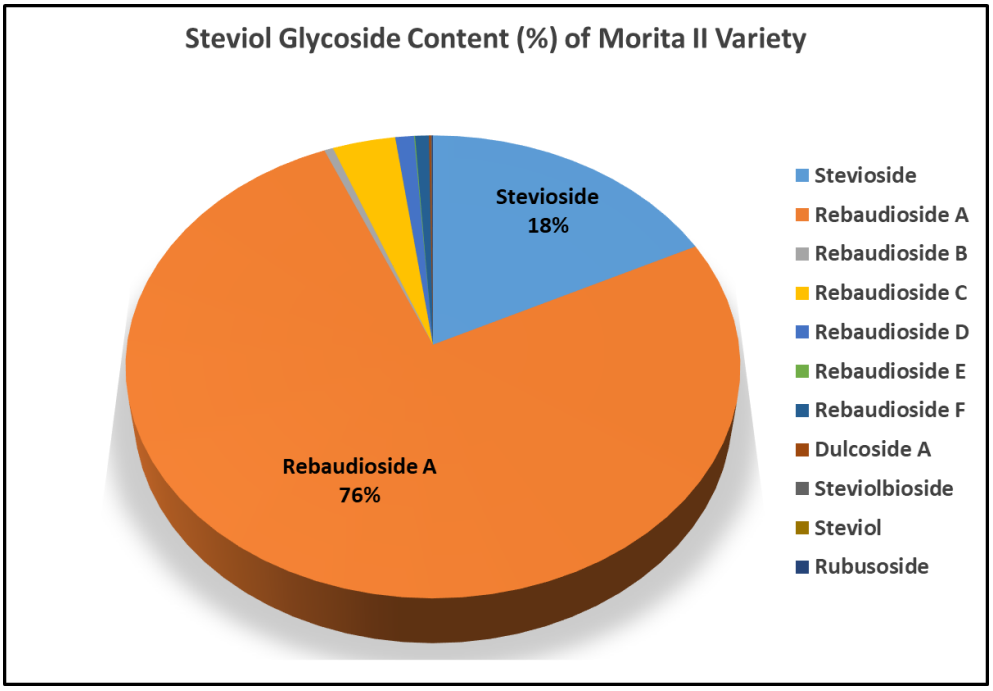

Figure 6. Steviol glycoside content of Morita II variety (%)

According to Figure 7, Reb A content of Criolla variety is approximately 40% of TSG. Stevioside is 54%. When the findings are compared; It has been determined that the Morita II variety has a better quality than Criolla variety.

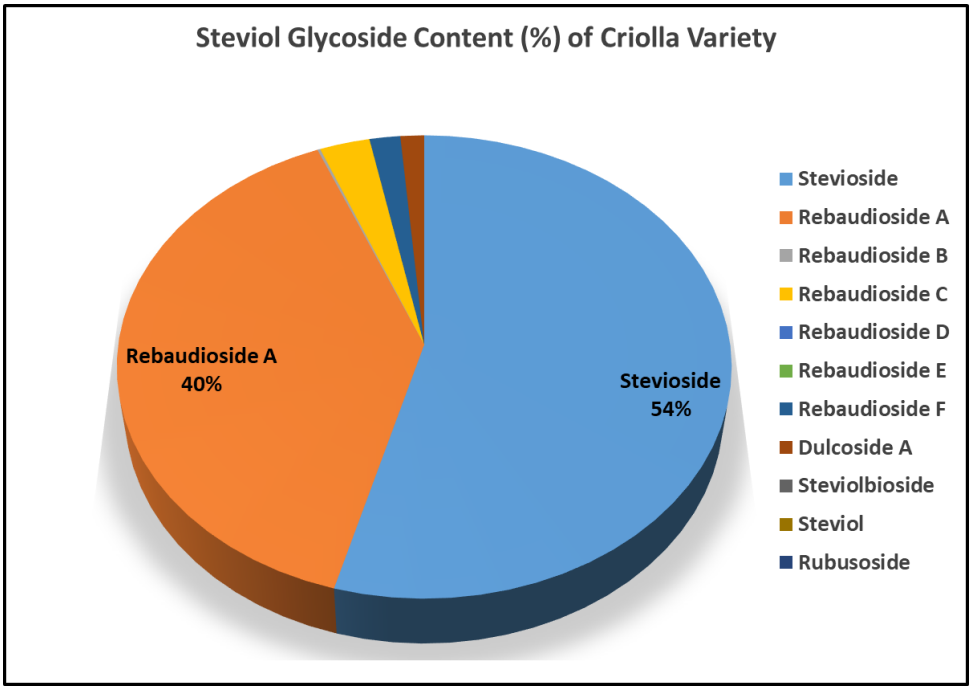

Figure 7. Steviol glycoside content of Criolla variety (%)

CONCLUSION

Stevia has no calories, and it is 250-520 times sweeter than sucrose in the same concentration. Other studies suggest that stevia might have extra health benefits. But a few studies show that replacing sugar with artificial or low-calorie sweeteners may not ultimately lead to weight loss in real life.

In this study; it has been determined that Criolla and Morita II stevia varieties are compatible with the climate and soil structure of our country. The leaves of Criolla and Morita II varieties can be harvested 2-3 times a year. Additionally, Morita II is a technologically high quality variety with its high content of TSG and Reb A content.

As a result; findings from this study reveal that stevia can easily be produced under the conditions of our country and that the products obtained will have superior qualities. Generally, the production of these imported products in our country will make a significant contribution to the Turkish economy.

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THE EFFECTS OF NANOPARTICLES ON THE EXPRESSION OF CATALASE ENZYME IN SHORT-TERM ADMINISTRATION ON BREAST CANCER CELLS

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ABSTRACT

Catalase is an enzyme found in almost all living organisms exposed to oxygen. Catalase enzyme, hydrogen peroxide separates water and oxygen. It's found in carrot and potatoes. Another task is to detect the peroxidase in foods. In this study, the therapeutic effects of nanoparticles on breast cancer cells were investigated. SiO_2, ZnO and Ag compounds and elements are used as nanoparticles. Our aim in this study is to investigate the chemo preventive effects of nanoparticles on direct breast cancer cells in 24-48-hour time period. After 24 to 48 hours of application of the above nanoparticles, the H2O2 uptake catalase activity is increased by using water in the cell and these effects are exerted through the expression of catalase enzyme. We quantified amount of catalase enzyme produced by measuring relative amount of RNA-cDNA by Quantitative PCR. As a result, compared with control cells, Ag nanoparticles decreased catalase activity in breast cancer cells compared to other 2 nanoparticles and increased 5 times in ZnO and 2.5 times in SiO2. These results show that the oxidative effect of Ag nanoparticles on breast cancer cells can be evaluated as a therapeutic agent with a high degree of oxidative effect.

Keywords: *Nanoparticule, catalase, cancer*

INTRODUCTION

Nanotechnology is science, engineering, and technology conducted at the nanoscale, which is about 1 to 100 nanometers. Nanoscience and nanotechnology are the study and application of extremely small things and can be used across all the other science fields, such as chemistry, biology, physics, materials science, and engineering (Logothetidis 2012).**Silicon dioxide:** chemical compound containing oxygen and silicon. The chemical symbol is SiO2. It has been known since the 16th century. It is used in many materials such as glass, concrete, tiles, porcelain. SiO_2 is in the form of crystalline forms (polymorph) rather than any material. There are 17 different crystal forms such as quartz, topaz and amethyst. There are some stones and quartz in the land. For this reason, it is spreading as dust. Long-term inhalation has been shown to increase lung cancer risk (McBrayer, Swanson et al. 1986). **Zinc oxide:** Has a bitter taste, white powder appearance, odorless. With carbon dioxide in the atmosphere has the property of absorbing ultraviolet rays. It is soluble in acids and alkalis but does not dissolve in water and alcohol. Zinc Oxide is a non-toxic substance but is highly flammable(Xia, Kovochich et al. 2008). **Silver:** ductile metal that reflects light very well. It shows a great resistance to oxidation in the atmosphere. it is to oxidize more difficult than copper and easier than gold. Standard electrode potential is 0,7978 V. It is resistant to acids and a few organic substances. But it is easily dissolved in nitric and concentrated hot sulfuric acid. It is especially used for burn and skin problems thanks to its curing properties(Sun and Xia 2002, Sondi, Salopek-Sondi et al. 2004, Rai, Yadav et al. 2009). Catalase, an enzyme that brings about (catalyzes) the reaction by

which hydrogen peroxide is decomposed to water and oxygen. Found extensively in organisms that live in the presence of oxygen, catalase prevents the accumulation of and protects cellular organelles and tissues from damage by peroxide, which is continuously produced by numerous metabolic reactions(Aebi 1974). In mammals, catalase is found predominantly in the liver. Our aim in this study is to investigate the chemo preventive effects of nanoparticles on direct breast cancer cells in 24-48-hour time period.

MATERIAL AND METHODS

Cells and Cell Culture MCF7 cells were kindly provided by TUTAGEM [6]. The progressed variants, were generated as described in previous publications [5,7]. The cells were cultured in minimal essential medium (Invitrogen, Carlsbad, CA) supplemented with 7.5% fetal bovine serum, 2.5% calf serum (Gemini Bioproducts, Woodland, CA), 500 U/ml penicillin, and 5000 U/ml streptomycin (Invitrogen) at 37jC in a 7% CO2 humidified environment.

The captured cells on each cap were lysed with 20 µl of extraction buffer for 30 minutes at 40°C, and centrifuged (12 000 RPM-Thermo S.) from the caps into microfuge tubes. Proteinase K buffer (Fermentase) was added to each 20 µl of cell extraction solution. The extraction solution was incubated at 55°C for 1 hour and subsequently the Proteinase K was inactivated (95°C for 10 minutes). Isolated RGCs were processed for RNA extraction using the Invitrogen RNA isolation kit (USA) according to the manufacturer's protocol. Briefly, the sample was loaded on a RNA spin column and washed several times. DNase (Qiagen, Valencia, CA) was added and the total cellular RNA was eluted from the column in 10 µl of elution buffer. RT was performed, according to the manufacturer's specifications (Invitrogen Corp., Carlsbad, CA), using 14 l of extracted RNA from each LCM sample and 200 U of Superscript RT polymerase (Invitrogen). To reduce the total volume of the cDNA samples, we precipitated the cDNA in ethanol. Briefly, 10 µl of 2 mol/L sodium acetate, 1 µl of 5 mg/ µl glycogen, and 30 µl of water were added and vortexed. Subsequently 500 µl of ice-cold 100% EtOH was added and the solution was placed on dry ice for 1 hour. The mixture was centrifuged for 45 minutes at 4°C (16,000 rpm). Finally the EtOH was removed and the pellet was allowed to dry before being resuspended in 10 µl of deionese water(Chan, Sepunaru et al. 2017).

After 24 to 48 hours of application of the above nanoparticles, the H2O2 uptake catalase activity is increased by using water in the cell and these effects are exerted through the expression of catalase enzyme. We quantified amount of catalase enzyme produced by measuring relative amount of RNA-cDNA by Quantitative PCR. Quantitative PCR experiments were carried out at the Technology Research Development Application and Research Center-Edirne/Turkey (TUTAGEM).

RESULTS

After 24 to 48 hours of application of the above nanoparticles, the H2O2 uptake catalase activity is increased by using water in the cell and these effects are exerted through the expression of catalase enzyme. We quantified amount of catalase enzyme produced by measuring relative amount of RNA-cDNA by Quantitative PCR. As a result, compared with control cells, Ag nanoparticles decreased catalase activity in breast cancer cells compared to other 2 nanoparticles and increased 5 times in ZnO and 2.5 times in SiO2 (Figure 1 and 2). These results show that the oxidative effect of Ag nanoparticles on breast cancer cells can be evaluated as a therapeutic agent with a high degree of oxidative effect.

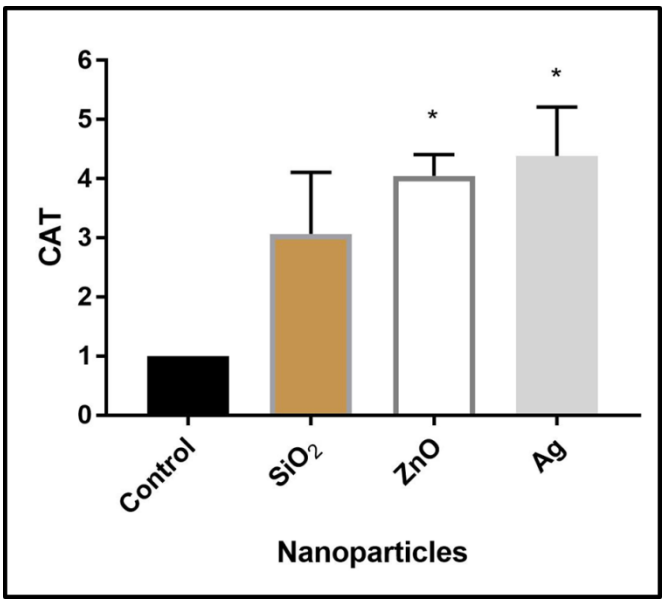

Figure 1: Catalase measurement after 48 hour.

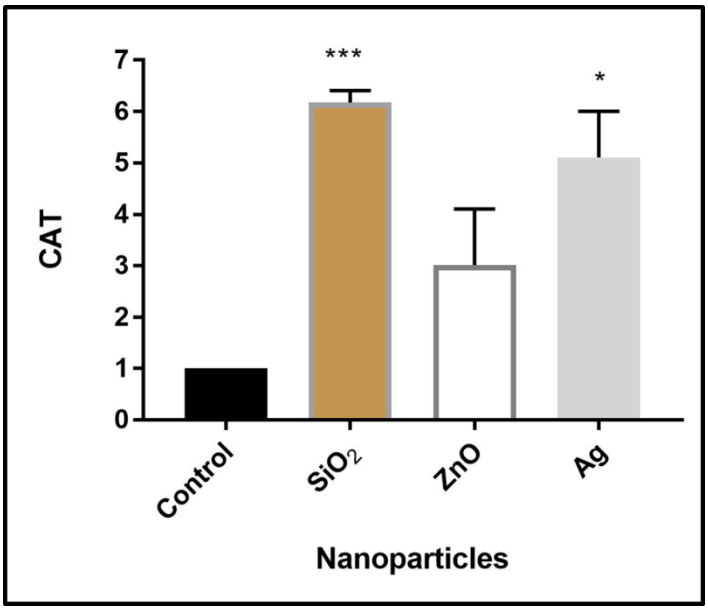

Figure 2: Catalase measurement after 48 hours.

DISCUSSIONS

Several malignant tumor or malignant neoplasm involve reactive oxygen species and implicate oxidative stress in disease mechanisms. Hydrogen peroxide (H_2O_2) formation due to mitochondrial superoxide leakage perpetuates oxidative stress in tissues. Catalase, an H_2O_2-degrading enzyme, thus remains an important antioxidant therapy target. But, catalase therapy is restricted by its labile nature and inadequate delivery. Here, a nanotechnology approach was evaluated using catalase-loaded, SiO2,

ZnO and Silver nanoparticles in human tissue protection against oxidative damage. Further, catalase-loaded NPs improved tissue recovery from H2O2 pre-exposure better than free catalase, suggesting possible applications in ameliorating stroke-relevant oxidative stress. Specific targeting of catalase-loaded NPs may find wide therapeutic applications for oxidative stress-associated acute and chronic tissue disorders against cancer.

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DETERMINATION OF NUCLEAR DNA CONTENT OF *LOLIUM* SPECIES BY FLOW CYTOMETRY METHOD AND ITS USAGE IN TAXONOMIC IDENTIFICATION

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ABSTRACT

The genus *Lolium* consists of 8 annual and perennial species. They are distributed through out temperate regions of the world. Two species in the genus, *L. perenne* and *L. italicum* are among the most important forage crops. *L. perenne* is also widely used in the creation of green areas. *Lolium* species are quite similar to each other morphologically. Interspecific hybridization is also often occur among the species. This close morphological similarity among *Lolium* spp. makes identification and classification of species a difficult problem for forage and turf grass scientists. Therefore, new methods are needed to facilitate the taxonomic identification and classification of *Lolium* species. Nuclear DNA content is the total amount of DNA included in the cell nucleus. The nuclear DNA content is stable among individuals of a species and cells of a single plant. However, it differs approximatelly 1000 folds in angiosperms. These characteristics of nuclear DNA content make it usefull in many areas of biology such as genetics and evolution. Today, flow cytometer is the method of choice in nuclear DNA content determination due to its accuracy, quickness and easiness. The objective of this study is to determine nuclear DNA content of 8 *Lolium* species by flow cytometry and to use the information for the taxonomic identification of species. Based on the results of the study, the mean 2C genome size of *Lolium* species varies from 7.76 pg to 4.87 pg. The differences between species are statistically significant.

Keywords: *Flow cytometry, Nuclear DNA content, Lolium, Taxonomy.*

INTRODUCTION

The genus *Lolium* consists of 8 annual and perennial species. Species of the genus are native to Europe, the North Atlantic Islands, temperate Asia, and north Africa, but they have been widely distributed to other geographic regions of the world (Terrell, 1968). Two species in the genus, *L. perenne* and *L. italicum* are among the most important forage crops. *L. perenne* is also widely used in the creation of green areas. *Lolium* species are quite similar to each other morphologically. Interspecific hybridization is also often occur among the species. This close morphological similarity among *Lolium* spp. makes identification and classification of species a difficult problem for forage and turf grass scientists (Polok, 2007). Therefore, new methods are needed to facilitate the taxonomic identification and classification of *Lolium* species. The nuclear DNA content is the total amount of DNA included in the nucleus. The nuclear DNA content is stable among individuals of a species and cells of a single plant. However, it differs approximatelly 1000 folds among the plant species (Bennett et al., 2000). Therefore, nuclear DNA content is species specific. These characteristics of genome size

make it usefull in many areas of biology such as genetics and evolution (Tuna, 2014). The objectives of this study was to determine nuclear DNA content of *Lolium* species by flow cytometer and use it in taxonomic identification of the species.

MATERIAL AND METHODS

One accessions for each of the 8 *Lolium* species and one hybrid were used in the study. Three genotypes were analysed separatelly for each accession. Samples were prepared by isolating nuclei from fresh leaf tissues by using Partec commercial kit (Fig 1). A protocol explained by Tuna (2014). *Vicia sativa* **(**cv karaelci**)** was used as internal standard. Propidium iodide was used as florescent dye. Nuclear DNA content was calculated using the linear relation between the ratio of 2C peak positions of *Lolium*/*Vicia* on histogram of fluorescence intensities (Fig 2 and 3).

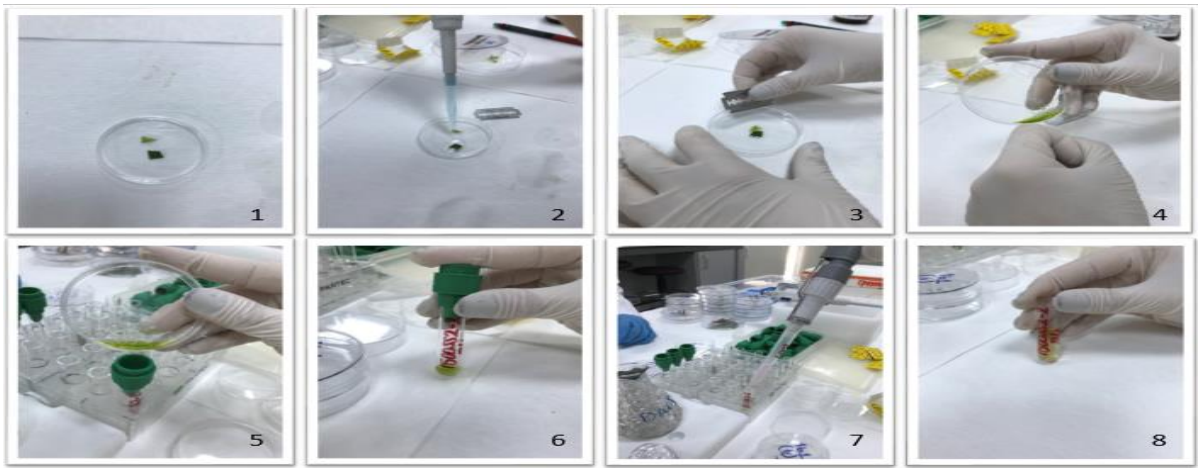

Fig 1. Preparation of the samples for nuclear DNA content analysis.

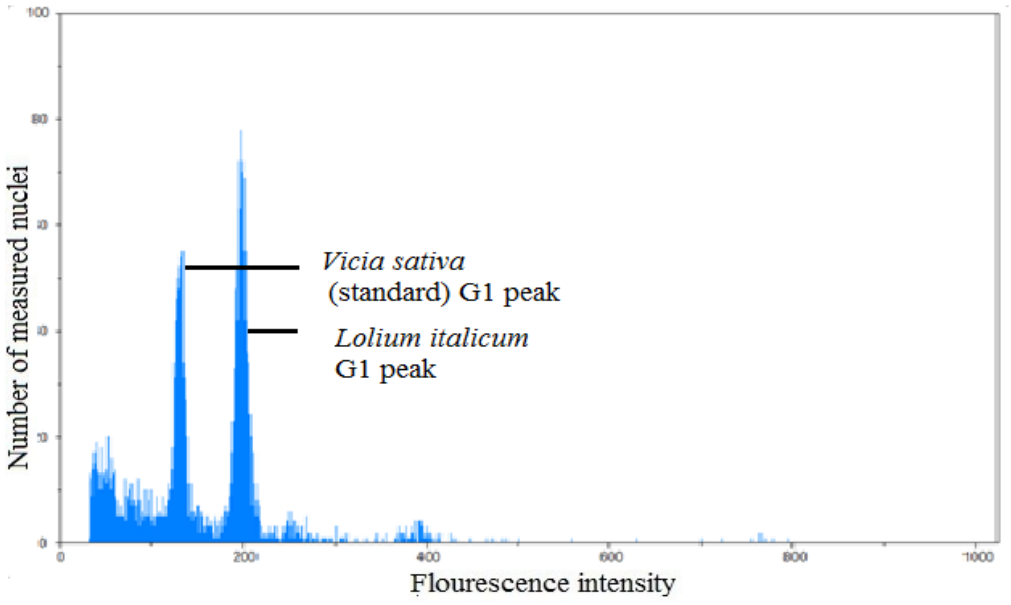

Fig 2. The relative positions of G1 peaks of sample (*L. italicum*) and standard (*Vicia sativa*), and mitotic chromosomes of sample (2n=14).

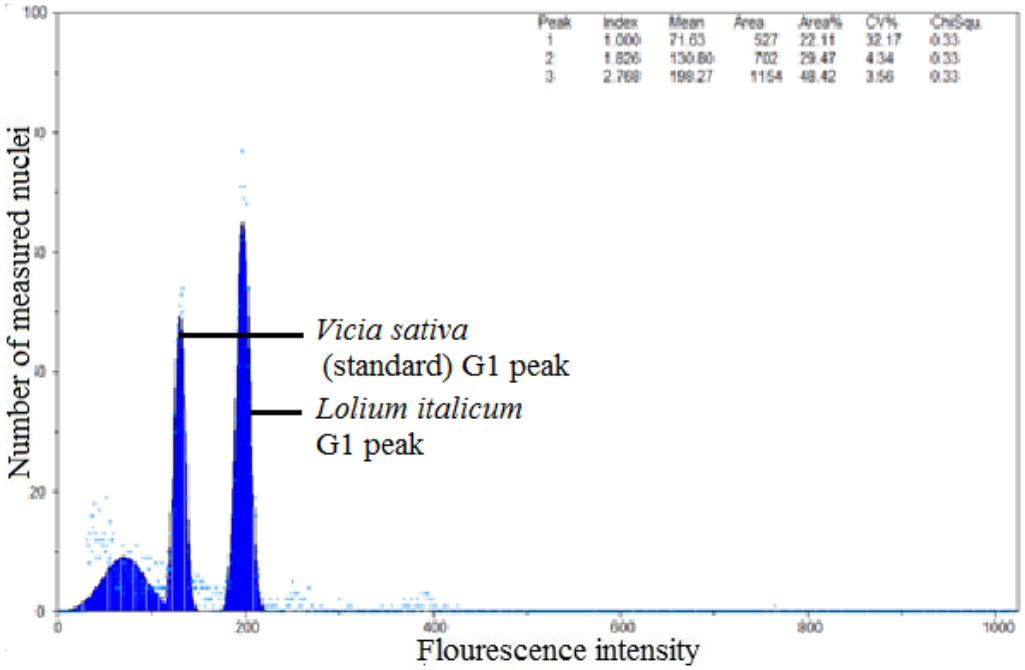

Fig 3. Appearance of histogram of fluorescence intensities after analysis by flomax computer program.

CALCULATION OF NUCLEAR DNA CONTENT

$$\frac{\text{Flourescent intensity of sample (G1 peak value)}}{\text{Fluorescent intensity of standard (G1 peak value)}} \times \text{DNA content of the standard as pg}$$

DNA content of *Lolium italicum*: (198.27/130.80) x 3.45 = 5.24 pg

RESULTS AND DISCUSSIONS

The results of nuclear DNA content analysis carried out on 8 *Lolium* species and a hybrid are presented in Table 1. According to the results obtained in the study, the mean 2C nuclear DNA content of *Lolium* species varies between 7.47 pg and 4.87 pg. L. rigidum had the lowest 2C nuclear DNA content within the *Lolium* genus while L. temelentum had the highest 2C nuclear DNA content. These results are the first reports on nuclear DNA content of *Lolium* species except economically important two species, *L. italicum* and *L. multiflorum*. The results obtained in this study is in agreement with previous results. The mean 2C nuclear DNA content differences observed among the *Lolium* species were statistically very significant.

In addition, the 2C mean nuclear DNA content of the two cultivated species of the genus, *L. perenne* (4.95 pg) and *L. italicum* (5.21 pg) had statistically different nuclear DNA content. However, 2C nuclear DNA content of *L. perenne* (4.95 pg) was not significantly different than some of the species as *L. rigidum* (4.87 pg), *L. subulatum* (4.93 pg) and *L. hybridium* (5.04 pg).

The mean 2C nuclear DNA content of *L. italicum* (5.21 pg), *L. canariense* (6.65 pg), and *L. persicum* species (7.03 pg) were statistically very significant from others included in the genus and each others. The mean 2C nuclear DNA content of *L. temelentum* (7.47 pg) and *L. remotum* (7.44 pg)

was significantly higher than all the other species included in the genus. But, the nuclear DNA content differences between these two species were insignificant.

Table 1. The mean 2C nuclear DNA content of the *Lolium* species (2C/pg)

Species name	DNA. Con. (Pg/2C)				SD	$T*S_x$	Conf. Interv.	
	1	2	3	Mean			Lower	Upper
L. rigidum	4.72	4.89	5.01	4.873	0.146	0.119	4.754	4.992
L. subulatum	4.89	4.9	5.02	4.936	0.072	0.059	4.878	4.996
L. perenne	4.87	4.91	5.09	4.956	0.117	0.096	4.861	5.052
L. hybridium	4.89	5.03	5.2	5.04	0.155	0.127	4.913	5.167
L. italicum	5.18	5.22	5.24	5.213	0.031	0.025	5.188	5.238
L. canariensis	6.6	6.67	6.69	6.653	0.047	0.039	6.615	6.692
L. persicum	6.75	7.12	7.23	7.033	0.251	0.206	6.828	7.239
L. remetum	7.41	7.44	7.49	7.446	0.040	0.033	7.414	7.480
L. temelentum	7.18	7.6	7.64	7.473	0.255	0.208	7.265	7.682

Conclusions

The mean 2C nuclear DNA contents of the *Lolium* species are significantly different. Therefore, the 2C nuclear DNA content of the *Lolium* species determined by flow cytometer can be used as a diagnostic tool to distinguish the species when it is needed. In addition, flow cytometer can also be used to confirm taxonomic identity and ploidy of the *Lolium* genetic resources before include them in research programs.

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MICROPROPAGATION OF *ANGELONIA ANGUSTIFOLIA* FROM STEM EXPLANTS

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ABSTRACT

The results of this study have established a micropropagation system for Angelonia angustifolia that will provide plant material of high-quality Angelonia plants for the commercial market. The study has been made for the possibility of introducing *Angelonia angustifolia* in culture by stem cuttings. Different variants of sterilization have been tested. The most successful variant was Var.2 / HgCl2 0.1% -5 min. It has the highest % sterile explants were reported - 84.7% and the highest percentage of live plants was 65.8%. Nodal explants of Angelonia angustifolia were cultured on MS basal medium and induced to form shoots when supplemented with different concentrations BAP(0.1mg/l, 0.3mg/l and 0.5mg/l). The MS medium supplemented with BAP (0.5mg/l) was the most effective, providing high multiplication factor (1,40) associated with a high number of shoots per explant (3,0±0,3 shoots/explant). The plants were rooted on MS medium without plant growth regulators or in combination with different concentrations of BAP (0.1mg/l, 0.3mg/l and 0.5mg/l). The highest percentage (90%) rooted plants were obtained when using BAP -0.1mg/l /. All plantlets survived acclimatization producing healthy plants in the greenhouse.

Key words: micropropagation, Angelonia angustifolia, sterilizing agents ACE-30% and $HgCl_2$, BAP

INTRODUCTION

The clonal micropropagation is an established tool for obtaining large amounts of disease-free, genetically identical and high-quality planting material of various plants. This approach is also successfully applied for producing ornamental plants. In vitro technquescan is a preferred method of breeding plant species due to the ability to breed the plant material for a short time regardless of the season and when using a limited site. It is also suitable for both rare and endangered species, thus protecting them against extinction, as well as sterile plants or plants that cannot retain their reproductive characteristics (Manolova, D et al. (2013), Manolova, D et al. (2014), Zaprianova, N., & Ivanova, I. (2016), Kaninski, A,et al. (2012), Datta K, Datta SK (1984), Maira, O., Alexander, M., & Vargas, T. E. (2010), George, E. F., Hall, M. A., & De Klerk, G. J. (2008).

Angelonia /*Angelonia angustifolia*/ (*Plantaginaceae*) is unpopular species for decorative gardening in Bulgaria. Originating from South America, it is a herbaceous plant with a height of 30 cm. The flowers are small, dyed in pink, purple and white, collected in inflorescence class. The plant has no scent and is not toxic. It is used for both gardening and for arrangement of individual containers, baskets and pots. Plants in this genus are known for their heat tolerance (Bruggeman,1957) and have been recently incorporated into active breeding programs in a number of major flower-breeding firms. However, little is known about the optimal temperature, irradiance level, photoperiod and growth-

retardant use for production of this species (Miller, A., & Armitage, A. M. (2002). The objective of this work was to develop a system for introducing Angelonia angustifolia into an in vitro culture by stem cuttings.The development of a propagation protocol tissue culture method will bring a well-balanced seedlings.

MATERIAL AND METHODS

Stem cuttings of Angelonia angustifolia were collected from 6 month-old plants grown at the greenhouse of the Institute of Ornamental Plants, Sofia.

Freshly harvested plant material was washed to remove the surface pollution and then was sterilized by soaking for 1 min with 70 % ethanol followed by one two sterilizing agents ACE-30% and HgCl2 0.1%. Finally it was rinsed three times in sterile distilled water.

Table 1. Variants of *Angelonia angustifolia* sterilization

Variant	$CH_2(OH)_5$	ACE 30%	$HgCl_2$ 0.1%	Sterile distilled water
Variant I	1min	-	2.5 min	1x5 min;1x10 min, 1x15 min
Variant II	1min	-	5 min	1x5 min;1x10 min, 1x15 min
Variant III	1min	-	10 min	1x5 min;1x10 min, 1x15 min
Variant IV	1min	5 min	-	1x5 min;1x10 min, 1x15 min
Variant V	1min	10 min	-	1x5 min;1x10 min, 1x15 min
Variant VI	1min	15 min	-	1x5 min;1x10 min, 1x15 min

After the sterilization the plant explants were laid out on MS medium with vitamins + 6 g/l agar + 30 g/l sucrose, with pH 5.7.

The following indicators of surface sterilization are reported: percentage of the explants with fungal contamination and sterile explants recorded at 7 and 14 days of incubation and the percentage of viable plants were reported. To determine the effect of cytokinin BAP on the Angelonia, multiplication factor nodal segments (1 cm in length) were pasted onto fresh culture medium MS basal with vitamins + 6 g / l agar + 30 g / l sucrose + BAP (0.1 mg / l; 0.3 mg / and 0.5 mg / L BAP) with pH 5.7. After 30 days, the multiplication factor and the size of the shoots were recorded. The multiplication factor is calculated by the formula: MK% = NxA / 100, where N - number of newly formed shoots, A-% multiplied plants (Tsvetkov, 1998).

In all the trials, media pH was adjusted to 5.7 with 0.1 N NaOH or 0.1 M HCl and autoclaved at 121 °C at 1.04 kg·cm-2 pressure for 20 min. Cultures were maintained under controlled conditions: room temperature 23 ± 2 °C, photoperiod 16/8 (day / night) and 50 μmol·m-2·s-1 light intensity provided by 40 W cool white fluorescent tubes (Philips, Bulgaria). The chemicals used in the experiments were purchased from Duchefa, The Netherlands. Data presented in table are mean values ± SE from two independent experiments with 10 plantlets per variant. T-test was used for statistical analysis (GraphPad Prizm).

RESULTS AND DISCUSSION

By comparing the action of sterilizing agents HgCl2 and ACE 30%, it was found that both can be used to introduce Angelonia angustifolia into an in vitro culture. From the tested variants, the most successful was Var.2 / HgCl2 0.1% -5 min. It showed the highest percentage of derived sterile explants - 84.7% and the highest percentage of live plants. The use of ACE also showed a relatively high percentage of 64.2% of sterilization (Table 2).

Table 2. Results of the introduction in in vitro culture of explants from stem cuttings from *Angelonia angustifolia*

Variant	I reporting /7 days/		II reporting /14 days/		
	explants with fungal contamination %	sterile explants %	explants with fungal contamination %	sterile explants %	sterile and viable plants %
Variant -1 /$HgCl_2$ 0.1 % -2.5min/	23,5	76,9	100,00	0	0
Variant -2 /$HgCl_2$ 0.1 % -5min/	8,5	91,5	15,3	84,7	65,8
Variant -3 /$HgCl_2$ 0.1 % -10min/	0	100,00	54.8	45,2	18.6
Variant -4 / ACE 30% - 5min/	4,6	95,4	23,3	76,7	34.28
Variant -5 / ACE 30% - 10min/	12,7	87,3	35,8	64,2	41.42
Variant -6 / ACE 30% - 15min/	35,3	64,7	50,71	49,3	5.7

After sterilization, the explants are placed on MS medium + 6 g / l agar-agar + 30 g / l sucrose, pH of the medium before autoclaving is 5.7. It influences favorably the growth of plants without signs of deviation from their normal development such as vitrification or chlorosis.

Most authors reported successfully cultivation of pelargonium in vitro on basal salt medium MS (Dunbar & Stephens, 1989; Hassanein & Dorion, 2005; Saxenaa et al., 2000). The MS basal nutrient medium was also successfully used (Martin and Perez, 1995) for the introduction of 5 wild species of the genus Limonium in in vitro culture. It was also cited as the most suitable for obtaining well-developed regenerants of the genus Artemisia (Hristova 2014, Liu et al., 2003) and Angelonia Salicariefolia (Datta K, Datta SK 1984). In many studies on micropropagation of ornamental plants, BAP is used alone in different concentrations to determine its effect on the crop multiplication factor (Nencheva, 2009, Bhosale et al., 2011, Hassan et al., 2013, Uzunova, 2014, Martin, C., Perez, C.

1995). Alone using a BAP at Aquilegiq hirta gives most quality cuttings at a concentration of 0,1 mg /l (Hassan, et al. 2013), while impatiens New Guinea best results in all tested varieties is using MS + 7 g / l agar + 30 g / l sucrose + 2 mg / l BAF (Nencheva, 2009).

BAP has a stimulating effect on the rate of multiplication, with the values of the studied parameters depending on the concentration applied. Low concentrations of BAP cause formation of a smaller number of shoots than the high. The largest number of lateral shoots (3,0±0,3,) was recorded in plants cultivated in MS medium supplemented with 0.5 mg/l-1-1 BAP for the *Angelonia angustifolia* (Table 3). The inclusion of BAP in the nutrient medium also affects the size of the formed shoots. The combination of MS basal salt medium and 0.5 mg/l-1-1 BAP led to formation many microplants. The resulting lower multiplication factor at low BAP concentrations is compensated for by the formation of larger size shoots. Highest height (9.7 ± 0.8 cm) was recorded in the explants cultivated in growth medium without growth regulators and at 0.1 mg / l BAP concentration (Table 3). The most well-developed root system was observed in Variant-2 / MS -0.1mg / l. Most of the micro plants grown on medium without growth regulators and at 0.1 mg / l BAP concentration formed roots and were fully prepared to adapt to conditions in vivo.

Table 3. Influence of tree concentrations of BAP on growth of explants of *Angelonia angustifolia*

Variant	Shoots /number	MK%	Height /cm	Root- length /cm/	Rooting %
Variant -1 control MS basal	2,0±0,5	0,12	9,7±0,8	2,0±0,1	90
Variant -2 / MS -0.1mg/l /	2,2±0,2*	0,83	9,4±0,8	2,4±0,2***	90
Variant -3 / MS -0.3mg/l /	2,5±0,4**	0,51	5.2±0,6**	0,9±0,2нс	50
Variant -4 / MS -0.5mg/l /	3,0±0,3***	1,40	4.5±0,2***	1,2±0,6нс	10

Successful acclimatization enhances optimal conditions for high plant survival, followed by growth and stabilization of the micropropagated plants. The acclimatization takes place in a greenhouse at an air temperature of 20-26 ° C and an air humidity of 80-90%. During the first two weeks the plants are overshadowed and sprinkled 4-5 times a day. After the second week the humidity gradually decreases to 60%. Full acclimatization occurs for about 4 weeks.

CONCLUSIONS

The research shows the following results: The highest number of sterile and vital explants is obtained with the usage of HgCl2 as sterilizing agent, applied at 0,1% concentration over a period of 5 minutes on the plant explants. (Variant 2). MS medium supplemented with BAP (0.5mg/l) was the most effective, providing high multiplication factor (1,40) associated with a high number of shoots per explant (3,0±0,3 shoots/explant). The resulting lower multiplication factor at low BAP concentrations is compensated by the formation of larger size shoots. Highest height (9.7 ± 0.8 cm) was recorded in the explants cultivated in growth medium without growth regulators and at 0.1 mg / l BAP concentration Plants were rooted on MS medium without plant growth regulators or in combination with different concentrations of BAP (0.1mg/l, 0.3mg/l and 0.5mg/l). The highest

percentage (90 %) rooted plants were obtained when using BAP -0.1mg/l / All plantlets survived acclimatization producing healthy plants in the greenhouse.

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ASSESSMENT OF THE RESPONSE OF POTATO VARIETIES TO ECOLOGICAL CONDITIONS IN NORTH IRAQ

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ABSTRACT

The ecological conditions in northern Iraq are suitable for potato cropping due to moderate climate; rainfall, suitable temperature, fertile soil, water availability and farmers skills. Potato is considered an important global food crop. So it is important to improve production and quality by; by selection of suitable and high productive varieties and usage of modern technology, such as irrigation and fertilization techniques to increase yield. This study was performed as a field experiment in two regions; Khazir and Kalakchi in north Iraq. Three varieties from Netherland were used these are: Laperla, Lanorma and Actrice. The results showed; that the Khazir region; produced the highest tuber number 113110 / dunum, total tuber weight 14.2959 tons / d and higher yield 14.2959 tons/d compared with 11.4513 tons/d in the Kalakh region, Laperla variety was significantly superior in yield which reached 15.8103 tons/d, while the lanorma variety gave 13.5440 tons/d and at last the Actrice variety which gave 9.2662 tons/d, the interaction between regions and varieties gave the highest yield (khazir+Laperla) which reached 16.7330 tons/d while the same variety yielded 14.8883 tons/d.

Key words: Potato, Response, Laperla, Lanorma, Actrice, Iraq.

INTRODUCTION

Potato crop *Solanum tuberosum L*. belong to the *Solanaceae* family. Wild potato species can be found in south America. [1]. Potato plays an important role in providing nutrient energy to humans through the provision of appropriate food to secure the nutrient requirements of the growing global population, in addition to cereal crops in a sustainable form such as; wheat, rice, corn, barley, triticale and pseudocereal crops such as; buckwheat, quinoa and amaranth …etc. Cereal crops in general are characterize by high prices and are subjected to global commercial speculations to supply, demands and commercial competition. While potato is not subjected to those speculations and competitions. Mass production of potato is common at the moment due to the short life of the crop. Their suitable price for the customer increased potato consumption. Potato cultivation in Iraq is facing many challenges, such as inadequate environmental conditions especially in the middle and southern regions, such as soil salinity, water scarcity, frequent pest infections, increase in production costs, traditional agriculture, lack of advanced technologies and the lack of advanced research centers for seed production [3]. Iraq imports 75,000 tons/year of potatoes from the neighboring countries to cover the local needs [4]. Moreover, Iraq import potato seeds from different sources such as: France, Italy and Holland to select the suitable varieties for the Iraqi environments with high productivity. In Ethiopia 16 varieties of potatoes were tested to select the highest productive varieties, the gera variety was superior which gave 53.97 t/h of marketable tubers [6]. Studies showed that dehydration of sliced fresh potato is one of the ways to prevent problems such as undesired sugar accumulation during storage and eventual browning on frying [8].

Dehydration of potato slices prevents the action of Amlolytic enzymes on starch. Certain characteristics should be available in potatoes to make it suitable for crisp industry these are: shallow eyes in the tuber, regular shape, light yellow color of the flesh, high dry matter, starch content, and low accumulation of sugars during storage [10]. The nutritional value of potato make it a very important crop as it contain 2.86% protein, 17.2% carbohydrates, 3.3 fibers, 0.0 % saturated oil. And 0.10% oil. Each 100 gm of potato give 78 calories and is a rich source of vitamin B6 and vitamin C which supply the body with 23% of daily needs. [11].

MATERIALS AND METHODS:

Factorial experiment of three potato varieties from Holland were used, they are: Laperla, Lanorma, from Den Hartigh company where imported by (Alrafidian land for agricultural techniques company) in Nineveh governorate in Iraq and Actrice variety from (Agro company plant), which were supplied from the Ministry of Agriculture in Iraq, for assessment of the response of these varieties to the ecological conditions in North Iraq. The three varieties were experimented in two regions; Alkhazir and Kalalchi. Complete randomized block design (CRBD) were used with three replications with six treatments and 18 experimental units, and 3x3=9 m^2 plots. sprinkler irrigation were used in addition to rain fall water. Dap NPK fertilizers were aded (18-46-0) at a rate of 40 kg / d .). The soil texture is a loamy-clay in both regions. The cultural practices were mechanically performed, such as; plowing, smoothening, furrowing and plotting . Seeds were planted on the 20 of February at a rate of 750 kg/d. The distance between furrows is 80 cm with 30 cm planting distances. The crop was harvested on the 15^{th}. of June at both regions. The samples were taken from the middle of the plots with an average of 10 plants from each plot to study the following traits: plant number/ d, tubers number/ d, total tuber weight (yield) / d , non marketable tubers No /d, non marketable Tubers weight ton/d, marketable tuber No. /d, marketable yield ton/d, according to the mathematical model by calculating the following traits; number of tuber/plant , weight of tubers/plant and the average tuber weight. Recorded data was statistically analyzed by SAS program and the means were compared under the 0.05 level according to Duncan's multiple range test for the studied traits as in text book [12].

RESULTS AND DISSCUSION

This study was carried out in two regions; Khazir and Kalakchi located in north Iraq, using three varieties; Laperla, Lanorma & Actrice with three replications. The two regions have nearly the same climate. with very warm & dry summer, with moderate wet and relatively cool winter. The rain falling is during the winter & spring seasons between November to May. With an average temperature of 28 – 30 C^0. with loamy - clay soil [13].The results in table (1) which represent the average results of the studied traits revealed that the Khazir region was significantly superior in the following traits; total tuber number 113110 / d, total tuber weight 14.2959 ton / d, marketable tuber number was 86073 tuber/d, marketable tuber weight was 13.5921 ton/ d,either there were no significant differences in plant number, nonmarketable tuber number between the two regions. Laperla variety gave significant superiority over the other varieties in terms of total tubers number 130443/ d, total tuber weight 15.8107 ton /d, nonmarketable tuber number 31777/ d, marketable tuber number 99332/d except plant number/d. table (2). The interaction between the two regions and the three varieties showed significant superiority of the Khazir region with Laperla variety in most studied traits especially the tuber number 140877 / d, and total tuber weight 16.7330 ton /d, table (3). The same result appeared in the Kalakchi area, as Laperla variety gave the highest yield 14.8883/d and tuber number number119999/d. It is worth to know from this study that the Khazir region was better than the Kakakchi region for their significant superiority in most traits, due to its suitable ecological conditions.

Also Laperla variety was significantly better than the others, may perhaps due to genetic composition of the variety which suit the weather conditions. It is also worth to note that the three varieties could be classified in order according to their yield: Laperla v. is the first and Lanorma v. is the second while Actrice v. Is the third respectively.

A mathematical model for potato production is been formulated from the values of table (2).

1- Tuber number / plant = Total tuber No. / Total plant No**.**

N = B / P

Whereas N= tuber number/plant

B= total tuber number

P= total plant number

For Lanorma variety N = 102221 / 10777.6 N= 9.5 **tuber/ plant**

For Laperla variety N =130443 / 10111.0 N = 12.90 =

For Actrice variety N = 77777 / 10666.5 N = 7.3 =

2- yield /plant =Total tuber weight / Total plant number

Y = W / P

Y= yield

W= total weight

P= plant number

For Lanorma variety Y = 13544 / 10777.6 Y = 1.256 kg/plant

For Laperla variety Y = 15810/ 10111.0 Y = 1.563 =

For Actrice variety Y = 9266 /10666.5 Y = 0.868 =

3- Average tuber weight = Total tuber weight / Total tuber number

Average T.W = T .T.W / T .T.No

Where: T.W; Tuber weight , T. T.W; Total tuber weight , T .T.No; Total tuber number.

For Lanorma v. T.W = 13.544/102221 = 132 gm

For Laperla v. T.W = 15.8107 / 130443 = 121 gm

For Actrice v. T.W = 9.2662 / 77777 = 119 gm

Through the application of the above mathematical model we were able to obtain the values of yield traits by using data in Table (2) . These values are used for the evaluation of the three experimented varieties; the average tuber number /plant for Lanorma (9.5), Laperla (12.90) and Actrice (7.3). The tuber weight per plant was calculated for the three varieties: Lanorma (1.256 kg/p), Laperla (1.556 kg/p) and Actric (0.868 kg/p). Finally, the average tuber weight was calculated of the three varieties;

Lanorma (132 gm/t), Laperla (121 gm/t) and Actric (119 gm/t). It is obvious that Lanorma gave the highest average tuber weight (132 gm/t); because of the large size of the Lanorma variety tubers. For the calculation of profit and maximum net benefits the following models is applied:

4- $F = \Sigma Pi\ Yi - C$

Where ; F; Profit , P ; Price .$, Y; Yield . ton , C ; Cost .$

5- Maximum net benefit (MNB)

$$MNB = \sum_{c} yc \times pc \times Ac - \sum_{c} c_1 w_x \times Q \times A_c$$

Where; y_c: crop yield , p_c:crop price , A_c;crop area , $c_1 w_x$:cost price Q:quantity price .

The production is considered as a function of the environmental factors, genetic factors, and crop & soil management factors, as expressed by the following mathematical model:

Production = Function of (Ecological factors + Genetic factors + management factors).

$P = \Sigma (E + G + M)$

Table 1. Average yield characters of the three varieties in khazir&kalakchi regions.

Experimental Region	Plant No./ d	Tuber No./ d	Total tuber weight in Tons/d	No.of non marketable tubers /d	weigh of non-marketable tubers ton/d	No. of marketable tubers /d	weigh of marketable ton /d
AlKHAZER	10592.4 A	113110 A	14.2959 A	27333 A	0.6666 A	86073 A	13.5921 A
KALAKCHI	10444.3 A	93851 B	11.4513 B	23926 A	0.6072 A	70370 B	10.8070 B

Means with the same letter are not significantly different at the 0.05 level.

Table 2. Average yield characters of the experimented varieties at both regions.

Variety	Plants No./ d	Tubers No./ d	Total tuber weight in tons /d (yield)	Tubers No./ of non marketable /d	weigh of non marketable tubers ton /d	No. of marketable Le tubers/d	weigh of marketable tubers ton /d
Lanorma	10777.6 A	102221 B	13.5440 B	26555 AB	0.6882 A	75666 B	12.8550 B
Laperla	10111.0 A	130443 A	15.8103 A	31777 A	0.6400 A	99332 A	15.1217 A
Actrice	10666.5 A	77777 C	9.2662 C	18555 B	0.5825 A	59666 B	8.6220 C

Means preceded by similar letters do not differ at the 0.05 level.

Table 3. The interaction between agricultural regions and varieties.

Experimental Region	Variety	Plant No./ d	Tuber No./ d	Total tuber weight ton/(yield)	No. of non marketable tubers /d	weigh of non marketable tubers ton /d	No. of marketable tubers /d	weigh of marketable tubers Ton /d
ALKHAZER	Lanorma	10444.3A	104888 B	13.9997 BC	26898 AB	0.6660 A	77999 B	12.333 AB
	Laperla	10222.1 A	140877 A	16.7330 A	33555 A	0.6900 A	107332 A	16.055 A
	Actrice	11111.0 A	93550 B	12.1550 C	21555 AB	0.6437 A	72888 B	11.389 C

KALAKCHI	Lanorma	11111.0 A	99555 B	13.0883 AB	26222AB	0.7103 A	73333 B	12.377 AB
	Laperla	9999.9 A	119999 AB	14.8883 A	30000 A	0.5900 A	91332 A	14.188 A
	Actrice	10222.1 A	61999 C	6.3773 C	15555 B	0.5213 A	4644 C	5.855 C

Means with similar letters are not significantly different at the 0.05 level.

Table 4. Indicate yield characters for varieties through a mathematical model.

Variety	Yield characters		
	Tuber number/plant	Yield kg/ plant	average tuber weight gm.
Lanorma	9.5	1.256	132
Laperla	12.90	1.556	121
Actrice	7.3	0.868	119

CONCLUSION

From the assessment of this experiment; it appear that the Khazir region gave better results than Kalakchi region for the three varieties due to suitability of the ecological conditions especially soil type, the Laperla variety was better than the other varieties, may be also due to genetic properties. Dunum = 50x50m = 2500 m^2 = ¼ = Hectare

RECOMMMENDATIONs:

1 - Necessity to use modern technologies in all processes of soil and crop services.

2- Expanding the scientific research centers all over the Iraqi governorates.

3- Providing technical (agricultural equipments) at subsidized prices to farmers.

4 - Application of sustainable agricultural methods to achieve healthy and eco-friendly products.

5 – Production of high yield & quality varieties such as resistance to pests, especially viruses.

6. Reduce the production costs due to low price of marketable potato in Iraq to ensure a profitable income for the farmers.

7 - Prevention of potato importation for local consumption to encourage national production.

8. Providing refrigerated stores and refrigerated transport for potato crop.

9 – Importation of potato high quality and productivity potato seeds.

10. Expanding the potato industry and processing to maintain profitable revenues.

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COLD STORAGE OF CARROTS GROWN IN KIRIKHAN (HATAY) REGION

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ABSTRACT

This study aimed to investigate quality changes in 'Nanco F1' variety carrots from Nantes group grown in Kırıkhan (Hatay) during cold storage. Harvested carrots were perforated bag, imperforated bag and modified atmosphere packaging (MAP) after washing with tap water and immersing in sodium hypochlorite containing 0.5% of chlorine, 3 minutes and stored at 0±0.5 °C and 90±5.0% relative humidity for 5 months are used analyzed every month. In addition to carrots were kept at 20±0.5 ºC and 75±5.0 % relative humidity for 7 days in order to similar shelf life. The weight loss, CO_2 concentrations in the bag, carrot color (L* and *h*°), appearance (1-9), rooting and sprouting rate and rooting and sprouting degree, incidence of fungal decay and physiological disorders, carrot firmness, total soluble solid content, pH value, titrable acid content and taste (1-9) were determined during shelf life and storage. In the light of our findings, weight loss in perforated bags was higher than imperforated bags and MAP bags. There was no difference between washing with tab water and soaking in sodium hypochloride in terms of weight loss. It was determined that 'Nanco F1' type carrots could be stored for 3 months at 0±0.5 °C and 90±5.0% relative humidity without losing much of the quality for local and distant markets. In order to reduce the weight loss in carrot, MAP application was necessary.

Key words: *Kırıkhan, carrot, MAP, storage, quality.*

INTRODUCTION

Carrot (*Daucus carota var. sativus*) is a member of the Umbelliferae-Apiaceae family. It is a native of Central Asia and Near East. It is a biennial vegetable produced with seeds and has edible roots (Yanmaz, 1994). In a study investigating quality losses in carrots, it was reported that the losses before and during the harvest were high. In particular, the 49.45% discard ratio in the first class carrots produced for domestic and international consumption is an indication that there are a number of problems even as early as the starting from breeding stage (Sermenli et al., 2014). The losses that occur during harvest, after harvest and during storage are of mechanical physiological and pathological origin. Mechanical and pathological losses can be minimized by careful handling of harvest and postharvest processes and providing hygienic conditions during the storage period up until the consumption stage (Halloran et al., 1997). The respiration rate of carrots is low, and they do not deteriorate quickly when compared to other fruits and vegetables, can be stored for a long time, they continue to grow after harvest and display undesirable physical changes (rooting and sprouting) after harvest, especially during storage process (Özdemir and Çandır, 2013). New technologies are needed to reduce the loss of taste and flavour, to prevent bitterness and to reduce fungal decay during storage while at the same time preserving quality. 5 kg of carrots wrapped with perforated polyethylene were stored at 5 °C and 85-90% relative humidity conditions for 4 months and rooting - sprouting rate and

rooting - sprouting degree were examined. This study reported that rooting and sprouting rate increased during storage, sprouting rate reached 100% at the 2nd month and rooting rate started at the 2nd month and reached 100% at the 4th month (Kasım et al., 2000). It is stated that a clean container should be used for storage purposes, temperature should be kept close to 0 °C and the relative humidity should not exceed 95% (Tülek and Dolar, 2011). Application of chlorine to harvested carrot fruits after pre-cooling is a practical method (Hurst, 1998). Surface sterilization is provided by this method. Washing the roots with water is an effective method to remove fungal spores. Washing the carrots with chlorinated water reduces the incidence of disease (Tülek and Dolar, 2011). The Modified Atmosphere Packing (MAP) technique is a product preservation and packaging method that meet the consumers' growing demand for safe, pure products with high nutritional value. The quality of the products can be preserved for a longer time and the shelf life can be extended with the selection of proper atmospheric composition, packaging material and storage conditions in MAP technique (Kader et al., 1989; Farber et al., 2003). The purpose of this study is to improve the storage conditions of 'Nanco F1' carrot variety from Nantes group carrots grown in Kırıkhan region of Hatay Province by using MAP technique. The aim of the study was to create alternatives for raising the income levels of regional producers and to make a contribution for meeting the needs of domestic and international consumption.

MATERIAL AND METHODS

In this study, 'Nanco F1' carrot variety in the Nantes group carrots grown in Kırıkhan region of Hatay province was used. The carrots were supplied from the production site of a producer (Sedef Tarım Ltd. Şti.) in Kırıkhan. The carrots were sorted and selected on the basis of a uniform size and appearance and absence of defects. Carrots were submerged in tap water and / or 0.5% sodium hypochlorite for 3 minutes. After the carrots were dried, they were put in 10 kg commercial perforated or imperforated bags and 5 kg MAP bags (PBTW: Perforated bag with washing tap water, PBSH: Perforated bag with immersing in sodium hypochlorite, IBTW: Imperforated bag with washing tap water, IBSH: Imperforated bag with immersing in sodium hypochlorite, MAPTW: MAP with washing tap water, MAPSH: MAP with immersing in sodium hypochlorite). They were then placed in cold storage at 0 °C and at 90-95% relative humidity for 5 months. The carrots were stored at 20 °C for 7 days each month to determine the shelf life of the fruit.

CO2 concentrations in MAP bags: CO2 concentrations in MAP and perforated bags were monitored per month with a needle attached to a portable gas analyser (PBI-Dansensor America Inc., USA) via the septum glued onto the package throughout storage and CO2 gas ratios are expressed as a percentage.

Weight loss: During storage, the carrots in the bags were weighed with a 0.2 g sensitive scale (AND GX-20K,Tokyo, Japan), with a weighing capacity of 16 kg. For the purposes of measuring shelf life, 30 carrots were individually numbered and weighed per month with a 0.01 g-sensitive precision scale (Ohaus Adventurer, USA). The weight loss was expressed as a percentage.

Appearance (1-9): Samples taken each month from carrots that were stored at storage and shelf conditions were evaluated according to hedonic scale. The scale ranges from 1 (worst) to 9 (best). "5" on the scale constitutes the limit of being marketable (Cliffe-Byrnes and O'Beirne, 2007).

Taste (1-9): The taste of samples taken each month from carrots that were stored at storage and shelf conditions was assessed by a panel of 10 people in accordance with the hedonic scale. The scale ranges from 1 (worst) to 9 (best). "5" on the scale constitutes the limit of being marketable (Cliffe-Byrnes and O'Beirne, 2007).

Incidence of fungal decay and physiological disorders: The carrots that were stored at storage and shelf conditions were evaluated individually, fungal deterioration and physiological deterioration were observed and the decay rates were expressed as a percentage.

Carrot colour (L* and h°): Throughout the shelf life and during monthly removal from the storage, carrots were used to take readings by using L* a* b* Minolta CR-300 model Chromometer colour measuring device in accordance with C.I.E. (Konica Minolta Sensing Inc., Osaka, Japan). Readings were taken at 5 cm below the head in both sides (McGuire, 1992).

Rooting and sprouting rate: The carrots that were stored at storage and shelf conditions were evaluated individually, the ratio of forming roots and shoots of carrots during storage was determined as a percentage.

Rooting and sprouting degree: The number and length of shoots and roots formed in carrots according to scale 0-5 were taken into account (Halloran et al. 1997). The length of shoots and roots was measured in cm with digital callipers and then evaluated in accordance to their scale values.

Carrot firmness: During storage conditions and shelf life, carrot firmness was measured with the help of a penetrometer (Effegi model FT 444, Italy) with a drilling head length of 6 mm. First a thin layer with an approximate diameter of 1 cm on both sides has been removed from 5 cm below the head of each carrot and the values were taken in kg power and then converted to Newton (N).

pH value: The pH level was measured with a digital pH meter (Thermo Fisher Scientific Inc., MA, USA) during the storage and shelf conditions.

Titrable acidity content: It was measured with the potentiometric method (Sadler, 1994) during storage and shelf life, and 5 ml sample obtained from the carrot juice was increased to 100 ml by adding distilled water and it was titrated with 0.1 N NaOH solution until reading 8.1 in the digital pH meter (Brand titrette, Germany) and the results were calculated as citric acid / g citric acid / 100 ml fruit juice.

Total soluble solid content (TSS): TSS was determined as a percentage with hand refractometer (Atago ATC-1E Model, Atago Co. Ltd., Tokyo, Japan) during storage and shelf life.

From the carrot samples taken out monthly during the storage and shelf conditions, 2 kg of carrots were analysed in 3 repetitions for each time and every application. The research is set in accordance with the "factorial experiment in a completely randomized block design" and the data acquired were analysed through SAS software (SAS Institute, Cary, N.C.) (SAS, 2018) and Tukey test ($p < 0.05$) was employed for comparison and the results are given in the tables.

RESULTS

The CO2 concentration in the imperforated bag and MAP was 17.17% at the end of the 5 month storage period in the 'Nanco F1' carrot variety. The application which yielded the highest increase in CO2 concentration was “imperforated bag with immersing in sodium hypochlorite” (23.33%) (Data not shown).

An increase in weight loss was detected when the storage time of the 'Nanco F1' variety carrots was prolonged and reached an average of 6.49% in the 5th month. Among the applications, weight loss was least in the “imperforated bag with washing tap water” application and most weight loss was observed in the perforated bag. As the shelf-life prolonged, the average weight loss increased from the initial 0.70% to average of 1.20% at the third month and then slightly decreased to 0.81% at the end of the fifth month. The greatest weight loss among applications during shelf life was observed in the application of MAP with washing tap water, and the lowest amount was observed in the application of imperforated bag with washing tap water (Table 1).

During the prolonged storage and during shelf life of the carrots, there has been a decrease in the appearance scores according to the 1-9 scale and at the end of the 4th month these scores fell below the acceptable limit of 5 (4.81). Among the applications, MAP applications yielded the best appearance scores during storage and shelf life and did not fall below the acceptable limit. As the storage time of the 'Nanco F1' variety carrots was extended and during shelf life, the taste scores decreased according to scale 1-9 and fell below the acceptable limit of 5 at the end of 4th month (average 4.81 and 4.98 respectively). Among the applications, MAP applications received the best taste scores during storage and shelf life and did not fall below the acceptable limit even at the end of storage (Table 1).

The fungal decay and physiological deterioration increased as the storage period of carrots prolonged, and at the end of the 5th month, it was 5.92% and 7.17% respectively. The greatest increase in fungal decay and physiological deterioration during storage has been observed in imperforated bag with immersing in sodium hypochlorite application. During the shelf life there were increases in fungal decay and reached 45.56% at the end of the fifth month. No physiological deterioration was detected in any application during shelf life (Table 1).

'Nanco F1' variety carrots have been reduced in brightness during storage. Among the applications, the maximum reduction in L* value of carrot colour was observed in perforated bag and imperforated bag with immersing in sodium hypochlorite applications; MAP application has increased the brightness of carrots. During the shelf life, carrots were similarly reduced in brightness (Table 1). There was statistically no difference between the applications at carrot colour h° value during storage, while there was an increase in initial value of carrot colour h° value. As the shelf-life prolonged, the carrot colour h° values increased compared to baseline, but the differences between the months were statistically similar (Table 1).

Table 1. Effects of treatments on weight loss (%), appearance (1-9), taste (1-9), fungal decay (%), physiological disorder (%), carrot color L* and h° values in 'Nanco F1' type carrots during 5 months of storage at 0 °C and 7 days of shelf life at 20 °C

Storage type	Weight loss (%)	Appearance (1-9)	Taste (1-9)	Fungal decay (%)	Physiological disorder (%)	L*	h°
Cold storage treatments							
PBTW	4.52 a	6.50 b	6.39 b	0.96 b	0.00 b	40.80 c	53.37 a
PBSH	5.11 a	6.65 b	6.45 b	0.96 b	0.00 b	41.76 bc	53.72 a
IBTW	2.65 c	6.74 b	6.22 b	1.12 b	0.00 b	43.26 b	55.34 a
IBSH	3.60 b	5.85 c	5.37 c	6.42 a	19.25 a	42.00 bc	54.26 a
MAPTW	4.29 b	7.24 a	7.52 a	0.60 b	0.00 b	52.31 a	52.96 a
MAPSH	3.64 b	7.30 a	7.41 a	0.42 b	0.00 b	53.20 a	53.76 a
Storage time (Months)							
0	---	9.00 a	9.00 a	---	---	47.76 bc	51.93 b
1	0.83 e	8.54 b	8.28 b	0.00 c	0.00 d	46.03 c	53.85 ab
2	2.77 d	7.95 c	7.85 c	0.28 bc	0.00 d	49.03 b	54.70 ab
3	4.10 c	5.81 d	5.74 d	0.74 bc	3.60 c	52.03 a	55.16 a
4	5.65 b	4.81 e	4.98 e	1.79 b	5.27 b	43.84 d	53.50 ab
5	6.49 a	4.17 f	3.50 f	5.92 a	7.17 a	34.63 e	54.29 ab
Shelf life treatments							
PBTW	0.74 de	6.26 c	6.35 c	11.11 bc	0.00 a	52.53 b	52.02 b
PBSH	0.90 bc	5.70 d	5.72 d	8.33 c	0.00 a	53.38 a	53.30 ab
IBTW	0.62 e	5.72 d	5.76 d	13.33 b	0.00 a	51.51c	53.35 ab
IBSH	0.84 cd	4.26 e	4.26 e	19.44 a	0.00 a	52.52 b	54.15 a
MAPTW	1.23 a	6.50 b	6.70 b	0.00 d	0.00 a	53.22 ab	53.70 ab
MAPSH	1.02 b	6.96 a	6.87 a	0.00 d	0.00 a	53.93 a	53.09 ab
Shelf life time (Months+days)							
0+7	0.70 d	8.78 a	8.78 a	0.00 c	0.00 a	56.64 a	50.28 b
1+7	0.86 bc	8.33 b	8.43 b	0.00 c	0.00 a	52.44 b	53.91 a
2+7	0.81 cd	6.63 c	6.83 c	0.00 c	0.00 a	52.07 b	53.47 a
3+7	1.20 a	5.24 d	5.22 d	1.11 c	0.00 a	51.00 c	54.10 a
4+7	0.98 b	3.93 e	3.91 e	5.56 b	0.00 a	52.36 b	54.07 a
5+7	0.81 cd	2.50 f	2.50 f	45.56 a	0.00 a	52.57 b	53.77 a

As the storage period of 'Nanco F1' variety carrots was prolonged, the rates of rooting and sprouting increased from the 2nd month and reached to an average 58.65% and 26.37% respectively at the end of the 5th month. In MAP applications, no rooting and sprouting occurred in the first 3 months. The greatest increase in the rate of rooting among applications was in perforated bag with immersion in sodium hypochlorite and the greatest increase in sprouting rate was in perforated bag with washing tap water applications, the least increase was in MAP applications. No rooting or sprouting occurred for 4 months in the shelf life, and at the end of the 5th month it was 13.89% and 23.33% respectively on average. The highest rate of rooting during shelf life was in MAP applications whereas the highest rate of sprouting occurred in MAP with immersing in sodium hypochlorite application. As the storage

time prolonged, there were increases in roots and sprouts. During the shelf life, rooting rate was 0.29% and sprouting rate was 0.53% at the end of the fifth month (Table 2).

Table 2. Effects of treatments on rooting and sprouting ratio (%), rooting and sprouting degree (0-5), carrot firmness (N) pH value and TSS (%) in 'Nanco F1' type carrots during 5 months of storage at 0 °C and 7 days of shelf life at 20 °C

Storage type	Rooting ratio (%)	Sprouting ratio (%)	Rooting degree (0-5)	Sprouting degree (0-5)	Carrot firmness (N)	pH value	TSS (%)
Cold storage treatments							
PBTW	44.31 c	23.02 a	1.00 a	0.45 ab	96.52 ab	6.18 b	9.62 b
PBSH	51.55 a	19.62 b	1.03 a	0.53 a	99.19 a	6.18 b	9.00 d
IBTW	46.90 b	16.05 c	1.05 a	0.33 ab	94.61 b	6.16 b	9.28 c
IBSH	20.67 d	20.67 b	0.55 b	0.50 ab	97.19 ab	6.20 b	8.52 e
MAPTW	16.60 f	6.92 e	0.51 b	0.28 b	93.99 b	6.40 a	10.09 a
MAPSH	18.86 e	8.86 d	0.70 b	0.27 b	98.64 a	6.43 a	9.78 b
Storage time (Months)							
0	---	---	0.00 e	0.00 d	99.05 c	6.05 d	8.27 e
1	0.00 e	0.00 e	0.00 e	0.00 d	101.01 b	6.20 c	8.78 d
2	12.92 d	12.78 d	0.27 d	0.30 c	105.03 a	6.18 c	9.46 c
3	40.16 c	16.86 c	0.96 c	0.33 bc	96.30 c	6.29 b	9.50 c
4	54.01 b	23.28 b	1.56 b	0.57 b	92.08 d	6.56 a	9.81 b
5	58.65 a	26.37 a	2.06 a	1.14 a	86.69 e	6.27 b	10.49 a
Shelf life treatments							
PBTW	0.00 c	5.00 b	0.00 c	0.16 a	97.04 a	6.17 b	9.21 c
PBSH	0.00 c	4.44 b	0.00 c	0.08 b	98.05 a	6.17 b	8.82 d
IBTW	2.78 b	0.56 c	0.06 b	0.01 c	87.25 c	6.20 a	7.96 e
IBSH	0.00 c	0.00 c	0.00 c	0.00 c	93.19 b	5.86 d	7.85 f
MAPTW	5.56 a	8.89 a	0.11 a	0.16 a	93.58 b	6.11 c	10.03 b
MAPSH	5.56 a	4.44 b	0.13 a	0.12 ab	97.84 a	6.19 a	10.14 a
Shelf life time (Months+days)							
0+7	0.00 b	0.00 b	0.00 b	0.00 b	101.01 a	6.04 d	8.93 c
1+7	0.00 b	0.00 b	0.00 b	0.00 b	99.44 ab	6.21 a	8.67 d
2+7	0.00 b	0.00 b	0.00 b	0.00 b	97.28 bc	6.15 b	9.16 b
3+7	0.00 b	0.00 b	0.00 b	0.00 b	95.03 c	6.10 c	9.64 a
4+7	0.00 b	0.00 b	0.00 b	0.00 b	90.02 d	6.10 c	9.08 b
5+7	13.89 a	23.33 a	0.29 a	0.53 a	85.02 e	6.11 c	8.52 e

As the storage period of the carrots grew longer, the carrot firmness which initially averaged 99.05 N showed some increases and decreases; and at the end of the fifth month, it decreased to 86.69 N. During shelf-life, carrot firmness which initially averaged 101.01 N, showed decreases and decreased to 85.02 N at the end of the fifth month. The application of perforated bags and MAP with immersion in sodium hypochlorite during storage and shelf life resulted in the highest carrot hardness (Table 2).

During storage and shelf life, the pH values of the carrots have increased. The highest pH value during storage was detected in MAP applications. The highest pH value during shelf life was detected in imperforated bag with washing tap water and MAP with immersing in sodium hypochlorite (Table 2).

The differences in storage and shelf-life periods and the effects of titratable acidity are statistically insignificant (Data not shown). The increase in the TSS content storage period, which was initially 8.27% for the 'Nanco F1' variety carrots, was found to be increased and it was 10.49% at the end of the 5th month. Among the applications, TSS was found to be the lowest in imperforated bag with immersing in sodium hypochlorite while it was highest in MAP with washing tap water application. The TSS content storage time, which was initially 8.93% during the shelf life, showed some decreases and increases and decreased to 8.52% at the end of the 5th month. As the shelf-life increased, the value of TSS which was initially averaged 8.93%, showed some decreases and increases; and it decreased slightly and the average was 8.84% at the end of the 5th month. Among the applications, TSS content was the highest in the application of MAP with washing tap water and the lowest in imperforated bag with washing tap water (Table 2).

DISCUSSION

The most proper packaging film for carrots; should preserve the gas composition and cause low condensation which prevents moisture loss during storage (Workneh et al., 2001). In general, if the weight loss rate exceeds 10% of the total weight of the product, the product may lose its ability to be marketable (Grierson and Wardowski, 1978). In none of our applications weight loss has exceeded 10% at the end of 5 months. All fruits and vegetables lose weight at different rates depending on the environment and the product (Halloran et al., 2002). Similar to previous studies, we found that there was weight loss at different rates in carrots (Tronsmo, 1989, Toivone et al., 1993; Kasım, 1994; Yanmaz et al., 1995; Kasım, 2001a; Kasım, 2001b; Karaca et al., 2008). Similar findings have been reported by different investigators, indicating that appearance scores are reduced during storage (Atasay, 1999; Kasım, 2001a; Kasım, 2001b). Similar findings have been reported by different investigators, indicating that taste scores reduce during storage (Atasay, 1999; Terzioğlu, 2000; Kasım, 2001b; Karaca et al., 2008). Similar to our findings, it has been reported by Karaca et al. (2008) that carrot colour L* value scores were reduced during storage. Unlike our findings, in another study the carrot colour h° value of the baby carrots was reduced in the MAP storage (Karaca et al., 2008). In a study conducted by Sulaeman et al., (2003), it has been reported that L* and h° values in carrot chips stored at 0-1 °C for 5 months did not go through any changes. In all of our applications, rooting and sprouting rates were observed, but unlike our findings, in a study conducted by Terzioğlu (2000), carrots were not rooted and sprouted in any of the applications during cold storage for 5 months. It has also been reported by various investigators that root and sprout rates have increased during storage (Kasım, 1994, Kasım, 2001a, Kasım, 2001b). It has been reported that the storage temperature should be between 0 and 1 °C in order to minimize the sprout rate during storage and the sprouting may develop within 1 to 3 months when stored at 5 to 10 °C (Fritz et al., 2013). It has been reported that stored carrots may become rotten and rot may develop within 1 to 3 months (Tülek and Dolar, 2011; Uysal, 2012; Fritz et al., 2013). Physiological deterioration in stored carrots are reported to be spotting, surface browning or oxidative discoloration, aggravation, cold damage, aging and taste alteration (Uysal, 2012; Fritz et al., 2013). In a study conducted on baby carrots, the pH-value increased during

storage, which is similar to our findings (Karaca et al., 2008). Unlike our findings, Koca (2006) reported that the pH value of carrots decreased at the end of storage in stored carrots. It has been reported that the increase in TSS content may be due to an increase in the metabolic activity of starch to sugars (Cemeroğlu et al., 2001). Similar to our findings Kasım (1994), Atasay (1999), Kasım (2001a), Kasım (2001b) ve Karaca et al. (2008) reported that TSS content increased during storage. Unlike our findings, Svanberg and Nyman (1997) and Koca (2006) found that there was a decrease in TSS in stored carrots.

CONCLUSIONS

In the light of our findings, weight loss in perforated bags was higher than imperforated bags and MAP bags. There was no difference between washing with tab water and soaking in sodium hypochloride in terms of weight loss. For carrots that are packaged after being washed with tab water with MAP and being soaked in sodium hypochloride, there was an increase in L* values in color and the appearance was preserved, and the rate of rooting and sprouting was less. It was determined that 'Nanco F1' type carrots could be stored for 3 months at 0°C and 85-90% relative humidity without losing much of the quality for local and distant markets. In order to reduce the weight loss in carrot, MAP application was necessary.

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INVESTIGATION OF ANTIMICROBIAL ACTIVITY OF PHYSALIS ALKEKENGI L. FRUIT EXTRACTS COLLECTED FROM CORUH VALLEY

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ABSTRACT

Physalis alkekengi L. is a plant that can be used for complementary and alternative medicine in Europe and China. Previous studies have reported that Physalis alkekengi L. has antifungal, anti-inflammatory, analgesic and anti-cough effects. During the studies, different parts of the plant such as crown and leaf were examined for antimicrobial activity. In this study, it was aimed to investigate the antimicrobial activity of the methanolic extract of Physalis alkekengi L. plant fruits collected from Coruh valley (Artvin / Turkey). The effect of the extract of Physalis alkekengi L. fruits was investigated by disk diffusion and liquid microdilution methods against reference strains containing 5 Gram negative, 6 Gram positive bacteria and Candida albicans. As a result of the study, it was determined that the methanolic extract of plant fruit had no effect against Candida albicans (MIC=4096 mg/ml). while it showed a high level of action against Bacillus subtilis and Staphylococcus epidermidis (MIC=128 mg/ml). In the disk diffusion method, the highest zone diameter was determined against Staphylococcus epidermidis and Staphylococcus aureus at concentrations of 40 and 50 µl. As a result of the study, it was determined that the extract of Physalis alkekengi L. fruit was not effective even at the highest concentration on Candida albicans, whereas it was found to have a partial effect on Pseudomonas aeruginosa, known to harbor multiple intrinsic and acquired resistance genes, and a high level of activity against Gram positive bacteria.

Keywords: *Physalis alkekengi, Plant Extract, Antimicrobial Activity*

INTRODUCTION

Since ancient times, human beings have used plants as a medical resource. Their use has led to the further development of modern pharmacologists to produce new compounds with additional benefits such as the potential for combating more resistant diseases and low toxicity. Between 1981 and 2010, naturally derived products and their imitators accounted for 70% of the new chemical compounds reported (Newman and Cragg, 2012). Although advances in technology, science and medicine today are quite dizzying, the fact that the dramatic spread of infectious diseases cannot be controlled is still seen as an important problem. World Health Organization (2004) stated that the infectious diseases continue to be the second most important cause of deaths worldwide. For this reason, the need for new antimicrobial agents is greater than ever. Microorganisms that have been living on the earth since millions of years have been able to adapt and develop to the changing nature throughout the ages. Plants are the largest known biochemical and pharmacy stores on our planet. These living stores can

produce an unlimited number of biochemical compounds (Cowan et al., 1999). Plants are rich in secondary metabolites with various antimicrobial properties such as saponins, tannins, alkaloids, alkenyl phenols, glycoloalkaloids, flavonoids, sesquiterpene lactones, terpenoids and phorbol esters, which have many defence mechanisms against micro and macro organisms (Lewis and Ausubel, 2006; Tiwari and Singh, 2009). The antimicrobial activity that plants possess is the result of a correlation between the plant extract and its major secondary metabolites, and as time progresses, the plants resist by producing secondary sequestered metabolites against different microorganisms (Parasites, Fungi, Bacteria and Viruses). The antimicrobial activity of different plants has been examined by many researchers around the world.

Physalis alkekengi (*P. alkekengi*) is a multi-annual plant classified as taxonomic in Solanaceae family and widely found in Chinese and World traditional medicine. All parts, including fruits of P. alkekengi plant, have been used for therapeutic purposes, including clinical use. Analysis of old medical research has shown that P. alkekengi is traditionally used for a long time for various illnesses such as sore throat, cough, eczema, hepatitis, urinary problems and tumors (Wiu et al, 2008; Kosalec et al., 2005; Huang et al., 2010). After further research, it was determined that the major components of this fraction contained physical and flavonoids. Other studies have shown that *P. alkekengi* strains have excellent antibacterial and / or anti-inflammatory activity. An antimicrobial study of this plant that grows in Turkey have been not carried out on particularly fruits are based on our best knowledge. Thus, in this study, it was aimed to investigate the antimicrobial activity of locally grown *P. alkekengi* fruits collected from the Coruh valley.

MATERIAL AND METHODS

Materials

P. alkekengi plant and fruits was collected from Coruh valley and used as a material after the verification the spices by Atatürk University Faculty of Agriculture.

Preparation of the extract

The fruits of *P. alkekengi* were grinded in a postal mortar after being dried outdoors. 30 g of ground fruits were taken and extracted in 300 ml of methanol by holding on a rotary shaker for 48 hours. Then, filtered with Whatman No: 1 filter paper. The extract was then concentrated to dryness under reduced pressure at 40 °C using a rotary evaporator. The ethanol solubilized extract was stored at 4 [deg.] C. for further processing.

Test microorganisms

In this study, *Candida albicans* ATCC 60913, *Escherichia coli* ATCC 25922, *Klebsiella pneumonia* ATCC 27736, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* ATCC 12453, *Bacillus cereus*, *Bacillus subtilis* ATCC 6633, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228 *Streptococcus agalactiae* ATCC 12986, a total of 11 different microorganisms have been used.

Preparation of Microorganism Cultures

The microorganisms used in the study were grown in Mueller Hinton Broth by incubation at 37 °C for 24 hours.

Microdilution method

The microdilution method is based on the method recommended by the Clinical and Laboratory Standards Institute. Briefly, bacterial strains were grown on Mueller-Hinton (MH) agar plates and

inoculum suspensions were prepared from bacteria cultured for 12 hours. 0.5 McFarland suspensions were prepared to obtain a standard turbidity and *P. alkekengi* fruit extract concentrations of serially diluted were added between 128 and 4096 mg/mL in MH water. Bacterial suspensions were incubated aerobically at 37 °C for 18 hours. Three replicate samples were run for each test concentration. A is used as a reference for bacteria as an antibiotic. The MIC is defined as the lowest concentration that prevents visible growth.

Agar disc diffusion method

Microorganisms were activated by inoculating the Mueller-Hinton (MH) agar and incubated for 24 hours at 37 °C. The appropriate suspension was then prepared (suspension blur was 108 cells / ml according to the McFarland standard) and spread on the Mueller Hinton agar medium by diffusion plate technique. For agar disc diffusion, the test compound was placed in discs (7 mm) in different amounts (10-50 µl) and then allowed to dry. The disc was then placed on a pre-inoculated agar plate. Finally, all agar plates were incubated at 37 °C for 24 hours. The experiment was performed 3 times under aseptic conditions. Microbial growth was determined by measuring the diameter of the inhibition zone and average values were obtained.

RESULTS

The antimicrobial effects of the fruits were observed as 128-4096 mg/mL MIC values against the tested microorganisms (Table 1).

Table 1. MIC values against microorganisms of *P. alkekengi* fruit extract

Reference Strains	MIC Values (mg/ml)
Bacillus subtilis ATCC 6633	128
Bacillus cereus ATCC	256
Candida albicans ATCC 60913	4096
Enterocooccus faecalis ATCC 29212	256
Escherichia coli ATCC 25922	256
Klebsiella pneumonia ATCC 27736	1024
Proteus mirabilis ATCC 12453	256
Pseudomonas aeruginosa ATCC 27853	256
Staphylococcus epidermidis 12228	128
Staphylococcus aureus ATCC 29213	256
Streptococcus agalactiae ATCC 12986	256

The MIC value was determined to be 4096 mg/ml for *Candida albicans* as shown in table 1. The same value was found to be 1024 mg/ml for *Klebsiella pneumonia*. The inhibitory composition was determined to be 256 m /ml for *Bacillus cereus*, *Enterococcus faecalis*, *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus agalactiae*. The results showed that *P. alkekengi* fruit extract had maximum activity against *Staphylococcus epidermidis* and *Bacillus subtilis* (128 mg/ml). Again, as shown in Table 2, the highest zone diameter was determined against *Staphylococcus epidermidis* according to the agar disc diffusion method (19 mm). At the highest dose (50 µl), zone diameters of 12, 13, 15, 15 and 18 mm against to *Pseudomonas*

aeruginosa, *Bacillus subtilis*, *Streptococcus agalactiae*, *Enterococcus faecalis* and *Staphylococcus aureus* were determined, respectively.

Table 2. Zone diameters of *P. alkekengi* fruit extract against to microorganism according to agar disc diffusion method

	Disc				
Reference Strains	**10µl**	**20µl**	**30µl**	**40µl**	**50µl**
Bacillus subtilis ATCC 6633	-	-	10mm	12mm	13mm
Bacillus cereus	-	-	-	-	-
Candida albicans ATCC 60913	-	-	-	-	-
Enterocooccus faecalis ATCC 29212	-	-	-	12mm	15mm
Escherichia coli ATCC 25922	-	-	-	-	-
Klebsiella pneumonia ATCC 27736	-	-	-	-	-
Proteus mirabilis ATCC 12453	-	-	-	-	-
Pseudomonas aeruginosa ATCC 27853	-	-	-	11mm	12mm
Staphylococcus epidermidis 12228	-	11mm	15mm	18mm	19mm
Staphylococcus aureus ATCC 29213	-	-	12mm	17mm	18mm
Streptococcus agalactiae ATCC 12986	-	-	12mm	14mm	15mm

DISCUSSION

Despite advances in antibiotics over the last 70 years, infectious diseases are an important cause of morbidity and mortality worldwide and account for nearly half of all deaths in tropical countries (Moellering et al., 2007). Therefore, medicines with new compounds that are likely to be an alternative to existing antimicrobial agents are needed. The antibacterial activity of *P. alkekengi* was evaluated by in vitro broth dilution and agar disk diffusion method. In a similar study, Helvaci et al. (2010) reported that the physalin D component obtained from *P. alkekengi* fruits had a MIC value of 32-128 µg/mL against different microorganisms. In another study (Li et al., 2018), antimicrobial effect of *P. alkekengi* var *franchetii* extracts was done by disk diffusion method. It has been reported that *Bacillus aeruginosus*, *Bacillus coli*, *Staphylococcus epidermidis*, *Staphylococcus pythogenesis*, and *Blastomyces albicans* could not be inhibited while extracts showed inhibition of *Staphylococcus aureus*, *alpha streptococcus*, *beta streptococcus*, and *bacillus subtilis.* In this study, it was suggested that effective antimicrobial component of *P. alkekengi* extracts is highly sensitive to *Staphylococcus aureus*, *alpha streptococcus*, and *beta streptococcus*. The MIC value of this plant was reported to be between 25 and 50 g/L. While the results are compatible with our data, it is thought that the difference arising from the MIC values is due to the difference in the calculation of the extraction method and amount of the extract.

In another study, Yang et al. (2016) reported that 3 different compounds from *P. alkekengi plants* showed highly antibacterial activity on *Bacillus subtilis* and *Escherichia coli*. Shu et al. (2016) reported that the *P. alkekengi* plant extract had antibacterial potency against *Staphylococcus aureus* and *Pseudomonas aeruginosa* between 0.825 to 1.65 and 1.65 to 13.20 mg / ml MIC values, respectively. In the same study, a sepsis model of *Staphylococcus aureus* and *Pseudomonas aeruginosa* was established in mice and the antibacterial activity of *P. alkekengi* in vivo was examined. At doses of 160, 320 and 640 mg/kg, the *P. alkekengi* extract reduced the mortality rates to 33.3%, 33.3% and 58.3% in mice infected with *S. aureus*, respectively. Similarly, it was reported

that at doses of 160, 320 and 640 mg/kg, *P. alkekengi* extract reduced the mortality to 50%, 58.3% and 58.3%, respectively, in mice infected with *P. aeruginosa*.

The increased resistance of bacteria to older compounds has resulted in a new understanding of health, such as newly discovered antibiotics from different sources, new semi-synthetic versions of the antibiotics, less-used antibiotics in the past, obtaining the novel narrow-spectrum derivatives that have not been developed before the plant extracts as an alternative to using of antibacterial drugs. The different components that are thought to have different antimicrobial activities, especially at different concentrations of different plants, are important in this regard. Plant based antimicrobials have therapeutic potential because they can serve this purpose. Because of the lesser side effects than synthetic antimicrobials, it is now necessary to further investigate plant-derived antimicrobials. Further research is needed to determine the identity of antibacterial compounds within these plants and to determine their full-effect spectrum.

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COLLECTION OF INDIRECT RESISTANCE TRAITS DISTURBANCE VIRULIFEROUS INSECTS TO ELONGATE THE PERIOD BEFORE THE FIRST INFECTION OF ZYMV

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ABSTRACT

Zucchini Yellow Mosaic Virus (ZYMV) is one of very important viruses that causes high yield loses of cucurbit in Iraq and the world. In a program of selection of indirect resistance traits for cucurbit viruses, four aspects of squash upper leaf shape and coloration (a- entire silver leaf coloration, b- white coloration of the leaf veins which appeared as white net, c- white spots between the main leaf veins branching on the upper leaf surface, d- downy upper leaf surface shape) were observed. Each of these aspects In one way or another might made repelling or fairing or disturbing viruliferous insects, and so lead to late the visiting of virus vector insects to the plants, and thus they delayed and diminished the infection percent, elongated the period of disease dissemination, and accordingly increased the health yield productivity period of plant. Previously the genotypes had possessed these aspects were selected and developed to pure lines of cucurbits indirect resistant origin, also number of genes responses of each character and their heritability nature were determined. This study aimed to introduce these aspect genotypes in a program of hybridization between every two of them to obtain the dual hybrids, which then crossed together to obtain the quadrilateral hybrid that have all the four aspects of upper leaf surface fluffy and coloration shapes. The coloration traits appeared successively each followed the other in genotypes, so the period of delaying of first infection elongated from 14 in control to about 33 days after control infection in the dual hybrids, and to about 55 days in the quadrilateral hybrid.

Key words: *vegetable breeding, virus resistance, cucurbits viruses, squash diseases, genetic resistance*

INTRODUCTION

Among several diseases infected cucurbits, those caused by viruses seriously affect the quality and quantity of yield, representing the highly limiting factor for these crops production (Aljanabi et al., 2017; Harris and Kring, 1964). More than 30 virus species can naturally infect cucurbit crops, but that belong to the genus *Potyvirus* were demonstrated to be the most important group (Finettisialer *et al*., 2012; Moura *et al*., 2005), and *Zucchini yellow mosaic potyvirus* (ZYMV) had been representing the most serious one of them. Aphids are the natural and efficient vector for virus species from the genus *Potyvirus* (Aljanabi et al., 2017; LIMA, 2015; Gal-on, 2007; Hrpaz, 1982; Kennedy, 1969; Kring.1964, 1969, 1972).

Genetic resistance is the most effective means to control diseases (Agrios, 2004), particularly that of virus. But in case of non-availability of direct resistance, pathologist may resort to the indirect traits, as the resistance to viruliferous insects or other vector organisms of the virus. It has been observed that some varieties of zucchini possess secondary indirect resistance characteristics, as the presence of the white spots coloration of squash leaves, or fluffy upper leaf shape or some other kinds of leaf coloration were detected to have the effect of repelling and fairing the viruliferous insects and so delaying their infection and led to reduce infections with viruses under field conditions (Aljanabi,

2014; Aljanabi et al., 2015a; Shifriss, 1982). Study of these traits pure lines effect on delaying infection of ZYMV was between 8-13 days and the infection percent reduced to about 46 –65 % (Aljanabi et al., 2015b). Generally these traits were found to be controlled by single dominant gene of two alleles for each one, so they were simply inherited and transmitted. (Aljanabi et al., 2015a; Shifriss, 1982).

This study aims to collect these indirect resistance traits genotypes of hairy surface and leaf coloration (full silvery, netty white, and spotted white) of some inbred lines of squash in dual and then quadrilateral hybrids to study their effectiveness on the infection delaying and the infection percent of ZYMV.

MATERIALS AND METHODS

Pure lines of fuzzy upper leaf surface and all forms of leaf coloration (Full silver, netty white, spotted white) which obtained in previous studies (Aljanabi et al., 2015a; Aljanabi et al., 2017) by continuous self-pollination, selection and purification of each aspect until stability or no new variations were observed. In this study collection of these indirect resistance traits in one genotype was achieved by a program of two stages, in the first one, each two shape pure lines (full silver and netty white) and (fluffy surface and spotted white) were crossed together to produce their dual hybrids. In the successive generations selection was done to the plants of each obtaining hybrid having the two interactive traits by self-pollination till their stability. In the second stage these selective stable genotypes having the two traits were crossed together to produce the quadrilateral hybrid that have the fourth interactive traits of all indirect resistance traits. Study of these hybrids genotypes having two to four aspects effectiveness on the delay of infection and its percent with the virus was culculated by the (Aljanabi, 2015b) following relations:

Character constant (Character efficiency) $= \frac{Character\ saving\ period}{Character\ appearance\ period\ mean}$..... (1)

Control lasting period $= No.of\ days\ to\ first\ infection\ before\ fruiting + No.of\ days\ to\ full\ infection\ dissimination$ ... (2)

Genotype saving period $= No.of\ days\ to\ first\ infection\ after\ control + No.of\ days\ to\ full\ infection\ dissimination$ ... (3)

Genotype saving degree $= \frac{genotype\ saving\ period - control\ lasting\ period}{genotype\ saving\ period} \times 100\%$..... (4)

Genotype saving efficiency = $saving\ degree \times character\ constant$ .... (5)

Health yield percent $= \frac{Health\ yield}{Total\ yield} \times 100\% = \frac{period\ of\ health\ yield}{period\ of\ total\ yield} \times 100\% = \frac{yield\ savig\ period}{period\ of\ total\ yield} \times 100\%$ ... (6)

RESULTS AND DISCUSSION

The four aspects of squash upper leaf shape and coloration [a- entire silver leaf coloration (Aljanabi , 2015a; Shiffriss, 1982), b- white coloration of the leaf veins appearing as white net(Aljanabi , 2015a), c- white spots between the leaf veins branching, d- downy upper leaf surface shape (Aljanabi , 2015a; Scarchuk, and Lent, 1965; Scott, D.M., and Riner, 1046)] of indirect virus resistance characters causing virulefirous insect disturbance and repelling were previously selected and developed to pure lines of squash indirect resistant origin in different genotypes (Aljanabi, 2014; Aljanabi et al., 2015a). These aspects were found to be controlled by one dominant gene of two alleles to each traits, so all traits were simply inherited except of the fluffy trait, as it was partially dominant,

so it lost some of their fluffy intensity when its genotype crossed with other haven't this trait (Aljanabi, 2014; Aljanabi et al., 2015a).

The two kinds of dual hybrids which obtained from the hybridization between every two inbred line genotypes (the full silver leaf with the netty white shape) as in (Fig. 1) and (fluffy surface with spotted white) as in (Fig. 2), to have two kinds of coloration shape, showed an increase in effectiveness of nuisance and drifting the insects away and keeping every hybrid plant intact for longer period, and this period increased more and more when the two dual crossed together to produce the quadrilateral hybrids having all the four aspects of upper shape and coloration (Fig. 3), as the period of delaying of the first infection was increased to the mean 14 days after control infection in the single genotype, and to 33 days in the dual hybrids and more to about 55 days in the quadrilateral hybrids having the four indirect resistance traits (Table 1), and accordingly the saving degree raised to 0.46 in the single genotype, and to 0.77, 0.89 , and the saving efficiency to 49.8, 66.6% in the dual and quadrilateral hybrids respectively, while the period of healthy yield were increased from 25 days in the single genotype, and to 33, 59 days in the dual and quadrilateral hybrids respectively, and so that reflecting on the disease dissemination period, as it increased gradually from 9 days in the single genotype, to 14 days in the dual, and more to 20 days quadrilateral hybrid. All that may due to the inconvenience or fairing or non-attracting of viruliferous insects to the plants might because of the disturbance of hairy surface or the reflection of light on the white spots so they disturb the insect and alienate it from the plant in a similar manner to its reflecting on aluminum covers which used by (Hrpaz, 1982; Kennedy, et al., 1969; Moore, et al.,1965; Kring, 1964, 1969, 1972) to repel aphids from plants, and accordingly that lead to late of visiting of virus vector insects to the plants, thus they delayed and reduce the incidence of viruses transmitted by this insect (Loebenstein, et al., 2006), and so diminished the infection percent and elongated the period of disease dissemination as (Loebenstein et al. 2006; Moore, et al.1965; Smith and Webb, 1969; Smith, et al.1964; Harris and Kring, 1964) found when used the reflecting mulches, and that increased the health yield productivity period of plants, and so the period was elongated more and more as the number of these traits increased in the genotype, and as they was regulated in appearing sequence in the genotype each followed the other as (Aljanabi et al., 2015b) found in some genotype, but they synchronized appearing in others.

Table 1. Relation of squash leaf coloration aspect number in genotype with the infection delay period, saving degree, efficiency, and healthy yield ratio of plants

Genotype leaf Coloration	Period of Character appearance (day)	Period of infection (day)	Period of Disease Dissemination (day)	Saving (lasting) period (day)	Character Constant %	Saving Degree	Saving Efficiency %	Health yield %
Full green leaf (Control)	0.0	4.0	3.0	7.0	0.0	0.0	0.0	3.0
Full silver leaf	8.2 – 20.2	12.0	9.0	21.0	92.8	0.46	46.0	14.0
Full silver × Netty white	28.0- 40.2	19.0	14.0	31.0	77.1	0.774	59.6	33.0
Spotted white × Fluffy surface	51- 70.8	28.3	14.0	42.3	59.7	0.834	59.8	38.3
Full silver to Netty to Spotted to Fluffy	65.0-90.0	47.0	20.0	67.0	74.4	0.895	66.6	55.0

Every number in table represents the average of three replicates.

Character constant (Character efficiency) = $\frac{Character\ saving\ period}{Character\ appearance\ period\ mean}$ ×100%..... (1)

Control lasting period = $No.of\ days\ \ to\ first\ infection\ before\ fruiting\ +$
$No.of\ days\ to\ full\ infection\ dissimination$ ... (2)

Genotype saving period = $No.of\ days\ to\ first\ infection\ after\ control +$
$No.of\ days\ to\ full\ infection\ dissimination$ ... (3)

Genotype saving degree = $\frac{genotype\ saving\ period-control\ lasting\ period}{genotype\ saving\ period}$ ..... (4)

Genotype saving efficiency = $saving\ degree \times character\ constant$ .... (5)

Health yield percent = $\frac{Healthy\ yield}{Total\ yield}$ ×100% = $\frac{period\ of\ healthy\ yield}{period\ of\ total\ yield} \times 100\% = \frac{yield\ savig\ period}{total\ yield\ period} \times$ 100% ... (6)

Full silver Netty white Full silver to netty white

Fig 1. Hybridization between the Full silver and Netty white coloration aspectes

Fluffy surface Spotted white Fluffy spotted white surface

Fig (2) Hybridization between the Fluffy surface and the spotted white coloration aspects

Full silver to netty white Fluffy spotted white spotted white Full silver netty white Fluffy

Fig 3. Hybridization between the two dual hybrids (Full silver to netty white) and (fluffy to spotted white) to produce the quadrilateral hybrid possess the (Full silver, netty white, Fluffy, spotted white) coloration aspects

CONCLUSION

Collection of indirect resistant traits of fluffy surface and leaf coloration in one hybrid genotype increase their effectiveness of disturbing and repelling viruliferous insects and so delay and reduce the virus infection and increased the disease dissemination period with the number of aspects in the hybrid.

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STEVIA: A NEW GENERATION NATURAL SWEETENER

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ABSTRACT

Nowadays, rapid changes in dietary habits, especially an increase in sugar consumption are the basic cause of human diseases like obesity, diabetes, tooth decay and cardiovascular diseases. Hence, utilization of natural sweetening agents other than sugar has been initiated around the world and more than 20 sweeteners are being used according to the priority and availability. One of such known natural sweeteners is *Stevia rebaudiana Bertoni* that belong to family *Asteraceae* and was discovered in 1887 in South America. More than 80% of Stevia species have been detected in North America and around 200 native species are found in South America. It is an endemic, perennial species that grows in moist environment with an average temperature of 25 °C. On one hand, where countries like Paraguay and Brazil used it as a sweetener and therapeutic agent, countries like Japan has been using it as a food additive over thirty years. Although its production is dominant in Mediterranean region of Turkey, some parts of the Aegean, Black Sea and Central Anatolian regions are known for its small-scale production. Despite the presence of several sweetening compounds, Stevioside is the main sweetening agent in the plant. Being an ideal natural product to replace sugar, *Stevia rebaudiana* has attracted many researchers because of its non-toxic and non-mutagenic nature with low calorie glycosides. Thus, we are working to determine the chemical properties of Stevia plant under different growth conditions so that its production can be increased. Specifically, we aim to determine the effect of Boron application on the changes in steviol glycoside content and the germination level of *Stevia rebaudiana* plant.

Keywords: *diabetes, glycosides, Stevia, stevioside, sweetener*

INTRODUCTION

Stevia rebaudiana Bertoni, known as sugar grass in Turkey, was first discovered by South American life scientist Antonio Bertoni in 1887 (Table 1). In 1905, after the Paraguayan chemist Dr.Rebaudi, this plant was named as *Stevia rebaudiana* (Yadav and Guleria, 2012). This plant is endemic and perennial in nature that grows at an average temperature of 25 °C. It is called as miracle grass as its glycosides provide 300 times more sweetness than saccharose and considered to be an ideal natural product that can be used instead of sugar (Anton et al., 2010; Puri et al., 2012).

Stevia leaves contain a natural complex evolved from eight sweet diterpene glycosides including isosteviol, stevioside, rebaudiosides (A, B, C, D, E, F), steviolbioside and dulcoside A (Rajasekaran et al., 2008; Goyal et al ., 2010). Stevia has a delicate and straight body with a deep rooted canopy of 30-50 cm. The taste of all green parts of the plant is sweet. The leaves are simple, thin with a length of 2-4 cm and of very different size and shape. The leaves in the dry state get a green-brown color with a darker upper side (Kinghorn 2003). Flowers are small, white, 15-17 mm wide and grow in clusters with a random distribution (Marsolais et al., 1998). Flowers start blooming atleast after 4 leaves are grown. Flowering lasts for about 1 month and during this period, flowers of

different developmental stages can be seen in the plant. Seeds, covered with a sensitive shell and protrusions, are 3 mm in length with 1000 grain weight of 0.15-0.3 g. They contain a small amount of endosperm that spread in the environment along with the wind (Ramesh et al., 2006). Generally, the seeds have low viability and great variation (Brandle et al., 1998).

Table 1. Classification of *Stevia rebaudiana*

Classification	
Kingdom	*Plantae*
Subkingdom	*Tracheobinta*
Superdivision	*Spermatophyta*
Division	*Magnoliophyta*
Class	*Magnoliopsida*
Subclass	*Asteridae*
Group	*Monochlamydae*
Order	*Asterales*
Family	*Asteraceae*
Subfamily	*Asteroideae*
Tribe	*Eupatorieae*
Genus	*Stevia*
Species	*rebaudiana*

Nowadays, rapid changes in dietary habits, especially the increase in sugar consumption, are the main causes of human diseases such as obesity, diabetes, tooth decay and cardiovascular diseases. However, Stevia is a natural and healthy alternative to sugar and artificial sweeteners. Therefore, we are working to determine the chemical properties of Stevia plant under different growth conditions so that its production can be increased. In this study, we aim to determine the effect of Boron application on changes in the steviol glycoside content and germination level of the *Stevia rebaudiana* plant.

MATERIAL AND METHODS

In this study, it was aimed to grow two different Stevia genotypes, Eirete F2 and Criolla in hydroponic conditions under different B dosages. The experiment was set up under controlled hydroponic environment with 3 replicates of each genotype and 10 plants per genotype were grown. Different treatments, Control, 0 ppm B and 10 ppm B were given to the plants.

RESULTS AND DISCUSSION

Importance of B element for the plants was understood during early 1900s after Katherine Warington released the results of their experiment on Phaseolus (Warington, 1923). In plants, Boron has important role in sugar transport, cell wall synthesis, lignification, formation of cell wall structure, carbohydrate metabolism, RNA metabolism, respiration, IAA (indolacetic acid) metabolism, phenol metabolism, pollen germination and regulates the structural and functional properties of biological membranes in pollen tube growth (Kacar ve Katkat, 1998).

This study was conducted with an aim of determining the effect of different Boron treatments on germination and increase the diterpene steviol glycoside content of two *Stevia rebaudiana* Bertoni genotypes. Boron deficiency and toxicity was observed in Stevia plants grown under 0 ppm B and 10 ppm B treatment, respectively. Spots and folded leaves were observed in grown leaves. Within the scope of this project, we will be able to promote healthy Stevia production in hoagland solution in a faster and homogeneous way.

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RHEOLOGICAL MODELING EVALUATION OF THE EFFECT OF TEMPERATURE ON DIFFERENT DILUTIONS OF HONEY

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ABSTRACT

Honey is the natural viscous food produced by honey bees from the nectar of plants. Honey available in Albanian market was characterized for rheological and physicochemical properties. Honey was serially diluted with different percentages of water (10 – 50 %). The density and viscosity of diluted honey were experimentally determined as a function of temperature (20 to 60°C). Viscosity is an important quality attribute of honey and there are various physical that influence this property. Several physicochemical and rheological characteristics of floral honey were investigated: density, pH, free acidity, ash, electrical conductivity, dynamic viscosity and kinematic viscosity. To study the temperature and dilution effect on viscosity Arrhenius, Abramovic and power law models were considered. The evaluations of the models were done by R^2 and mean absolute percentage error. According to the results Abramovic model provides a good description of honey viscosity as a function of the combined effects of temperature.

Key words: Honey, density, viscosity, temperature, Arrhenius model.

INTRODUCTION

Honey is a sweet, viscous food substance produced by bees and some related insects (Crane E., *1990).* Bees produce honey from the sugary secretions of plants (floral nectar) or other insects (aphid honeydew) through regurgitation, enzymatic activity, and water evaporation. Honey is stored in wax structures called honeycombs (Crane E., *1990 and* Crane, E., Walker, P., & Day, R. 1984). It is composed of mainly carbohydrates such as monosaccharides (glucose and fructose) and oligosaccharides like sucrose, maltose, melezitose, and raffinose. Pure honey also contains proteins, fats, water, vitamins and minerals (Nwalor, J.U., Babalola, F.U. and Anidiobu, V.O. 2018). It is reputed to have a diverse set of nutritional and medicinal benefits. Honey production constitutes 1.4% of livestock production in Albania. Data show that one of the most important developments during this period (until 2016) was the increased number of bee hives. By the end of 2016, there were 303,000 bee hives, so 4000 ton honey production. Meanwhile, in the end of 2014 Albanian beekeepers counted a total of 261,000 bee hives and total production of honey was 3000 ton. This indicator along with honey production figures confirm that honey is one of the main farm products measured in tons. Honey production increased by 25 percent. Most of the honey bee colonies are found in Korca, Vlore, Fier, Durres, and in the southeastern region (Institute of Statistics INSTAT, 2017). The purpose of this work was characterized physicochemical and rheological properties, of pure and diluted honey sample from south region. Honey was serially diluted with different percentages of water (5%, 10%, 15%, 20%, and 25 %). Furthermore, this study explores the efficacy of rheological characterization properties to adulterated or fake honey. The physicochemical characterizations of honey need time and expensive procedures, while rheological properties determination is more efficiency (Bogdanov, 2009; Akhtar et al., 2014). The composition and properties of honey vary with the floral and honeydew sources utilized by honeybees, as well as by regional and climatic conditions (Lazaridou et al. 2004). Studies on rheological behaviors of honeys, like other fluid foods, are important for applications

related to handling, storage, processing, quality control, and sensory analysis (Yoo, 2004). Moreover, honey rheology has been correlated with its chemical composition (Gómez-Díaz et al. 2009, Bakier 2007, Buba et al., 2013;). The rheological properties of honey vary, depending on water content, the type of flora used to produce it (pasturage), temperature, and the proportion of the specific sugars it contains. Honey is a natural viscous food well known for its high nutritional and prophylactic values (T. Sato and G.Miyata, 2000). The low moisture content protects honey from microbiological activity and thus it can be preserved for longer periods (AL-Naji and Hujazy, 1982; Cantarelli et al., 2008; and El-Metwally, 2015). Viscosity is an important quality attribute of honey and there are various physical as well as biochemical factors such as temperature, moisture content, and presence of crystals, colloids, and sugars that influence this property (Sudhanshu Saxena, Lata Panicker, and Satyendra Gautam, 2014). Most of the honeys were reported to have Newtonian fluid like characteristics, whereas, some honey has been reported as non-Newtonian fluids (Witczak M., Juszczak L., and Gałkowska D., 2011).

MATERIAL AND METHODS

Honey available in Albanian market, from south region was characterized for rheological and physicochemical properties. Honey was serially diluted with different percentages of water (5%, 10%, 15%, 20%, and 25 %), with a volume of 500 ml. The density and viscosity of diluted honey were experimentally determined as a function of temperature (20°C, 25°C, 30°C, 35°C, 40°C, 45°C, 50°C, 55°C, and 60°C). Physicochemical and rheological characteristics of honey were analyzed: density, pH, free acidity, ash, electrical conductivity, dynamic viscosity and kinematic viscosity. The pH (PHS-3CW Microprocessor pH/ mV METER) and the electric conductivity (DDS-120W Microprocessor Conductivity Meter) were measured. Viscosity and temperature of honey samples were measured using the Digital Viscometer Model NDJ-5S with accuracy ±1%. The SP (1-4) spindle was operated at 6, 12, 30, and 60 rpm. In addition to the dynamic viscosity and density, kinematic viscosity by the formula was also determined. Density and viscosity are very sensitive to temperature, studied and the dependence between them. The effect of temperature on the kinematic viscosity of liquid is described by means of the Arrhenius equation as:

$$\mu = \mu_{\infty,T} \exp\left(\frac{E_a}{RT}\right) \qquad (1)$$

Where μ is the dynamic viscosity in Pa.s, $\mu_{\infty,T}$ is the viscosity at infinite-temperature in Pa.s, E_a is the exponential constant that is known as activation energy (J/mol); R is the gas constant (8.31 J/mol.K) and T is the absolute temperature Kelvin (Clements C. et al.; 2006, Ahmad M. F.; 2009, Giap S. G. E.; 2010).

Abramovic also used three-constant formula known as Andrade equations that are represented in the following equations:

$$Ln\mu = A + \frac{B}{T} + \frac{C}{T^2} \qquad (2)$$

Where μ is the dynamic viscosity in Pa.s, T is the temperature in Kelvin. A, B and C are constants.
Power Law Model

$$\mu = k(T - T_{ref})^n \qquad (3)$$

Where k and n are constants, T_{ref} is reference temperature of 273.15 K (Fasina O.O. and Colley Z., 2008).

The mean absolute percentage error (MA%E), which indicates the deviance of the observed values from the calculated, was calculated using the following formula:

$$MAPE^{a} = \frac{\left|\sum_{1=1}^{n}\left(\frac{A_0 - A_C}{A_0}\right)\right|}{n} \cdot 100 \qquad (4)$$

Where A_0 is the observed value, A_C is the calculated value, and n represents the number of pairs of samples.

RESULTS AND DISCUSSION

This study focused on the effect of water and temperature on the rheological behavior of honey. Rheology is used as a quality parameter for many food products and is also an important property for honey (Bera A., Almeida-Muradian L. B., and Sabato S. F., 2009). Several physicochemical and rheological characteristics of pure and diluted honey were investigated: density, pH, free acidity, ash, electrical conductivity, dynamic viscosity and kinematic viscosity. Table 1, shows the **p**hysicochemical and rheological properties of pure honey (H). Honey is acidic (pH 4.1), within the European range 3.7- 4.5. The ash content of our pure honey was 0.02%. The ash content of honey is mainly mineral trace elements: calcium, copper, iron, magnesium, manganese, potassium, sodium, and chlorides, phosphates, silicates and sulphates. The color of our pure honey was dark. Dark color shows is very rich in minerals. These trace amounts of minerals may be important for human nutrition (Al M. L., Daniel D., Moise A., Bobis O., Laslo L., and Bogdanov S., 2009). Our honey samples showed electrical conductivity 0.1363 mS/cm. This measurement depends on the ash and acid content of honey; the lower ash and acid content, the lower the resulting conductivity. Free acidity was 10 meq/Kg. Figure 1 shows the effect of temperature on density and electric conductivity of pure honey (H). Results showed that temperature has significant influence on physical parameters. Electric conductivity rise (0.1283 – 0.1713 mS) and density decrease with temperature rise (1.4622 – 1.4601 g/cm^3).

Table 1: Physicochemical properties of pure honey (H)

Honey	Density (g/cm^3) (25C)	pH	Free acidity (meq/Kg)	Ash (%)	Electric conductivity (mS/cm) (25C)	Viscosity dynamic (mPa.s) (25C)	Kinematic viscosity (mm^2/s) (25C)
H	1.46185	4.1	10	0.03	0.1363	68800	47064

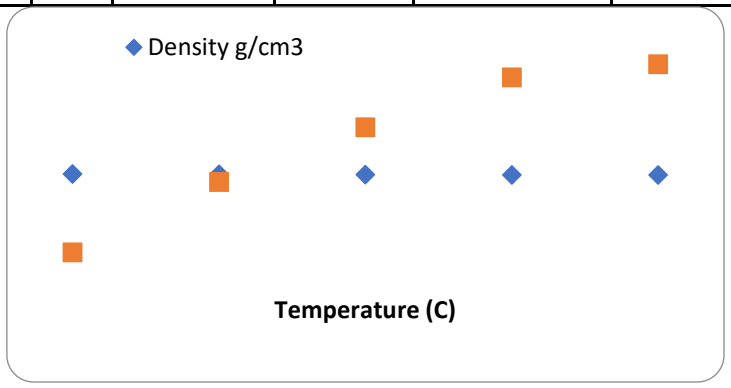

Figure 1: Effect of temperature on density and electric conductivity of pure honey (H)

Figure 2 shows effect of temperature on dynamic and kinematic viscosities of pure honey (H). Results showed that temperature has significant influence on viscosities. The kinematic viscosity (mm^2/s) requires knowledge of mass density of the liquid, at that temperature and pressure. It is defined as ratio of dynamic viscosity (mPa.s) to density (g/cm^3). The viscosities of honey were measured at temperatures ranging from 25C to 60C and found to decrease with increase in the temperature. Temperature dependent decrease in viscosity of honey may be attributed to reduced molecular friction and hydrodynamic forces (Saxena S., Panicker L., and Gautam S., 2014).

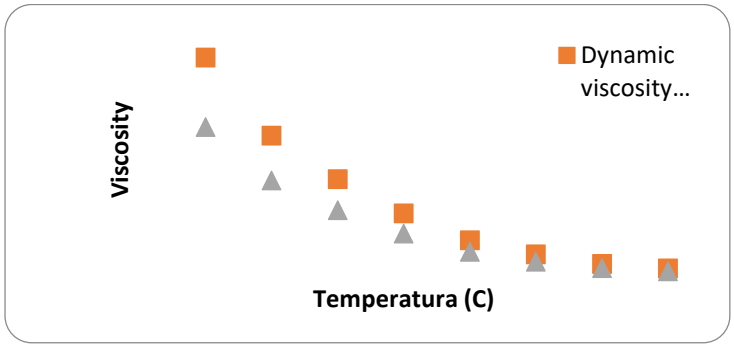

Figure 2: Effect of temperature on dynamic and kinematic viscosity of pure honey (H)

The experimental data of pure and diluted honey, for dynamic viscosity (Pa.s) fitting by Arrhenius model are shown in Figure 3. The dependence of the honey viscosities to temperature was modeled by using Arrhenius Equation (Eq. 1). Table 2 shows the results of parameters for Arrhenius model, correlation coefficient (R^2) and mean absolute percentage error ($MAPE^a$).

Table 2: Parameters for Arrhenius model

	a	Ea (kJ/mol)	R^2	$MAPE^a$
H	3.04699E-12	76.42	0.994	0.3285
H1	3.64280E-13	74.52	0.9987	0.0880
H2	5.03027E-12	65.47	0.995	0.0269
H3	6.97733E-12	63.38	0.99	0.5305
H4	2.21555E-10	51.60	0.9922	0.2847
H5	4.72675E-10	48.53	0.995	0.1445

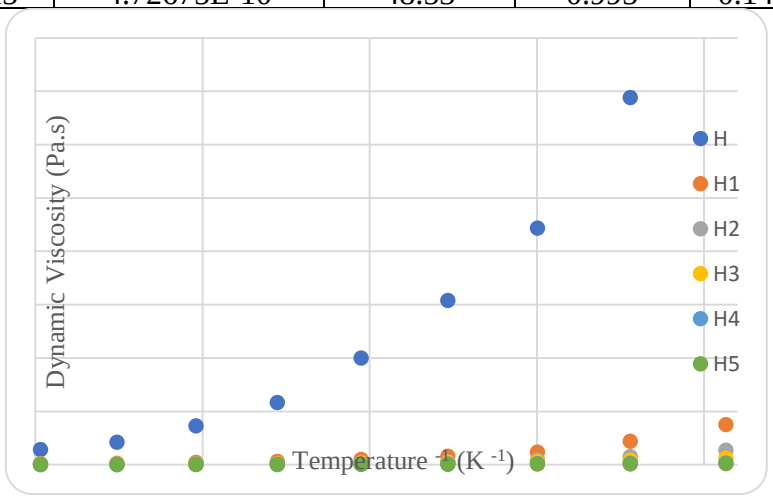

Figure 3: Effect of temperature on dynamic viscosity of different diluted honey and fitting of model to experimental data by Arrhenius equation

Arrhenius model was suitable to describe temperature dependence of the viscosity for all investigated honeys. In all cases the determination coefficient (R^2) exceeded values >0.99. The mean absolute percentage error (MAPE[a]) (Equation 4) for pure and diluted honey range from 0.02 – 0.53 %, was below 10%. The values of activation energies of the analyzed honeys ranged from 48.53 - 76.42 kJ/mol, which describe the sensitivity of viscosity to temperature changes.

Figure 4 shows the honey ln μ (Pa.s) dependence of the (1/T) and the fitting of the Abramovic model to experimental data (Equation 2). Table 3 resumes the results of Abramovic parameters, correlation coefficient (R^2) and mean absolute percentage error (MAPE[a]).

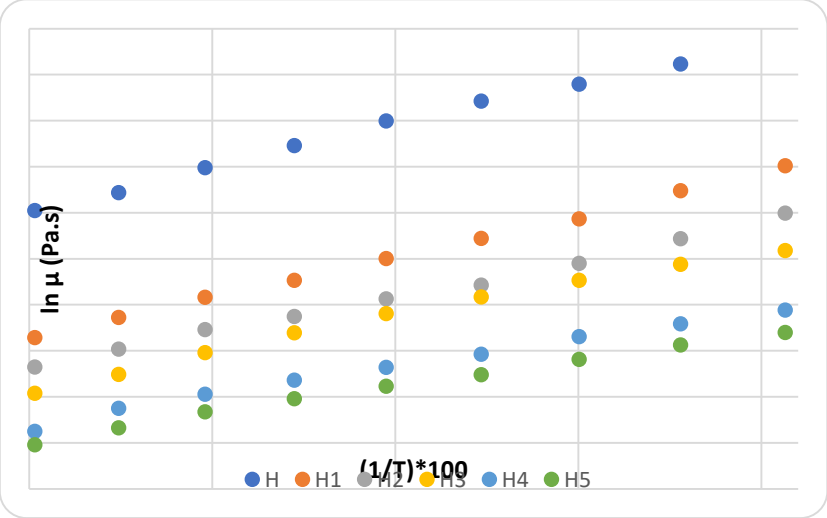

Figure 4: Honey viscosities dependence of temperature and fitting of model to Experimental data by Abramovic equation

Table 3: Parameters for Abramovic model

	A	B	C	R^2	MAPE[a]
H	-88.516	482.59	-614.47	0.9987	0.06
H1	-13.452	-5.2207	147.97	0.9991	3.57
H2	5.3465	-117.16	305.52	0.997	7.43
H3	-92.88	496.06	-654.56	0.9998	0.13
H4	-59.617	295.67	-364.21	0.9968	0.01
H5	-51.557	246.36	-293.08	0.9985	0.01

According to our experimental data, Abramovic equation was the best fit of dynamic viscosity of honey samples, where the determination coefficient (R^2) exceeded values> 0.995. The mean absolute percentage error ranges from 0.01 to 7.43 for pure and diluted honey were below 10%.

Figure 5 shows the dynamic viscosity (Pa.s) versus temperature (C), for pure and diluted honey. Experimental values were processed according to the Law Power model, equation 3. In the table below are presented k, n constants, correlations coefficient and mean absolute percentage error.

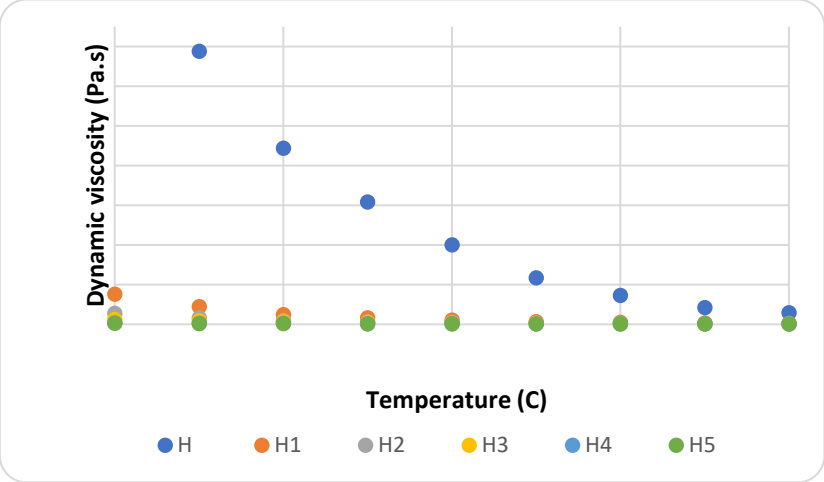

Figure 5: Honey viscosities dependence of temperature and fitting of model to Experimental data by Law Power equation

Table 4: Parameters for Law Power

	k	n	R^2	MAPE[a]
H	12852097.23	-3.69	0.97	2.2256
H1	222979	-3.375	0.99	0.8761
H2	22090	-2.976	0.9932	0.3221
H3	8053.3	-2.83	0.9534	2.3287
H4	420.15	-2.314	0.9638	1.1309
H5	168.65	-2.178	0.9687	1.1531

Law Power equation was not the best fit of dynamic viscosity of honey samples, because the correlation coefficients were under 0.99. The mean absolute percentage error ranges from 0.3 to 2.2 for pure and diluted honey.

CONCLUSIONS

The temperature affects the viscosity of honeys at temperatures between 20– 60°C, with viscosity decreasing with increase in temperature. The viscosities for pure honey (H) from 20-60 C, were higher than the values of diluted honey viscosities. The value of correlation coefficient and mean absolute percentage error indicates that the model fits satisfactorily to experimental data. In according with these results, we can conclude that the mathematical model adopted provides a good description of honey viscosity as a function of temperature. For Arrhenius and Abramovic models, the correlation coefficient obtained from the non-linear regression procedure was greater than 0.99. However, comparisons of the calculated data indicate that the temperature-dependence of viscosity for the honey samples was best described by the Abramovic model.

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IN VITRO REGENERATION IN SELECTED *CUCURBITA SPP.* GERMPLASM

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ABSTRACT

The genus *Cucurbita* is an important vegetable food and crop in around the world and Turkey is the 7th biggest squash (*Cucurbita pepo* L.) producer in terms of annual production ranged 300 to 400 thousand tons. Cucurbita species constitutes 20% of the total vegetable production in the world and 31% of the vegetable production in Turkey. *In vitro* techniques are very helpful for acceleration of breeding studies, quick mass production, cloning and disease-free plant production. In this study, we aimed to develop suitable protocols for direct and indirect regeneration of *Cucurbita* hybrid variety through different explant for 5 different hybrid squash lines which obtained from the Trace Agricultural Research Institute. Seeds were surface sterilized by immersion in 70% ethanol solution for 3 minutes and keeping in 15% bleaching solution (with 2-3 drop of Tween 20) for 15 minutes. Sterilized seeds were cultured on MS (Murashige and Skoog) medium with 30 gr/l sucrose. Explants were isolated from 15-21 days in vivo grown plants by collecting cotyledon, leaf, node, internode, shoot tip and hypocotyl explants and were prepared in sterile cabin. Inoculation of explants was made singly per culture vessel in solid MS (Murashige and Skoog) supplemented with different concentrations and combinations of BAP (1mg/ml,2 mg/ml, 3 mg/ml) and 30 gr/l sucrose. Cultures were assessed according to callusing, shooting and rooting. Among different concentrations, 3.0 mg/l BAP showed best response for In vitro regenerated shoots induction. In vitro regenerated shoots were rooted well in strength MS (Murashige and Skoog) with no plant growth regulator and MS (Murashige and Skoog) with 1 mg/l IBA and micro plants were acclimatized successfully in natural condition.

Key words: *In vitro regeneration, Cucurbita, explant type.*

INTRODUCTION

Cucurbita is an important tropical vegetable grown all around the world. Cucurbita fruits are rich in carbohydrate, protein, lipid, fiber and vitamins (especially B and C). Also vegetable-oil from Cucurbita seeds is popular in some areas. Cucurbita seeds can be consumed as snack (Kurtar et al., 2002). The genus cucurbita is native to the Americas. Turkey is an important producer for area and its production is increasing year by year. Annual vegetable production of Turkey is 25.671.517 tons and 31% of the production is *Cucurbita* spp (7.658.000 tons) in 2017. Summer squash constitutes 300-400 thousand tons of this production (FAO 2017). Despite its economic importance, this species has been otherwise little improved by biotechnology (Gaba et al., 2004).

In vitro methods are frequently used for accelerating the breeding studies, obtaining transgenic lines, mutation studies and production of grafted seedlings. Producing of parent lines for *Cucurbita* spp. could last for 8-10 years with conventional methods (Kurtar, 2017). On the other hand *in vitro* methods is used effectively in breeding programs of many species due to both shortening the

reproduction period and decreasing the cost and labor. Regeneration in C. pepo has been reported via somatic embryogenesis (Kintzios et al., 2002; Urbanek et al., 2004). Additionally, regeneration in squash has been recently reported by direct shoot organogenesis for the first time (Ananthakrishnan et al., 2003), but only for three cultivars.

As a wide range of genetic variation occurs within *Cucurbita pepo*, for the widely disparate representatives of this species could be expected to reveal genetic differences in the ability to regenerate *in vitro*. The aim of the study is to optimize an in vitro regeneration procedure for locally important summer squash lines. In this study 5 different hybrids were used as plant material (Ardendo, Anjelina, Torpido, Roni, Sena Hanım) which supplied by Thrace Agricultural Research Institute. Cotyledons, hypocotyls, nodes, internodes and shoot tips were used as explant.

MATERIAL AND METHODS

Material

Hybrid seeds were provided by Thrace Agricultural Research Institute. Cotyledon nods, hypocotyls, nodes, internodes and shoot tips were used as explant.

Surface sterilization and In vitro culture

Surface sterilization started with the washing the seeds under tap water for three hours. Then seeds were treated with 70% ethanol solution for three minutes. After that, seeds were treated with 15% sodium hypochloride solution with two three drop of Tween 20 for 15 minutes. Then seeds were washed with sterile distilled water theree four 3-4 times. Seed coats were removed before culture and seeds were placed on MS (Murashige and Skoog) medium without plant growth regulators. Cultures were stored in dark for 24 hours then were placed in culture room at 26C^{o} with 16/8 hours photoperiod, under 6500 lux lightning (Obembe et al., 2017).

Culture mediums and In vitro regenerations

MS (Murashige and Skoog) medium with no PGR (Plant Growth Regulators) is used for germination of seeds. For shoot induction, MS (Murashige and Skoog) with different concentrations of BAP (6-Benzylaminopurine) was used (1 mg/L, 2 mg/L, 3 mg/L). Regenerated shoots were rooted on MS (Murashige and Skoog) with no PGR (Plant Growth Regulators) (Obembe, 2017). All culture mediums prepared with 2,7 g/L phytagel and 3% sucrose and pH was adjusted to 5,7. Culture mediums were autoclaved 3 hours with 121 ^{o}C for sterilization. Effect of genotype, BAP concentration and explant type was evaluated on callus and shoot regeneration.

Acclimatization

Successfully rooted plantlets are potted for acclimatization on sterile commercial soil. Pots are covered with stretch film for prevent moist loss. In two weeks, stretch films were removed gradually (Lee et all., 2003).

RESULTS

In vitro regenerations were succesfully achieved on selected *Cucurbita pepo* L. genotypes. Regenerations were obtained from all genotypes, and the highest regeneration rates were observed on Ardendo. Cotyledon node and shoot apex were the most successful explant type for callus and shoot regenerations. Shoot regeneration rates for cotyledon node, shoot apex, node, internode and hypocotyl was 95%, 80%, 22.5%, 0% and 0% respectively. Callus regeneration rates was 86%, 96%, 96%, 6.6% and 41.23% respectively.

Culture medium with 1 mg/L BAP (K1) and 3 mg/L BAP (K3) had higher regeneration rates with compared to culture medium with 2 mg/L BAP (K2). K1, K2 and K3 shoot regeneration rates were 67.56%, 41.3% and 55.55% respectively and callus regeneration rates were 78.37%, 69.56% and 77.77%.

Only shoots from Shoot apex and cotyledon node could be rooted. Rooting were made at MS (Murashige and Skoog) without PGR (Plant Growth Regulators). Shoot apex was gave the highest root regeneration rate.

DISCUSSION

In vitro regenerations were succesfully achieved on selected *Cucurbita pepo* L. genotypes. The highest shoot regenerations were obtained from Ardendo and Torpedo. Cotyledon nodes were one hundred percent regenerated in K1 (MS + 1 mg/L BAP) and K2 (MS plas+ two mg/L BAP) mediums. The highest regenerations were obtained from K1 medium (MS + one mg per/L BAP). Regenerated shoots from shoot apexes and cotyledon nodes were successfully rooted on MS (Murashige and Skoog) without PGR (Plant Growth Regulators) .

In this study, similarly with the studies made before, we couldn't obtain shoot regenerations from hypocotyls (Abrie et al., 2001; Ananthakrishnan et al 2003). Also Ananthkrishnan (2003) was obtained the highest regeneration rate on kotyledon node and this result is parallel with our findings.

CONCLUSIONS

In this study, tissue culture systems are provided in the plant and tissue culture optimization is provided for micropropagation. This study is made as a pre-study. More detailed studies are being carried out.

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MORPHOLOGICAL EVALUATION AND DNA BARCODING IN PLANTS

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ABSTRACT

Identification of plant species is very important because of the basic research in taxonomy, cataloguing hidden diversity, improving environmental monitoring, sustaining natural resources, protecting endangered species, etc. Plant species are identified using a variety of methods, including analyses of morphologic characteristics and molecular genetic variations. In spite of the effects of air and soil conditions on plant growth, plant morphology is an easy and convenient method for identifying different plant species. The classical use of morphological traits for species identification has several limitations. So, identification of species can be completed by using more reliable molecular methods such as DNA barcoding approach representing the best solution for identifying species when their morphology is of limited use by being faster and cheaper. In this study, morphological characterization and DNA barcoding analysis were performed together to identify Colchicum species reference to horticulture, ornamental and generation of metabolites for pharmaceutical industries. In this study, 168 *Colchicum* L. genotypes of the wide variety of 49 Colchicum species in Turkey 35 of which are endemic and 16 candidate Colchicum species were characterized with important 38 morphological traits. As a result of the morphological measurements, Colchicum technical certificate based on UPOV was prepared and color catalog was created. When the morphological measurement results were evaluated, it was determined that morphological characterization is inadequate for identifying of species. For this reason, *rbcL, matK* and *trnH-psbA* chloroplast genes were used to determine Colchicum species using DNA barcoding method. The discrimination power of these genes were compared to each other. As a result, it was determined that the *matK* barcode gene region was more successful than *rbcL* and *trnH-psbA* gene regions in discriminating species. In comparison morphological and DNA barcoding data obtained in this study, DNA barcoding method was found to be more successful and easier for identifying Colchicum species.

Keywords: *Morphological characterization, DNA barcoding, Colchicum* L.

INTRODUCTION

The bulbous ornamental plant *Colchicum* L. belongs the family Colchicaceae that has 49 taxa and 35 of them are known endemic in Turkey and it has medicinal value of having colchicine (Persson, 2007; Ghosh et al., 2008). The kind of confusion and synonym excess of the Colchicum plant put this plant into a taxonomically difficult position. Since the information on Colchicum species is extremely limited, taxonomic classifications have not been completed yet. Considering this negative situation of the Colchicum plant, it is necessary to carry out more morphological characterization supported by molecular studies.

The effectiveness and reliability of the methods used to determine the species is highly relevant to systematics research. Morphological characters remain an important tool for phylogenetic studies, even in the current age of molecular systematics. Using of objective definers based on the morphology

of reliable characters can be useful in confirming and evaluating similarities and uniformity, differences between plant populations. Identification of the variation in quantitative and qualitative characters is important for plant breeding programs and usually, these characters have been used for cultivar characterization and determination by UPOV (The International Union for the Protection of New Varieties of Plants). Classical morphological features have some limitations in species definition (Valentini, 2009). Thus, a high level of expertise is often required to correctly identify species with the accuracy required in ecological studies. Identification of species can be completed by using more reliable molecular methods such as DNA barcoding. This approach might currently represent the best solution for identifying species when their morphology is of limited use (Ahrens, 2007). Even if morphological identification of a species is possible, DNA barcoding might enhance biodiversity inventories by being faster and cheaper, and by overcoming the taxonomic impediment

DNA barcodes use short sequence diversity and standard gene regions to help identify species (Hebert et al., 2003a; Savolainen et al., 2005). Thus, it can be used as biological barcodes to enable the identification of DNA sequence differences in small parts of the genomes of organisms to the species level at the species level, and a universal type identification key to identify these species by means of matching the DNA sequences in the DNA sequences and the DNA sequences in unidentified species where it is not possible to obtain morphological characteristics it is possible (Sass et al., 2007; Aravind et al., 2007).

In this study, it was utilized from morphological characterization and DNA barcoding method to identify 168 genotypes (16 new candidate species include) belongs to *Colchicum* L.

MATERIAL AND METHODS

Plant material

As a plant material, 168 genotypes of belong to 49 *Colchicum* L. species and 16 new candidate species were provided by Republic of Turkey Ministry of Agriculture and Livestock General Directorate of Agricultural Research and Policy, Atatürk Central Research Institute of Gardening Plants in Yalova.

Morphological characterization

For identification of the 49 Colchicum species, morphological characterization was performed using measurement of morphological characters and results were scored. These morphological characters were selected according to "The International Union for the Protection of New Varieties of Plants" (UPOV) guidelines for Tulip and Lilium genus. Also, according to "Pantone" color scale, color of the tepal, peduncle, anther, leaf, corm and fruit capsule were determined and color catalog of *Colchicum* L. species was prepared.

Molecular characterization

To identify the Colchicum genotypes, multi-marker DNA barcoding method, proposed as universal DNA-based tool for species identification was used. For this aim *rbcL*, *matK* and *trnH-psbA* chloroplast genes were chosen which were suggested by "CBOL - The Consortium for the Barcode of Life Plant Working Group". For DNA barcoding, as first, the isolation of genomic DNA using the leaves of *Colchicum* L. was performed by CTAB (Doyle and Doyle, 1987) method. PCR analyses were performed by using genomic DNA of all genotypes and primers of these barcode genes. Obtained PCR products were sequenced. Using these sequence information, DNA barcodes were obtained and

sequences of *Colchicum* L. genotypes were sent to the NCBI database by the BOLD system and the access number was obtained for each genotype.

RESULTS

Morphological characterization

Characterization of 168 *Colchicum* L. genotypes was performed by measuring 38 morphological characters. As a result, the color catalog was prepared for Colchicum species to identify the differences between Colchicum species using color cods for autumn and spring *Colchicum* species. Also, using morphological characters, UPOV technical description document for Colchicum species was created (unpublished data).

Molecular characterization

Using the *rbcL*, *matK* and *trnH-psbA* barcode genes, the results of the analysis (phylogenetic tree, Structure, PCA) for the identification of 168 *Colchicum* L. genotypes by DNA barcoding were compared between each of these genes. Structure, PCA analysis of the phylogenetic tree performed were found to be consistent with each other. After assessing the barcoding results of all barcode genes together, *matK* barcode gene was found to be more successful than *rbcL* and *trnH-psbA* barcode genes for the identification of the *Colchicum* L. species (unpublished data).

As a result of this study, DNA sequence data of genotypes staying out candidate species were entered into BOLD (The Barcode of Life Data) database and DNA barcodes of each genotype belong to the three barcode genes (*rbcL*, *matK* and *trnH-psbA*) were created.

DISCUSSION

It is known that morphological features are very important and this basic information help to accurately identify the plants considering the medicinal importance of Colchicum species and many synonyms reported. With morphological characterization studies that were performed using 168 *Colchicum* L. genotypes belong to flora of Turkey will contribute to the understanding of the diversity of the Colchicum species. Morphological character measurements of Colchicum species were used to evaluate the differences between the species. Technical certificate based on UPOV requirements for Colchicum species using morphological parameters and the color catalog were prepared to simplify the determination of Colchicum species. Since this study was performed for the first time in the Colchicum plant, the data obtained from this study is very important for the development of DNA barcodes specific to the Colchicum plant with the addition of analysis of more conserved gene regions.

CONCLUSIONS

As a conclusion, the Colchicum plant, which is the material of this study, has been used as an ornamental plant for the treatment of some diseases especially in medicine because of colchicine alkaloid, so it has a great economic value. Through all these studies, the taxonomical classification of the genus Colchicum will help to correct the wrong synonyms and to understand the evolution of the genome structure and relationships. In this way, conservation and sustainable use of plant material at the DNA level will be ensured to contribute to the conservation of gene resources and the transformation of these resources into economic value.

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INDUCTION OF PARTHENOGENETIC HAPLOID EMBRYOS AND PLANTS AFTER POLLINATION BY IRRADIATED POLLEN IN SUNFLOWER

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ABSTRACT

The method of irradiated pollen induced parthenogenesis followed by in vitro culture of immature embryos as an efficient method for production of sunflower (*Helianthus annuus* L.) doubled haploids. The influence of gamma ray doses and genotypes on the induction of haploid embryos obtained by irradiated pollen technique was studied in sunflower. The female flowers were were bagged before flowering to avoid to avoid undesirable pollinations. The pollen was collected from the pollen sources on alternate days and stored at 4^{o}C till irradiation and pollen was irradiated with gamma-rays at the doses of 500, 750 and 1000 Gy. Only bagged and opened female flowers were pollinated. Pollination was done by hand by brushing the stigmas with irradiated pollen, and thereafter the pollinated flowers were re-bagged to eliminate contamination by foreign pollen. All pollinations experiments were conducted in the field. Embryos were rescued from 12 to 20 days old and cultured in magenta boxes containing solid A, FM and D medium. The conditions of the experiment, best results were obtained in inactivation of the pollen with 750 and 1000 Gy. After in vitro culture, a total of 6 haploid plantlets were obtained. To determine the ploidy level of in vitro obtained from rescued embryos and subjected to ploidy analysis using flow cytometry technique. As a result of the present study, haploid embryos and haploid plants were obtained, with haploid production strongly influenced by gamma ray doses, embryo stages and genotypes. This research has been supported by TUBITAK TOVAG (Project No: 214O274) and Marmara University Research Foundation-BAPKO (Project No: FEN-C-YLP-081117-0627).

Keywords: *Sunflower, Haploid plant, Irradiated pollen, Embryo rescue.*

INTRODUCTION

Sunflower (*Helianthus annuus* L.), which has an important share in world vegetable oil production, is the main raw material of our oil industry and the most cultivated oil crop in Turkey (Kaya, 2004). Hybrid sunflower lines preferred by produces due to their high yield performance, quality and uniformity. However, the development of inbred lines by self pollination is time consuming even if immature embryo rescue techniques may reduce by half the time required (Zhong et al., 1995). Haploidization techniques facilitate the production of pure lines from heterozygous plants in a single generation and represent significant advantages for breeders and geneticists.

The results from studies with anther culture (Bohorova et al. 1985; Yang et al., 1990; Gürel et al., 1991a; Pugliesi et al., 1993; Thengane et al., 1994; Zhong et al., 1995; Saji and Sujatha, 1998), gynogenesis (Yang et al., 1985; Gelebart and San, 1987), and microspore culture (Gürel et al., 1991b; Coumans and Zhong, 1995). Sunflower has been recalcitrant to all in vitro culture techniques, that makes challenging to establish haploid techniques (Friedt, 1992; Yang et al., 1995; Hahne, 2001).

Parthenogenic haploid induction could be advantageous method when androgenesis and gynogenesis are not successful. *In situ* parthenogenetic induction is widely used for all haploid induction studies, in which germinated plants have maternal genotype. This method can be generalized as maternal embryo induction with pseudo fertilization.

Induction of maternal haploid embryos by pollination with irradiated pollen has been used successfully in many species (Mishra et al., 2014). Todorova et al. (1997) used irradiated pollen-induced parthenogenesis obtained the number of agronomically useful fertile DH lines. However not all the species respond well enough to the haploidization techniques. To test this approach for production of haploid plants in sunflower we extended this technique to sunflower genotypes from Turkey's National Sunflower Breeding Program.

MATERIALS AND METHODS

The study was carried on with 16 different sunflower lines (G1, G2, G3, ..., G16) which supplied by Thrace Agricultural Research Institute (TARI). All plants were grown in TARI fields. Planting, emasculation and pollination stages were carried out in the fields of Thrace Agricultural Research Institute by Dr. Göksel Evci and İbrahim Yılmaz. Flower heads, except CMS lines, emasculated daily by hand early in the morning, before anthesis. Pollen collected from different sunflower lines and mixed before irradiation treatment. Pollen were treated by gamma irradiation (Cobalt 60 source) at doses 500 Gy, 750 Gy and 1000 Gy. 81 flower-heads were pollinated with irradiated pollen and bagged again. 12-20 days after pollination flower heads were harvested and were brought to laboratory. The embryo stage determined before cultivation.

Seeds were extracted after determination of embryo stage. Then seeds were treated 20 minutes with 20% commercial bleaching solution with 2-3 drops of Tween 20 in sterile bottles. Bottles were gently shaken during the application. After that seeds were washed with sterile distilled water 5-6 times until all the detergent foams gone (Dagustu, 2010). Three different embryo rescue procedures were used (A - Aspiroz, 1988; FM - Freyssinet, 1988; D - Dagustu, 2010). Culture mediums were autoclaved for 3 hours at 121^{o}C

After surface sterilization embryo sacs were removed and embryos were cultured with endosperm. Equal number of embryos were attempted to cultured on mediums that prepared according to three different embryo rescue procedure (which coded A, D, FM). Cultured embryos were placed in the culture room at illuminance of 6000 lux with a photo-period of 16/8 h and at 25°C. Embryos in contaminated cultures were planted directly to soil.

In vitro germinated embryos with strong shoots and roots were planted to the soil for acclimatization (Figure 1).

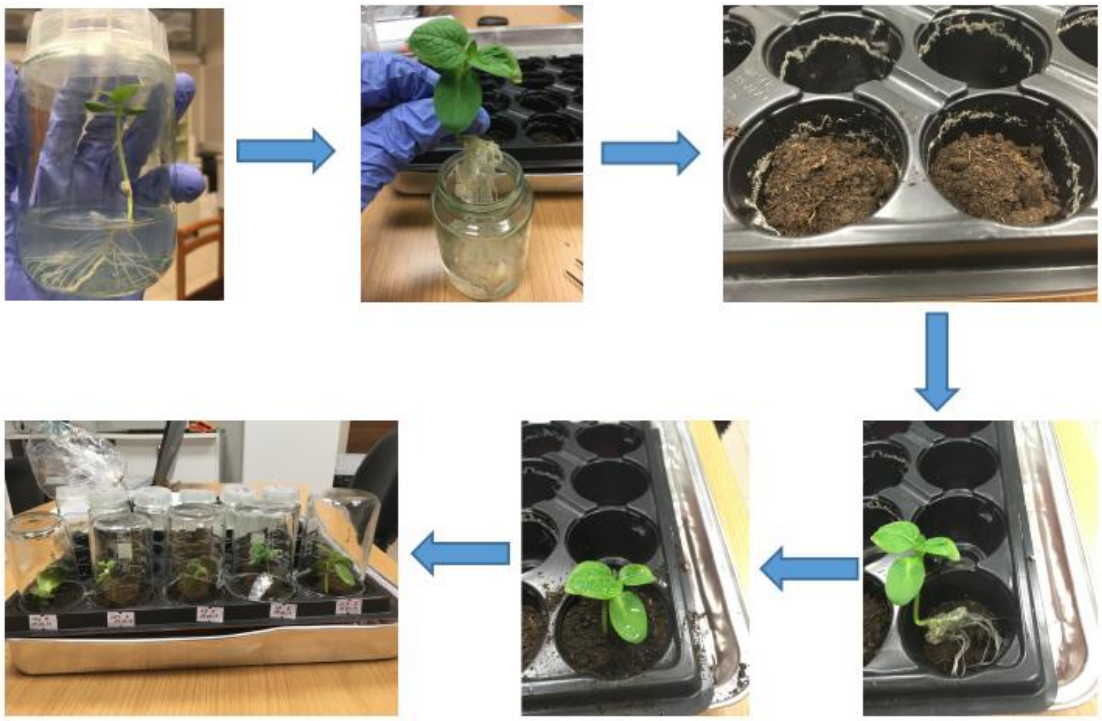

Figure 1. Acclimatization stages of germinated plantlets.

The ploidy of the obtained plants was determined with flow-cytometry and chromosome analyze using root apical meristem cells.

RESULTS

Haploid plantlets were detected from G9 and G15. Fertile diploid plantlets were selfed and seeds were obtained from G5, G6, G8, G9 and G11. Biochemical tests are continued for detection of spontaneous haploidization on this lines. Embryo rescue protocols were compared to their germination efficiency. The highest germination rate was obtained from D media. Also effect of the irradiation doses were evaluated. It was determined that 1000 Gy and 750 Gy irradiation doses were suitable.

DISCUSSION

Haploid induction by irradiated pollen appears to be an applicable approach for rapid production of haploid plants in sunflower and with this method seems useful for accelerate breeding line production. Embryo germinations were obtained from almost all breeding lines but the effectiveness of the method highly depends on the genotype specificity of the recipient.

Flower-heads were harvested 12-16 days after pollination with irradiated pollen. Microscope observations were shown that most of the seeds had embryos but the regeneration rate was low for relatively weak seeds. To increase the regeneration rate harvesting time expanded to 21 days and only the stronger embryos were cultured and germination rates were rose. This method change was made our results similar to Todorova's results. Todorova (1997) obtained 1107 plants from 2279 embryos and had 48,5% germination rate.

CONCLUSIONS

The effectiveness of method highly dependent on the genotype of the recipient line. At the beginning of the study, flower heads harvested 12-16 days after pollination but in this early stage embryo in different development stages were observed but survival ratio was very low. To prevent this issue, we were extended the waiting period until 21 days and were waited for embryos to be more mature. Only embryos with relatively more endosperm were regenerated and create healthy plants.

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COMPARATIVE ANALYSIS OF DIFFERENT IN VITRO HEAVY METAL CONTAMINATION IN SUNFLOWER

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Abstract

Sunflower (*Helianthus annuus* L.) is cultivated as a food and feed crop as well as for bioenergy production, and it has an important role as a plant. Growing sunflower plants have shown the potential to absorb various metal contaminants apart from industrial applications of dry sunflower biomass. The plant tissue culture techniques can be used in study of metal tolerance of a plant by exposing it in culture media containing known quantities of the specific heavy metal. In this study, sunflower plants were exposed to the MS medium having concentrations nickel-Ni, lead-Pb, copper-Cu and cadmium-Cd in 10 ppms and their genotoxic effects were evaluated by flow cytometry analysis and morphological observations comparison with seeds cultivated in heavy metal-free MS medium during 21 days. Flow cytometry analysis showed that the roots were more affected plant tissues than leaves for all heavy metal treatments on sunflower. The results show the highest genotoxic effect was obtained for the 10 ppm Pb treatment in leaf tissue while the highest genotoxic effect was obtained for the 10 ppm Cd treatment in root tissue. As a result of morphological observations, Cd (10 ppm) is much more abnormalities having percentage than Pb, Cu and Ni, as it induced more black nodules in the roots of all treated plants unlike the control group. Also, Cd is induced the lowest germination percentage (76,83 %), root length (2,8 cm), stem length (2,5 cm), total plant length (5,44 cm) and stem/root rate (3,87). Both flow and morphological results will be useful in environmental monitoring of the genotoxicity of metals and with the help of tissue culture experiments the potential of plant for heavy metal stress can be studied easily.

Key words: *Sunflower, heavy metal contamination, flow analysis.*

INTRODUCTION

Sunflower, that Latin name is *Helianthus annuus* L., is get to name from Greek origin words meaning "helios" is sun, "anthus" is flower. Sunflower is one of 67 species belonging to Helianthus genus which found in the family Compositae (Asteraceae) of Asterales (Berglund, 2007; Hu et al., 2010). Sunflower is grown in moist and containing rich organic substance soil and sunflower is plant of too arid climate and half arid climate. Homeland of sunflower known as Peru and Mexica (Ġlisulu, 1973). Cultivated sunflower (*Helianthus annuus* L.) is a globally important oilseed, food, and ornamental crop plant. The regions made of sunflower cultivation in Turkey is Thrace, South Marmara, Aegean, Central Anatolia, Çukurova and Black Sea Region. Most of sunflower is produced in Turkey Thrace-Marmara Region. Turkey's exports of sunflower seeds for oil is much smaller than imports (Ministry of Customs and Trade, 2018). Therefore, sunflower production in Turkey, it is not

enough for consumption. In this study, the effects of heavy metal contamination on sunflower agriculture are highlighted.

Heavy metal contamination is a relatively recent concern but an ancient problem as technological advances like the discovery of fire or ore-mining and more importantly the industrial revolution has contributed to continuously and exponentially increase metal pollution in the environment. Even worse, as heavy metals are not degraded by living organisms, they can easily accumulate to harmful levels. From the many forms of heavy metal toxicity, genotoxicity poses one of the major threats to organisms. Damage to the DNA can have severe repercussion to cells such as mutagenesis or deregulation of the cell replication machinery, among others, which could ultimately result in tumorigenesis and death. Heavy metals are passed to the water sources by the dissolution of heavy metals in the soil of industrial wastes or acid rain, and thus the heavy metals in the composition and reaching river, lake and underground waters of dissolved heavy metals (Rodriguez et al., 2011). Heavy metals cause a decrease in yield and quality in plant production as they affect the events such as root and stem growth, germination and photosynthesis rate, enzyme activities, protein synthesis and ion uptake (Zengin et al., 2006; Asri et al., 2007). In this study, the effects of heavy metals on sunflower plants were examined in plant tissue culture.

MATERIAL AND METHODS

Plant Material

Sunflower seeds (TARSAN-1018) were provided by Thrace Agricultural Research Institute (TARI).

Plant Tissue Culture

The control group contains MS (Murashige and Skoog), sucrose and phytagel, as shown in Table 1. In the heavy metal group there are additionally 10 ppm of the related heavy metal.

Table 1. Preparation of MS medium containing different in vitro heavy metals

Murashige and Skoog Basal Medium (MS, 1962)				
Control	Cd	Pb	Ni	Cu
-	10 ppm (1 liter)	10 ppm (1 liter)	10 ppm (1 liter)	10 ppm (1 liter)
4.4 g MS vitamin not including	4.4 g MS vitamin not including	4.4 g MS vitamin not including	4.4 g MS vitamin not including	4.4 g MS vitamin not including
30 g sucrose	30 g sucrose	30 g sucrose	30 g sucrose	30 g sucrose
2.3 g phytagel	2.3 g phytagel	2.3 g phytagel	2.3 g phytagel	2.3 g phytagel

These studies were carried out in sterile conditions in the biosafety cabinet. Sunflower seeds were kept in tap water for 1 hour. The seeds were surface sterilized in 20 % commercial bleach with a few drops of detergent for 20 minutes. Then they were rinsed three times in sterile distilled water. Later the seeds were placed on sterile drying paper and dried. Eight seeds were cultured in each culture box. After cultured, the tissue culture boxes are wrapped with aluminum foil for germination. After

germination they were placed in the plant growth cabinet. For the analysis, 21-day seedlings were used (Figure 1).

(A) **(B)**

Figure 1. Culture conditions day 1 (A), day 21 (B).

RESULTS

Morphological Analyzes

Morphological analyzes were performed on day 21 (Figure 2).

Figure 2. 21 day seedlings. Respectively Control, Ni, Pb, Cu, Cd.

Percentage of germination (%), root length (cm), stem length (cm), total plant length (cm) and stem/root rate were calculated. Control group is induced the highest germination percentage (89,02 %), root length (5,68 cm), stem length (5,32 cm), total plant length (11 cm) and stem/root rate (3). Cadmium (Cd) is induced the lowest germination percentage (76,83 %), root length (2,8 cm), stem length (2,5 cm), total plant length (5,44 cm) and stem/root rate (3,87).

Significant, distinctive morphological characteristics of each culture medium were noted (Figure 3). The obtained data were analyzed in comparison with the control group.

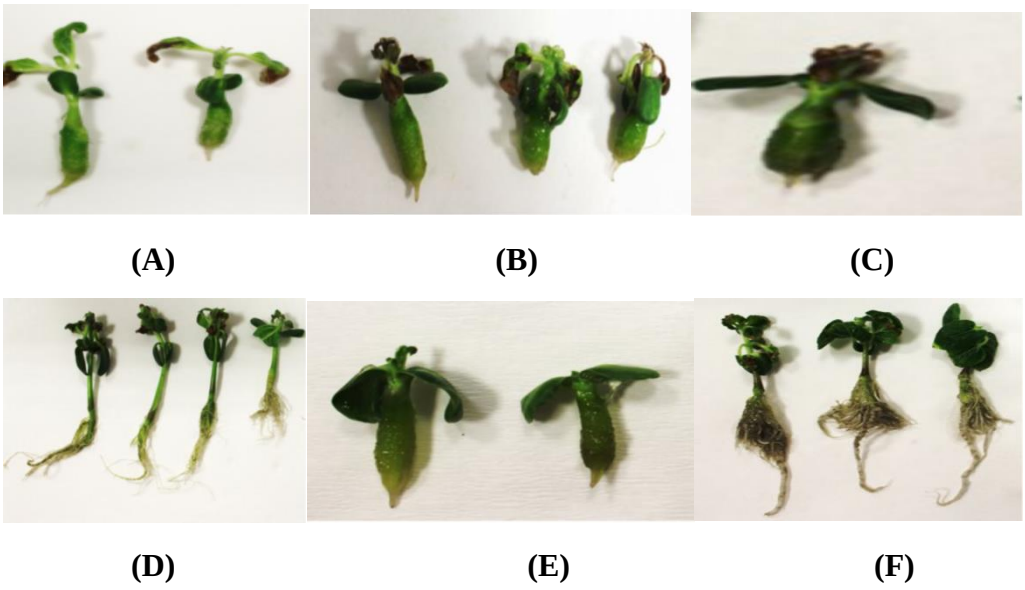

Figure 3. Plants that are exposed to nickel heavy metal have swollen hypocotyl in the majority, unlike the control (A), in the same way, plants that are exposed to copper heavy metal have swollen hypocotyl in the majority, unlike the control (B), most of the plants exposed to lead heavy metal have swollen hypocotyls such as nickel and copper and plants that are exposed to lead heavy metal haven't growth of shoot in the majority, unlike the control (C,D), plants that are exposed to cadmium heavy metal have swollen hypocotyl in the majority, like other medium. Also, unlike other medium, the roots of plants exposed to cadmium heavy metal have black nodules and it has become quite thick (E,F).

Flow Cytometry Analysis

Roots

Heavy metal contamination was also measured by flow cytometry. The lowest genotoxic effect in the roots was obtained in the control group. The highest genotoxic effect in the roots was obtained in the 10 ppm cadmium (Cd) treatment (Figure 4).

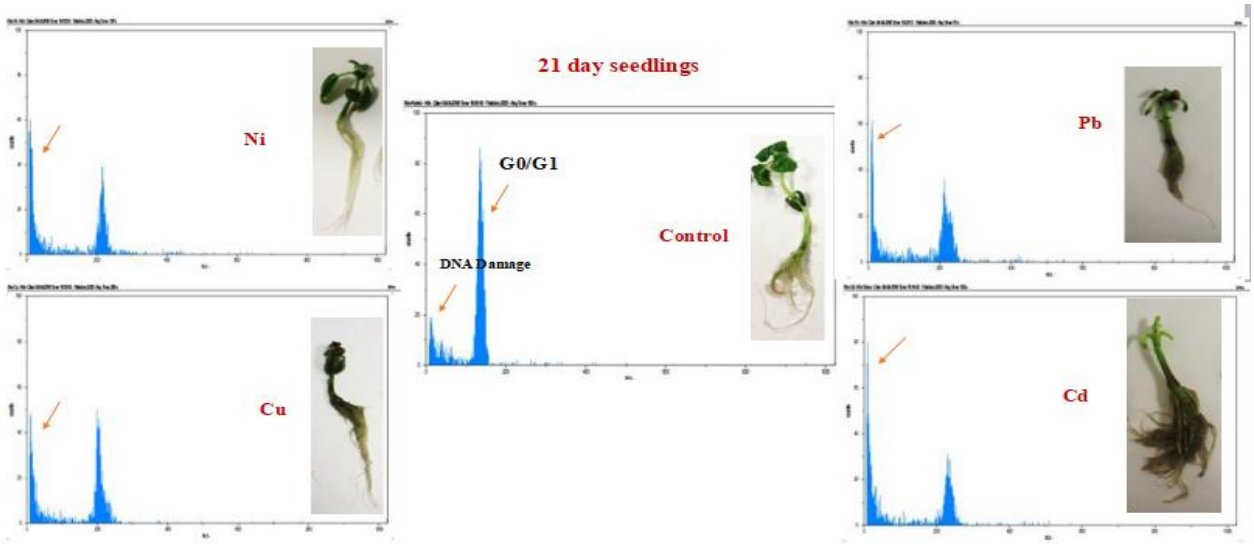

Figure 4. Flow cytometry measurements in roots.

Leaves

As in the roots, the lowest genotoxic effect in the leafs was obtained in the control group. The highest genotoxic effect in the leafs was obtained in the 10 ppm lead (Pb) treatment (Figure 5).

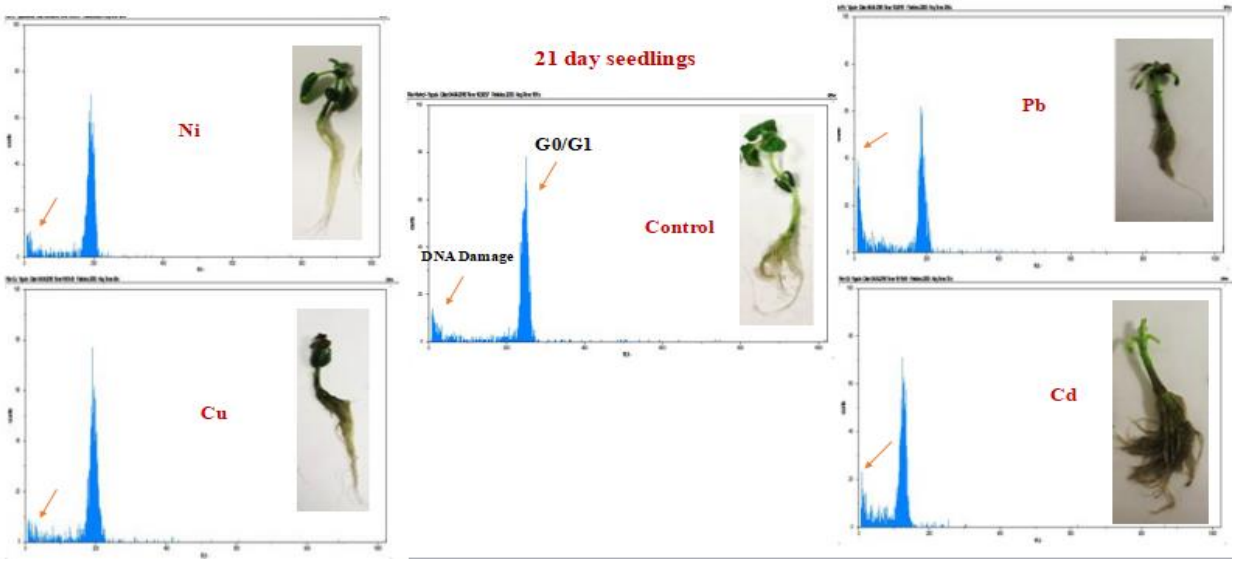

Figure 5. Flow cytometry measurements in leaves.

DISCUSSION

The heavy metal contamination of the oily seeds causes high concentrations of various metals in the crude oils obtained from these seeds (Alpaslan et al., 2001). Cadmium is known to reduce the fidelity and quantity of DNA synthesis and cause chromosome breakage (Degraeve, 1981). Cadmium is much more easily taken up by plants than other heavy metals (Stoeppler, 1991) and this may explain its toxicity at low concentrations. Copper toxicosis in plants is less usual than in animals or humans (Scheinberg, 1991). To compare the toxicity of some heavy metals, six metal, aluminum (AI), cadmium (Cd), copper (Cu), nickel (Ni), lead (Pb) and zinc (Zn) effects of *Helianthus annuus* plant working in Chakravarty et al. high toxicity was found by both high and low concentrations of Pb, while Zn was found to be the least toxic. According to the results, Cu and Zn Al showed less damage than Cd, Pb and Ni (Chakravarty et al, 1992). In the results of Alaboudi et al., Cd and Pb were reported to have negative effects on shoot and root length in sunflower plants (Alaboudi et al., 2018).

In this study, as a result of morphological observations, control group is induced the highest percentage of germination, root length, stem length and total plant length. On the other hand, cadmium is induced the lowest percentage of germination, root length, stem length and total plant length. Nickel are the heavy metal that cause the least damage.

Flow cytometry analysis showed that the roots were more affected plant tissues than leaves for all heavy metal treatments on sunflower. Because the roots are exposed to heavy metals before the leaves. According to the results of flow cytometry, copper is the heavy metal that causes the least damage to both roots and leaves, as in the results of Chakravarty et al.

CONCLUSIONS

With the help of tissue culture systems in the direction of our results, the potency of the sunflower plant for heavy metal stress can be easily examined.

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THE PERFORMANCE OF SOME HYBRID RICE CULTIVARS IN EDIRNE CONDITIONS

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ABSTRACT

This study was carried out to test performance of some *japonica* hybrid rice cultivars at Uzunköprü and Edirne conditions in 2015. Six japonica type rice cultivars and three self-pollinated check were used as a material. To obtain seedlings, seeds were planted at 3, May, 2015. Seedlings were transplanted at 4th of June and 7th of June to field in Edirne and Uzunköprü respectively. 4-5 seedlings were transplanted at 16.7 cm within rows and 30 cm between rows. Experiment was conducted with three replications at Randomized Complete Block Design (RCB). 180 kg/ha N and 80 kg/ha P_2O_5 fertilizer were applied. Line TL2015-02 had the lowest days to flowering and days to maturity at the both locations, followed by check varieties Edirne and Osmancık-97 respectively. LiaO 8/ you 5 had highest plant height and longest panicle length. TL2015-2 and TL2105-3 had the highest fertile panicle per square meter. In Edirne location Liao/ you62 rice cultivar had the highest pady yield with 10, 102 t/ ha and followed by TL2015-3 with 9,098 t/ ha. All hybrid rice cultivars had better yield than check cultivars except TL2105-2. In Uzunköprü location TL2105-3 rice lines had 9,772 t/ ha and it was followed by Liao 8/ you 5 with 9, 390 t/ ha. In Uzunköprü location all hybrid rice cultivars had better yield than that of check cultivars.

Key words: Edirne, hybrid rice, performance, yield,

INTRODUCTION

Because of being a self-pollinated crop, the flower structure and high seed use, hybrid breeding in rice has started very late. On the other hand, especially in China, hybrid paddy rice production increased to over 50% with great success. After these successful efforts in China, especially private sector breeding institutions have entered hybrid rice breeding activities in Asia and America in every year. However, hybrid rice is new in Europe and some varieties of American seed companies are cultivated in small quantities in Europe countries and but there is no hybrid rice production in Turkey.

While there is no hybrid rice production, more than 90 % hybrid seed is used in some crops (sunflower, corn, many vegetables etc.) in Turkey. The reason is that high seed rate at direct seeding which traditionally used in Turkey and low heterosis in japonica type rice.

Heterosis in rice was first reported in 1926 (Jones, 1926). Due to the self-pollinated plant and flower structure, it was very difficult to develop a hybrid rice at the beginning. The first hybrid paddy rice was registered in China in 1976 and after this date the cultivation areas have increased rapidly. In 1991, more than half of paddy rice sown in China was hybrid rice. Most of the rice varieties planted in Europe is not hybrid, but the F_1 hybrid rice varieties especially in the southern states of the United

States has reached 35 %. On the other hand, F_1 hybrid rice is cultivated in many Asian countries and research and breeding studies in various private companies in the USA, including European ones continue with collaboration with IRRI.

Heterosis is reported that in rice root system, panicle and grain size, larger photosynthesis area, more photosynthetic activity, distribution of photosynthesis products and grain yield, etc. (Yuan and Fu, 1995). In the experiments at IRRI, some F_1 hybrid rice lines had high yield than the best control cultivars by 22% (Yuan and Virmani, 1988) and some F1 hybrid lines exhibited more yield between 29.3% and 46.6% over than traditional cultivars in China in 1981-1990 (Yuan and Fu 1995). Furthermore, the varieties grown in China were tested in the USA in 1983-1984 they showed higher yield by 14-19% then US cultivars (Yuan and Virmani, 1988). In another study conducted in Turkey, average heterosis and heterobeltiosis per plant in seed yield was found as 21.2% and 15.2%, respectively in rice (Surek and Korkut, 1996).

The first trials to develop the F1 hybrid varieties utilizing from advantage of heterosis were performed in rice in India in 1962 (Richhari A) in China in 1966 (Yuan, 1966), in USA 1966 (Stanselve and Craigmiles) in Japan in 1966 (Shinjyo and O'Mura, 1966) and in IRRI in 1972 (Athwal and Virmani, 1972). However, these efforts could not be realized because of being self-pollination and the flower structure.

On the other hand, Chinese researchers started to develop F_1 hybrid rice in 1964 with a 3-line system (CMS, maintainer and restorer line) and registered the first F_1 hybrid rice in 1973, identifying F hybrids showing higher heterosis in 1974 and the first F_1 hybrid rice lines were distributed to farmers. In 1976, F_1 hybrid rice was planted in 150 thousand ha area (Yuan and Virmani, 1988). In China, F_1 hybrid paddy rice varieties were cultivated in regions from tropical the northern 18º latitude Hainan Island to cold areas the Liaoning District in the northern 43º latitudes (Yuan and Virmani, 1988).

The first F_1 hybrid varieties were developed with the application in three-line system and in this hybrid system, CMS (Cytoplasmic Male Sterility) genes was obtained from wild lines via backcross (Yuan and Fu 1995). The first CMS resources to cause male infertility in rice was firstly reported in 1954 (Weeraratne, 1954, Sampath and Mohanty, 1954) and then CMS genes was firstly transferred to cultural types in Japan (Shinjyo and O'mura, 1966). Later, different researchers obtained then transferred CMS from different sources (Erickson, 1969, Carnahan et al., 1972, Athwal and Virmani, 1972, Cheng and Huang, 1979). Among these CMS sources, the most stable and totally male sterile one was developed by Shinjyo and O Virmura (1966) then this CMS source was widely used in China (Yuan and Virmani 1988). Additionally, the CMS source obtained from *Oryza sativa f. stimmelma* called WA type (Lin and Yuan, 1980, Yuan, 1972) was used in IRRI and other national programs for male sterility in China (Yuan and Virmani, 1988).

On the other hand, restorer genes were mainly found in wild rice with tropical and subtropical *indica* types. However, there are almost no restorer genes in Japanese types so the restorer gene of the *Japonica* type restorer lines transferred from the IR8 lines developed by IRRI was used commercially in China (Yuan and Fu 1995). Nearly 20 % of the cultivated and breeding lines developed in IRRI have been reported that having WA gene restoring cytoplasm (Yuan and Vimmani, 1988).

Rice is a self-pollinated plant due to its floral structure but, cross pollination ranged from 0-6.8% in the cultivated varieties (Sahadevan and Namboodiri, 1963) and varied from 16.5% to 100% in wild forms (Sakai and Narise, 1959, Oka and Morishhima, 1967). On the other hand, the rate of cross pollination in CMS lines ranges from 0 to 44% (Athwal and Virmani, 1972) but cross pollination rate was observed in CMS lines between 3 to 43% in IRRI studies (Yuan and Virmani, 1988). The cross pollination rate varies with the floral structure and it is related to the size of the stigma, its output and the time of the flowers' exposure affect. Flowers remain open for 1 to 3 hours and not open again after closing in rice (Taillebois and Guimaraes, 1988). For higher seed development in F_1 rice CMS lines, 2-3 m/ s wind speed is required to keep more than 50% seed development (Namai and Kato, 1988). In the F1 hybrid rice and parental line seed production, two times gibberellic acid application was recommended to increase seed development when first 10-15% of flowering and after 2 days (Virmani and Sharma, 1993).

The highest opening rate of rice flowers varies between 9 and 11.30 in the morning, but there are some differences between species. While *O. globerrima* opens 60 % of flowers at 9 am in a sunny day, *O. Sativa* type IR 36 line opens less than 5 % of flowers at this time (Yuan and Virmani, 1988). For higher seed development in CMS lines; the panicles should be as higher as possible from the flag leaf, should be at least 10 paniclelets in the panicle, the flowers should be opened for at least 45 minutes, the stigma should come out in the blossoming flowers and the pollens could be taken at least for 5-7 days.

After the three-line system, two sensitive and temperature-sensitive male infertility have been developed for hybrids in China (Yuan and Fu 1995). Since there are two lines in this system, F_1 seed production is cheaper. For example, the temperature-sensitive genetic male sterile lines vary according to the lines of flowering time but they are completely male sterile at 23-29 Cº. Single-line F_1 system could be possible also that reduces the cost of hybrid paddy rice breeding such as using multi-year paddy rice use, a large number of F_1 hybrid paddy reproduction from somatic cells, apomixis, etc. From these methods, major researches have been carried out on apomixis and these studies started in the 1980s in China and Lu52 (*Japonica*), Alixisini (*Japonica*), Shuang 13 (*indica*) and Shuang 3 (*indica*) facultative lines were developed (Yuan and Fu, 1995).

F_1 paddy rice breeding activities were started in 1964 in China, 1976 in North Korea, 1979 in the Philippines, 1980 in the USA, 1981 in India, 1982 in Indonesia, 1982 in the Republic of Korea, 1982 in Brazil, 1983 in Thailand, 1985 in Vietnam, 1988 in IRRI, 1988 in Egypt, 1988 in Sri Lanka, 1990 in France, 1990 in Colombia. In addition to state institutions in these countries, especially in some countries (USA, Japan, China, India, Philippines etc.) private sector firms have started F_1 hybrid rice breeding programs too (Virmani 1994).

Three early CMS hybrid IRRI rice lines (Reimei, IR682776, IR68283) were tried but their seed development rates were lower in field conditions in Turkey. Additionally, two temperature sensitive lines (Norin Pl 12 and IR 68948-4-12-3-7-B) were also tested in yield trials and satisfactory results were obtained from these lines in Edirne, Turkey conditions (Beşer and Sürek, 1999).

This study was carried out to test some japonica hybrid cultivars, improved with three-line system, at two locations at Edirne and Uzunköprü, in Turkey. This study is the first japonica hybrid yield trial in Turkey.

MATERIAL AND METHOD

The experiment was conducted with six japonica hybrid rice varieties obtained from China and 3 controls. Seeds were planted at seed bad on May 9, 2015, then transplanted to field on June 4, 2015 in Uzunköprü and in 7 June, 2015 in Edirne locations. The experiment was conducted according to the randomized block trial design with 3 replications and the seedlings were transplanted as 30 cm in between rows and 6.7 cm within rows. 4-5 seedlings were transplanted per each hill. The trial was fertilized with 18 kg / da N and 8 kg / P2O5. The plants were harvested by hand. Measurements and observations were done for days to flowering, days to maturity, Plant height, panicle length, panicle per square meter and yield.

RESULTS AND DISCUSSION

The varieties tested in the experiment were adapted to Edirne conditions and it was observed that they could grow in Edirne conditions without any problems according to their days to flowering and days to maturity characteristics. For days to flowering, TL2015-02 was the earliest in both locations, followed by the Osmancık-97 and Edirne varieties. Days to flowering of other varieties were closer to the standard varieties. For days to maturity, similar results were obtained with days to flowering and the TL2015-02 had the earliest days to maturity, and standard varieties followed it respectively (Table 1 and 2).

For plant height, Liao8/you5 hybrid was the highest hybrid after check variety Edirne, while the other hybrid varieties were shorter than the standard varieties (Table 1 and 2) in both locations. Shorter plant height is needed to prevent lodging in rice production.

Table 1. Performance of hybrid rice cultivars in Edirne location

Lines	Days to flowering (days)	Days to Maturity (days)	Plant Height (cm)	Panicle Length (cm)	Panicle per square meter	Seed Yield (t/ha)
Osmancık-97(Std)	85,6 d	121,6 cd	101,3 b	15,6 cd	268,6 d	8,259 bc
Edirne (Std)	84,3 d	121,6 cd	109,1a	17,1 bc	292,3 cd	7,426 c
TL 2015-01	94,0 a	132,0 a	92,5 c	16,0 cd	288,3 cd	8,658 abc
TL 2015-02	80,6 e	119,6 d	89,1 c	16,6 bc	339,0 a	7,677 bc
TL 2015-03	94,3 a	126,3 bc	94,4 c	16,4 bc	326,6 ab	9,985 a
TL 2015-04	95,3 a	131,0 ab	94,1 c	16,8 bc	295,7 bcd	8,636 abc
TG-1 (Check)	91,6 b	128,6 ab	89,2 c	14,1 d	272,0 d	8,362,7 bc
Liao 8/you5	88,6 c	131,3 ab	103,1 b	21,5 a	283,3 cd	9,098 ab
Liao /you62	90,3 b	129,0 ab	94,3 c	18,3 b	310,3 abc	10,102 a
CV (%)	1,10	2,32	3,22	7,28	6,49	10,60
LSD (%5)	1,65	5,06	5,32	2,12	33,34	1,586

Liao8/you5 variety had the highest panicle length followed by Liao /you62 in two locations. The Edirne standard variety had a longer panicle length than the other hybrids except Liao8 / you5 and Liao / you62 in both locations (Table 1 and 2). For panicles per the square meters TL2015-2 and

TL2105-3 hybrid varieties had higher number of panicles in both locations and exhibited a higher potential for tillering.

Liao / you62 hybrid rice variety had highest paddy grain yield in Edirne location with 10,102 t/ ha, it was followed by TL2015-3 hybrid variety with 9,098 t/ ha paddy yield. The standard Edirne and Osmancık-97 varieties had lower yields than hybrid varieties tested except TL2105-2 hybrid in Edirne location. In Uzunköprü location, TL2105-3 had the highest yield e with 9,772 t/ha paddy yield, it was followed by Liao8/you 5 with 9,390 t/ha. In this location, all hybrids tested had higher yields than check verieties. (Table 1 and 2).

Table 2. Performance of hybrid rice cultivars in Uzunköprü location

Lines	Days to flowering (days)	Days to Maturity (days)	Plant Height (cm)	Panicle Length (cm)	Panicle per square meter	Seed Yield (t/ha)
Osmancık-97 (Std)	84,7 d	121 d	85,3 ab	13,0 d	322,7 bc	7,260 c
Edirne (Std)	83,3 d	120 de	89,2 a	15,7 bc	278,7 d	6,297 d
TL 2015-01	92,7 a	130,3 a	83,7 bc	15,7 bc	360,7 ab	7,461 c
TL 2015-02	80,3 e	118 e	80,6 c	15,6 bc	377,0 a	8,885 b
TL 2015-03	93,3 a	124,3 c	83,1 bc	14,1 cd	360,3 ab	9,772 a
TL 2015-04	94,3 a	130,0 ab	82,3 bc	16,0 b	283,0 cd	8,968 ab
TG-1 (Check)	89,3 b	127,7 b	81,0 bc	12,9 d	300,0 cd	7,326 c
Liao 8/you5	87,0 c	129,0 ab	89,6 a	19,2 a	297,3 cd	9,390 ab
Liao /you62	89,0 b	130,0 ab	85,4 ab	15,9 b	310,0 cd	8,899,7 b
CV (%)	1,25	1,23	3,12	6,66	7,79	5,77
LSD (%5)	1,86	2,66	4,56	1,75	43,26	0,820

CONCLUSIONS

This study was the first japonica hybrid experiments was reported in Turkey. In this study, it has been found that hybrid rice varieties had better performances than standards. it seems that there is potential to grow Chinese japonica hybrids in Turkey. But the one thousand kernel size of hybrid varieties used in the study were small than the check varieties and thousand kernel size is one of the important quality characteristics in Turkey for customer.

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CARCINOGENESIS OF HIGH-RISK HUMAN PAPILLOMA VIRUS (HPV) IN PATIENTS WITH CERVICAL ABNORMALITIES

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ABSTRACT

A total number of 90 subjects were included. 70 of them are Iraqi patients with cervical abnormalities and 20 apparently healthy women. The patients and healthy were aged between 25-55 years. Buccal swab-Pap smear were collected from each women under supervision of gynecologist. Real Time PCR for qualitative detection and genotyping of Human Papilloma virus High Risk was done. The results also revealed that out of 70 cases, (30) patients were positive to HPV. Carcinoma showed the highest HPV infected samples (100%) followed by HSIL (50%), LSIL (38.89%), ASC-US (35%) and non specific cervicitis (20%). Multi HPVgenotypes were detected in all grades and the most common high-risk HPV types among them is the genotype 16 (26.67%). These results revealed that HPV genotype 16 plays a role in cervical cancer development since it exist in ISIL, HSIL and carcinoma.

INTRODUCTION

Human papillomaviruses (HPV) is considered a major risk factor for the development of cervical cancer (Stanley, 2012). This virus is of different genotypes and according to the associations with neoplasms, HPV classified into low risk genotypes such as HPV 6, 11, 42, 43 and 44 that are associated with benign genital warts and high risk types such as HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 and 70 that are associated with the development of cancer (Woodman *et al*., 2007). High risk HPV genotypes nucleic acid has been found in the vast majority of squamous cell carcinomas and adenocarcinomas of the cervix. Additionally, different studies showed HPV DNA in cervical intraepithelial neoplasia (Kuhn *et al*., 2000). There is a great variation in the prevalence of HPV and the prevalent genotypes depending upon patient age, cytology stage, and other risk factor. PAP test is cheap, simple and most commonly used technique for cervical cancer and preinvasive cervical lesions screening. Atypical squamous cells (ASC) is a term that refers to inflammatory, reactive and reparative processes which are atypical and of higher level and whose quality and quantity is insufficient to be classified as cervical intraepithelial lesions (CIN)(Solomon and Nayar 2004; Nayar and Wilbur 2015). The major cause of CIN is chronic infection of the cervix with the sexually transmitted HPV, especially the high-risk HPV types 16 or 18. Over 200 types of HPV have been identified (Anttila *et al*., 2001). About a dozen of these types appear to cause cervical dysplasia and may lead to the development of cervical cancer (Van Baars *et al.,* 2012). The current study aimed to explore the association of HPV and cytology grads of the cervical abnormalities.

MATERIALS AND METHODS

Subjects:

A total number of 90 subjects were included. 70 of them are Iraqi patients with cervical abnormalities and 20 apparently healthy women. Patients and healthy were referred to the surgical pathology department of the teaching laboratories in the Medical City Teaching Hospital and Al-Elweya Teaching Hospital, Baghdad, Iraq from April 2017 to February 2018. The patients and healthy were aged between 25-55 years. Buccal swab-Pap smear were collected from each women under supervision of gynecologist.

Molecular analysis:

Real Time PCR for qualitative detection and genotyping of Human Papilloma virus High Risk was done using *HPV* HCR genotype-titre-RT PCR kit (AmpliSens® /Russia) and according to the company instruction (Table-1). They were four channel for read four dyes (FAM, JOE , ROX and Cy5) to detect HPV genotyping, showed in table-1. Results are interpreted by the crossing (or not-crossing) the fluorescence curve with the threshold line set at the specific level that corresponds to the presence (or absence) of a *Ct* value of the DNA sample. RT-PCR condition for HPV and genes were listed in table-2.

Statistical Analysis

The Statistical Analysis System- SAS (2012) program was used to effect of difference factors in study parameters. Chi-square test was used to significant compare between percentage and Least significant difference –LSD test (ANOVA) was used to significant compare between means in this study.

Table -1: Amplification program for HR-HPV

Step	Temperature C°	Time	Cycles
1	95	15 min	1
2	95	5 s	5
	60	20 s	
	72	15 s	
3	95	5 s	40
	60	20s fluorescence acquiring	
	72	15 s	

Table 2 : HPV genotypes detection dyes

Matrix for comparison			
FAM	JOE	ROX	Cy5
16	31	18	IC
39	45	59	IC
33	35	68	56
58	52	66	51

RESULTS AND DISCUSSION

The high cytological grads among the cervical abnormalities samples were Asc-us, 20(28.57%) and ISIL, 18(25.72%) followed by HSIL,16(22.85), Non specific cervicitis, 10(14.29%) and carcinoma,6(8.57%). The results also revealed that out of 70 cases, (30) patients were positive to HPV. Carcinoma showed the highest HPV infected samples (100%) followed by HSIL (50%), LSIL (38.89%), ASC-US (35%) and non specific cervicitis (20%) (Table-3). Multi HPVgenotypes were detected in all grades and the most common high-risk HPV types among them is the genotype 16 (26.67%) (Table-4, Table-5). These results revealed that HPV genotype 16 plays a role in cervical cancer development since it exist in ISIL, HSIL and carcinoma. This study shows the importance of monitoring women with ASCUS lesion for early detection of lesion that can progress to higher level of change such as cervical cancer. Infection with HPV16, either as a single infection or multiple infections with other hrHPV genotypes, is associated with a high incidence of high-grade disease. In Iraq, several studies declared an association of HPV with cervical carcinoma such as Mohammed Ali (2001), Al-Azzawi (2006) and Al-Shwaikh (2006) who found that between 25% to 33% of cervical abnormalities were positive for HPV.

A prospective study to evaluate the prevalence of HPV types in Asian women with invasive cervical cancer showed that HPV-16, -18, -52, -45, -58, -33, or -31 were strongly associated with cervical cancer (Lisa *et al*., 2018). HPV-16 as a cause of cervical dysplasia and cervical cancer was also demonstrated by others (Stewart and Wild, 2014; Bose *et al*., 2005). Several pathological studies also demonstrated the interference between multiple HPV type Interactions between different HPV types during their life cycle may affect the propagation, pathogenesis, and evolution of HPVs (Mori *et al*., 2014). These are categorized of high-risk viruses (HR-HPV), according to their oncogenic potential, and the HR-HPV (16, 58, 59) found in all cytology grads (carcinoma, HSIL, LSIL and ASC-US). According to cervical lesions observed by Papillomavirus types- a research in Europe study on Portugal women, 12 HPV types were associated with high grade cervical lesion, of which especially HPV-16, -18, -31, -33, -35, -45, -51, -52, and -58 (Monsonego *et al.*,2012). HPV-16 as a cause of cervical dysplasia and cervical cancer was also demonstrated by others (Stewart and Wild, 2014; Bose *et al*., 2005). High risk of HPV are associated with progression of lesions to invasive cervical cancer.

Common high-risk types of genital HPV include types 16, 18, 31, 33, 35, 39, 45,51, 52, 56, 58, 59, 68, 73, and 82 (Bell *et al.*, 2007). High-risk HPV, multiple high-risk HPV and HPV 16 infections are significantly associated with anal cytological abnormalities.

ASC-US Atypical squamous cells of undetermined significance , LSIL: Low-grade squamous intraepithelial lesion and HSIL High-grade squamous intraepithelial lesion.

Table 3: Correlation between cytopathology grad and HPV infection.

Cytopathology		HPV		P-value	
		Positive	Negative		
Carcinoma	N	6	0		
	%	100.00	0.00	0.0001	15.00 **
HSIL (High grade SIL)	N	8	8	1.00	NS
	%	50.00	50.00		
LSIL (Low grade SIL)	N	7	11		
	%	38.89	61.11	0.0002	10.036 **
ASC-US	N	7	13		
	%	35.00	65.00	0.0001	12.064 **
Non specific cervicitis	N	2	8		
	%	20.00	80.00	0.0001	13.250 **

Table 4: Positive results of high-risk HPV genotyping real time PCR assay.

Genotype of HPV	Number	Percentage (%)	Genotype of HPV	Number	Percentage (%)
16	8	26.67	56	4	13.33
66	3	10.00	58	3	10.00
Chi-square value	---	9.026 **	Other HPV genotypes	5	3.33-6.76
P-value	---	0.0002	** (P<0.01)		

Table 5: Detection of HR HPV genotype according to cytopathology

Cytopathology	No.	HPV (+) Genotype
Carcinoma	6 (12.00%)	16,18
HSIL	8(16.00%)	59,16,58,68,45
LSIL	7 (14.00%)	66,39,58,59 51,16
ASC-US	9 (18.00%)	68 ,51,56,66,59,58
Normal	20 (40.00%)	Negative
LSD value	Chi-Square = 9.331 **	---

Almost all (99.7%) cervical cancer cases are result of persistent infection with high-risk type HPV (Wright, 2014). There are 15 high-risk (oncogenic) HPV, with just two, 16 and 18, responsible for 70% of all cervical cancers (Frumovitz, 2013). Such conclusion was also noticed in the current study where the high risk HPV 16 and 18 were detected in cervical carcinoma. HPV commonly spreads through sexual contact; it can spread without sex, by skin-to-skin contact with an infected area of body (Wright, 2014; Wright *et al*.,2007). Weak immune system is another risk factor. As a result of HIV or by drugs causing suppression of immune response put women at high risk for HPV infection (ACS, 2014). Woman with mother/sister having cervical cancer has 2-3 times risk of developing cervical cancer than women without family history (Frumovitz, 2013).

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SINGLE AND COMBINED EFFECT OF BOTH FUNGICIDES TRICYCLAZOLE AND CYPROCONAZOLE OF FUNGUS ALTERNARIA ALTERNATE

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ABSTRACT

In a study on *Alternaria alternata*, which was isolated from the salad plant *Beta vulgaris subsp. cicla*, the minimum inhibitory concentrations of Tricyclazole and Cyproconazole were measured. The minimum inhibitory concentration of the Tricyclazole was 750 µg/ml while for Cyproconazole was 0.2 µg/ml. Spontaneous mutants were also isolated for each fungicide, it turns out that the frequency of spontaneous resistant mutants of Cyproconazole is 30.8×10^{-4} Which is less than half frequency of the Tricyclazole (66×10^{-4}) .In order to study the effect of the fungicides mixture, the minimum inhibitory concentration was measured. It was about 0.15 µg/ml of Cyproconazole when Tricyclazole 75 µg/ml, and when the Tricyclazole was increased to 250 µg/ml, it required a lethal concentration of Cyproconazole which was 0.1 µg/ml in the mixture, proving an inverse relationship between the effects of the pesticides in their mixture. The spontaneous resistant mutants of the mixture were isolated and calculated at lethal concentrations in their mixture in two successive experiments for three replicates were performed. The first experiment gave a frequency of 134.1×10^{-4} while the second experiment gave 24.5×10^{-4} .The resistant mutants were also distinguished in the color of conidia because they were affected by the active substances of these fungicides even though they were in the same mixture. We conclude from the above that the process of mixing pesticides is a process that not only leads to positive results as it accelerates the process of killing fungus pathogen, but at the same time lead to the growth or emergence of resistance between pathogenic fungi of plants, which may cause results contrary to the expected.

Key Word: *Tricyclazole, Cyproconazole,* Fungicides mixture, Spontaneous resistant mutants, *Alternaria alternate*

INTRODUCTION

The economic losses caused by the infection of plant pathogens are frequent and severe. Therefore, the last century has witnessed a significant increase in the global production of pesticides. The pesticides in general and fungicides in particular have become one of the technological inputs to increase agricultural production (Fisher, 2012). However, a wide range of resistance strains have emerged, these strains have acquired new genes and have enabled fungi to possess some mechanisms of overcoming the toxic effect of the pesticide due to the excessive and indiscriminate use of chemical control (Klaassen et al., 2012; Fisher et al.,2012) .The effectiveness of mixing two or more pesticides is one of the methods of eliminating the increasing resistance to fungicides, a subject that has been widely discussed and intensified since the late 1970s. Some researchers prefer mixing, while others reject mixtures largely as a tactic to eliminate resistant strains (Shaw,2006).

In this study two types of fungicides were used, the first is Tricyclazole, it's structural formula is shown in (Figure 1-A), while molecular formula is [5-methyl-1,2,4-triazole(3,4-b) benzothiazole, code

number EL-291], it is a systematic fungicide used to control blast disease on rice (Froyd et al.,1976). Tricyclazole is a melanin inhibitor that does not affect growth effectively (Kurahashi, 2001). Therefore, it has been classified with a group of fungicides that inhibit the bio-building of melanin (FRAC Code, 2018).

The second fungicide is the Atemi®, its active ingredient is Cyproconazole that has molecular formula [alpha-(4-chlorophenyl)- alpha-(1-cyclopropylethyl)-1h-1,2,4-triazole-1-ethanol] and its structural formula is shown in (Fig: 1-B),it is a systematic fungicide from triazole group (Buchenauer, 1987) which is economically important agricultural compound and it is widely used against crop diseases such as wheat, barley, fruit vegetables, cereals, and seeds or as human antimycotic therapeutics (Buchenauer, 1987; Bolčič–Tavčar and Vračko, 2009; FRAC Code, 2018). The goal of this pesticide is to remove the instance of carbon atom 14 in cytochrome P450 sterol 14 alpha-demethylase, an enzyme required for the biosynthesis of ergosterol in fungi which is the bio-building process of cell membrane (Dahl et al., 1987).

Therefore, the current study aims to test the appearance of the spontaneous mutants of each \fungicide alone and then comparing these frequencies of mutants during fungus exposure to a mixture of both fungicides.

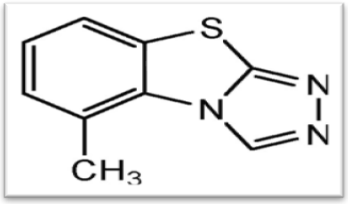
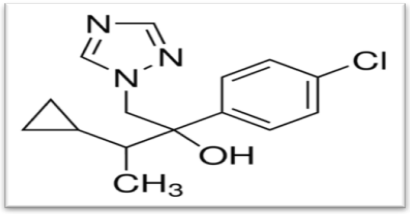


Figure (1):A;Tricyclazole



Figure(1): B: Cyproconazole

MATERIALS AND METHODS

Isolation and Diagnosis of fungus

The fungus was isolated from black leaves containing black spots of Salad plant *Beta vulgaris subsp. cicla*, which was cut, washed, sterilized and then cultured on the (PDA) potato dextrose agar media at 28 °c. Then, the fungal colonies were sub cultured, examined under the microscope. The conidia and morphological characteristics were observed according to (Pitt& Hocking, 2009).

Media and culture conditions

The (M) minimal medium was used to isolate the resistant spontaneous mutants Depending on (Caten, 1979). The solution (D) Sodium deoxycholate was also used to obtain Specific, distinct and individual colonies with a concentration of 400 µg/ml (Mackintosh& Pritchard, 1963). PDA was used to make the fungus grows in a rich and dense manner.

Fungicide solutions

The minimum amount of tricyclazole was dissolved in concentrated 95% ethanol alcohol and then the solution was supplied to a certain size so that the concentration of the stock solution has been 2500 µg/ml,

the tricyclazole fungicide was obtained in the form of 98% purity through the correspondences with the Chinese company Shanghai Redbrillian Chemical Co., Ltd... The Cyproconazole fungicide was dissolved in distilled water because it is an emulsified form and then added at the concentrations under study. This fungicide from a Syngenta Comp. /Swiss.

Measurment of minimal inhibitory concentration (mic)

Mic was measured for each fungicide alone and their mixture using the minimal medium (M) that contain progressive concentrations which were measured depending on the active ingredient for each fungicide.

Resistant mutant isolation

The isolation of spontaneous resistant mutations was done by inoculation 10 petri dishes that were containing (MD) medium as well as the fungicide with concentration higher than lethal for each fungicide alone by inoculation of 10^0conidial suspension(un diluted susp.) that was already prepared at rate 0.1 ml for each petri dish .three petri dish contain (md) medium were also inoculated by the conidial suspension with 10^{-4} dilution at rate of 0.1 ml for each petri dish in order to numerate the living cells.

Calculations and statistical analysis

The inhibition percentage was calculated after four days from inoculation by calculate the mean of colonies diameters that were growing around the inoculating point in each petri dish which then were compared with the diameter of colonies that were growing on the (MD) medium which was didn't contain fungicide and represented the control. The inhibition percentage was calculated:

$$\boldsymbol{inhibition\ precentage} = \frac{\boldsymbol{spntaneouus\ treatment\ average - fungicide\ treatment\ average}}{\boldsymbol{spntaneouus\ treatment\ average}} \times \mathbf{100}$$

The frequency of spontaneous resistant mutants was estimated through:

$$\boldsymbol{mutants\ freqauency} = \frac{\boldsymbol{mutants\ number\ for\ each\ treatment}}{\boldsymbol{expected\ population\ size\ for\ that\ treatment}} \times \mathbf{100}$$

Expected population size was estimated from conidial suspension for both treatments:

Expected population size =mean of colonies no. on (MD) plates × dilution factor × control plates no.

Statistical analysis was done for the results using t-test at level of 0.05>P (Cochran and Cox, 1957) in order to explain the differences between mixture fungicides treatment and fungicied treatment alone.

RESULTS

Isolation and Diagnosis of fungus

Figure 2 showed the *Alternaria* leaf spot of salad plant, while (figure3) showed the microscopic characteristics of *Alternaria alternata* conidia.

Figure (2): *Alternaria* leaf spot of salad plant

Figure (3): *Alternaria alternata* Conidia

Minimal inhibitory concentration (MIC) of each fungicide alone

This study found inhibition percentage of Tricyclazole was 100% at concentration750 µg/ml and sometimes 650 µg/ml.

Table1: Diameter of the colonies (cm) for *Alternaria alternata* growing on the M medium containing mounting concentrations of Tricyclazole

Inhibition Percentage	(Average M)	Colony Diameter				**Tricyclazole µg/ml**
		R4	R3	R2	R1	
0	2	2	2.2	1.8	2	0
64	0.75	0.9	0.8	0.5	0.7	50
75	0.5	0.6	0.4	0.4	0.6	75
86.5	0.27	0.1	0.1	0.4	0.5	250
96.2	0.075	0.0	0.1	0.1	0.1	500
98.7	0.025	0.0	0.0	0.0	0.1	650
100	0.0	0.0	0.0	0.0	0.0	750

Wherase the MIC of cyproconazole reached to 0.2 µg/ml as follow:

Table 2: Diameter of the colonies (cm) for *Alternaria alternata* growing on the M medium containing mounting concentrations of cyproconazole

Inhibition Percentage	(Average M)	Colony Diameter				Cyproconazole µg/ml
		R4	R3	R2	R1	
0	2.0	2.1	2	1.9	2.2	0
60	0.8	0.8	0.9	0.7	0.8	0.02
62.5	0.75	0.5	0.9	0.8	0.8	0.04
82.5	0.35	0.5	0.4	0.2	0.3	0.06
90	0.2	0.0	0.1	0.2	0.5	0.1
97.5	0.05	0.0	0.0	0.1	0.1	0.17
100	0.0	0.0	0.0	0.0	0.0	0.2

Minimal inhibitory concentration (MIC) of mixture

The process of measuring the minimum inhibitory concentration of the fungicide mixture was a little complicated process.to measure the MIC of mixture two experiments were done. However, the under lethal concentration of both fungicides were used in both experiments.in the first one when a constant concentration of tricyclazole which is 75 µg/ml was used, it is found that killing fungal growth requires

an increase in cypro concentration from 0.04 to 0.15 µg/ml in order to find the MIC of mixture (Table 3).while in the second experiment a constant concentration of tricyclazole which is 250 µg/ml was used, and it is found that killing fungal growth requires less concentration of cypro that reached about 0.1 µg/ml (Table 4).

Table 3: minimal inhibitory concentration for fungicide mixture when Tricyclazole conc. is 75 µg/ml.

Inhibition Percentage	Average	Colony Diameter(cm)			Cyproconazole µg/ml	Tricyclazole µg/ml
		R3	R2	R1		
0	1.76	1.5	2	1.8	0	0
26.13	1.3	1.3	1.3	1.4	0.04	75
85.22	0.26	0.4	0.2	0.2	0.1	75
100	0.0	0.0	0.0	0.0	0.15	75

Table 4: minimal inhibitory concentration for fungicide mixture when Tricyclazole conc. Is 250 µg/ml.

Inhibition Percentage	Average	Colony Diameter(cm)			Cyproconazole µg/ml	Tricyclazole µg/ml
		R3	R2	R1		
0	1.76	1.6	1.5	2.2	0	0
73.8	0.46	0.5	0.4	0.5	0.04	250
92.61	0.13	0.1	0.2	0.1	0.07	250
100	0.0	0.0	0.0	0.0	0.1	250

Mutant frequencies for each fungicide separately

It is noted from (Table 5) that the average frequency of spontaneous mutants of the Cyproconazole fungicide alone was 30.8. Which is less than that of Tricyclazole which that means the average frequency of spontaneous mutants of Tricyclazole is more than twice of that of Cyproconazole.

Table 5: mutant frequencies for each pesticide separately.

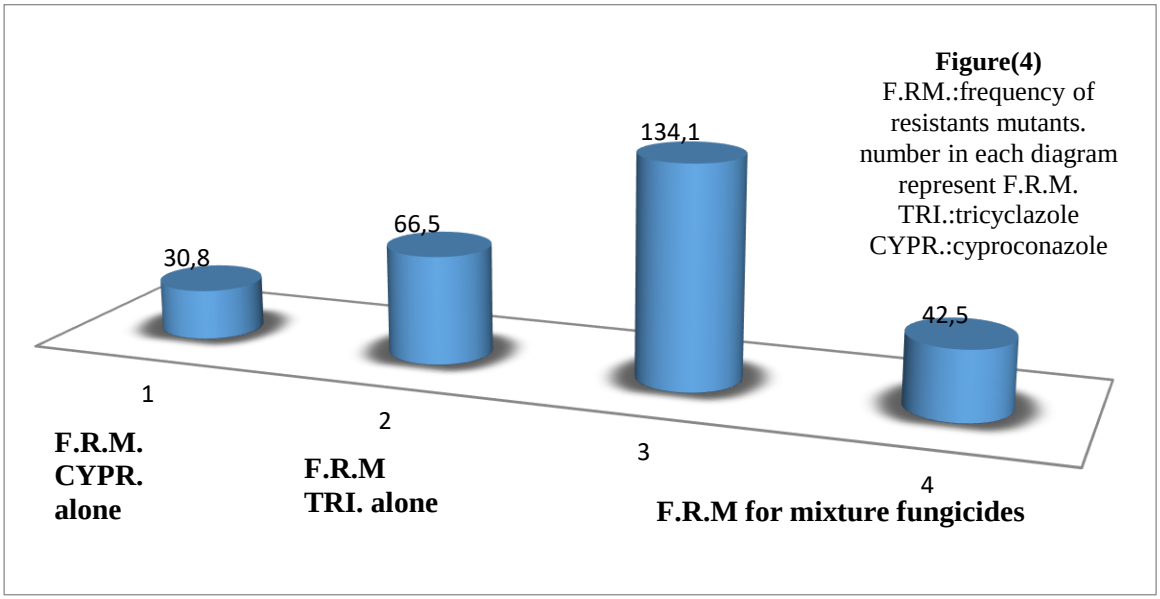
Standard error SE	Variation S	Mutant frequency× 10^4 average	Fungicide µg/ml
0.87	1.5	30.8	Cyproconazole 0.2
0.4	0.7	66.5	Tricyclazole 750

Mutant frequencies of fungicides mixture

two different experiments were done in order to measure the average frequency of resistant spontaneous mutants of fungicides mixture.in the first experiment When the concentration of Tricyclazole was 75 µg/ml and concentration of cyproconazole was 1.5 µg/ml the mutants frequency was 134.1(Table 6 and figure 4) but in the second experiment When the concentration of Tricyclazole was 250 µg/ml and concentration of cyproconazole was 0.1µg/ml the mutants frequency was 42.5which is less than that of the first experiment (Table 6 and figure 4).

Table 6: frequency of mutants to mixture of pesticides.

t value	Standard error SE	Variation S	Mutant frequency $\times 10^4$ average	Cyproconazole µg/ml	Tricyclazole µg/ml
47.6	1.39	2.4	134.1	0.15	75
17.39	1.33	2.3	42.5	0.1	250



Figure(4)
F.RM.:frequency of resistants mutants. number in each diagram represent F.R.M.
TRI.:tricyclazole
CYPR.:cyproconazole

Colour of resistant mutants

Resistant mutants of fungicides mixture were different in color Colonies (Figure 5) that are that resistant to Tricyclazole appeared in white color. While those that are resistant to cyproconazole still retain their black color

Figure (5): Diference in color of resistant mutants of fungicides mixture.

DISCUSSION

Isolation and Diagnosis of fungus

The *Alternaria alternata* fungus was isolated and it was confirmed that the fungus was *Alternaria alternata* through the conidia properties depending on (Pitt& Hocking, 2009).

Minimal inhibitory concentration (MIC) of each fungicide alone

Full inhibiting of fungus growth requires very high concentrations of Tricyclazole when compared to Cyproconazole fungicide which was the very low concentrations (Table 1,2), Sharma et al. observed this in 2013 when he treated cumin that was infected by *Alternaria* with Tricyclazole which had moderate or little effect in comparing with triazole fungicide. which is a very high concentration in comparison with the inhibitory concentration of mycelium and conidia growth of the fungus *Piricularia oryazae*, that was about 200 and 300 µg / mL for inhibition respectively (froyd et al., 1976).

MIC of cyproconazole was similar to that of difenoconazole a fungicide from triazole group also which is highly effective against potato early blight disease caused by *Alternaria Solani* (Horsfield et al., 2010) .other study demonstrated that the difenoconazole was very effective against the growth and germination of *Alternaria* spores in compared with a variety of fungicides (alum et al., 2017). Other laboratory experiments have shown that a fungicide from the same group of triazole, Propiconazole, is very effective in controlling the *A. alternata* which is very sensitive to it, and is also from a triazole group (Duba et al., 2018).

The great difference between these two fungicides in MIC is in fact due to the completely different working mechanism towards the cell of fungi, as the first fungicide targets a compound of secondary metabolites that has no major effect on the vitality of the fungus (Hopwood, 1997; Kimura and Tsuge, 1993). Melanin is not necessary for growth or development, but it enhances the competitiveness among species and provides protecting against various environmental stresses to the organisms that produce (Jacobson, 2000).

While the triazole fungicides are targeting an important vital process that takes place within fungal cell membrane, the process of building the ergesterol which is an essential component of the cell membrane, thus inhibiting the growth of mycelium fungus (Panwar et al., 2013).

Minimal inhibitory concentration (MIC) of mixture

The results in (Table 3, 4) indicate an inverse relationship between the two lethal concentrations of both fungicides when used as a single mixture. This is may be due to a cooperative or complementary action between the two fungicides, so that if the concentration of one of them increases, thus the lethal concentration of the other fungicide decreases. This makes the mixture of fungicides significantly effective in eliminating fungal diseases in plants. Also this was found by Zamani-Noor and Knüfer in (2018) when they mixed two pesticides together, one of them returning to the triazole group (tebuconazole), noting the severity of the elimination of fungal diseases.

Mutant frequencies for each fungicide separately

The reason of low spontaneous mutant's frequency of Cyproconazole that the Cyproconazole is from triazole group which is a highly effective pesticides against *Alternaria*(Kiran et. al.,2018), but it does not hide the possibility of developing resistance even if it is low (Fisher et al.,2012).

Mutant frequencies of fungicides mixture

From the statistic (Table 6), it was found that the calculated value of T is higher than the value of T-table at the level of probability 0.05 or 0.01 for mixture resistance mutants in comparison with the frequency average of mutants in the case of Tricyclazole alone. The results of the mixture resistant were divided into two categories: The first one demonstrated that; when the concentration of Tricyclazole fungicide was 75 µg/ml the frequency of Resistant mutations then became 134.1 (Table 6), there was a significant difference in the resistance which was higher than that in the case of using of the aforementioned fungicide alone When its concentration was 750 µg/ml; the frequency of

resistant mutations changed to 66.5 (Table 5). second section demonstrated that; when the concentration of Tricyclazole was 250 µg/ml T value will be significant This may be due to the high concentration of Tricyclazole compared to the first resistance to the mixture, possibly due to its increased effect on melanin, which is the resistance component of this fungal pathogen (Kawamura et al., 1999) this lead to increase in the cyproconazole effect even if its concentration was low consequently the number of mutants decreased. . This has been confirmed by some studies on the possibility of reducing the resistance of fungi by mixing two or more pesticides (Dinakaran et al., 2012), But these results do not prevent the emergence of resistance against the mixture of fungicides that was very high as recorded in the first experiment mentioned above of the mixture.

Color of resistant mutants

Mutants that have resistance against the mixture of fungicides were different in color. Colonies that are that resistant to Tricyclazole appeared in white color, while those that are resistant to cyproconazole still retain their black color as in the case when the mutants resisted each fungicide alone. This indicates the separate impact of each fungicide alone. Which may means that there is no fusion or interaction between the active ingredients of the both fungicides,in spite of they were present in the same mixture, although there was a difference in the lower inhibitory concentration when mixed together and the different numbers of resulted frequencies mutants.

CONCLUSIONS

This study indicates the evolution of resistant strains of pathogenic fungi in the case of mixing the fungicides, regardless of the fact that it is possible to cause a 100% kill rate in this mixture for both fungicides with low concentrations that are less than their lethal concentration when used separately. It can be seen through the experiment of mixing fungicide that, it is a complex process and not as easy as some may think it. It depends on the biology of the organism to be combated, as well as on the study the mechanism of work of each fungicide and the study of lethal and under the lethal concentrations. It depends on conducting many experiments before the mixing process.

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A CONTRIBUTION TO THE FAUNA OF MICROCHELONUS SZÉPLIGETI, 1908 (HYMENOPTERA: BRACONIDAE: CHELONINAE) OF ÇANAKKALE PROVINCE

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ABSTRACT

Çanakkale province consists of land on both sides of the Dardanelles and is naturally bordered by Koru Mountain on the Gelibolu peninsula in the north, the Marmara Sea in the north-east, the Aegean Sea in the north-west and west, and Kaz Mountain and the Aegean Sea in the south. This study area consists of forests, agricultural fields and meadows. The study was carried out in spring, summer and autumn periods between the years 2008-2016 various localities and habitats of Çanakkale Province. This study increases about ten times the number of localities of *Microchelonus* Szépligeti, 1908 (Hymenoptera: Braconidae: Cheloninae) previously known from the Çanakkale province of Turkey. Samples collected from short plants using standard insect sweeping nets were transferred into tubes containing 70% ethanol and labeled following their preparations according to museum techniques. Members of Cheloninae are solitary koinobiont egglarval endoparasitoids on Lepidoptera (especially Tortricoidea and Pyraloidea), Diptera, Hymenoptera and Coleoptera, and therefore are potentially very important biological control agents to be used against pest insects. Eight solitary egg-larval endoparasitoid *Microhelonus* species were collected from pastures, vegetable garden, crop fields, orchards, pine and mixed forests at different altitudes in Çanakkale province. The distributions of the determined species in Turkey as well as their general distributions were given and discussed zoogeographically. For each species its chorotype was reported.

Keywords: *Microchelonus*, Hymenoptera, Cheloninae, Fauna, Turkey

INTRODUCTION

Çanakkale province has a European (Thrace) and an Asian (Anatolia) part. The European part is formed by the Gallipoli (Gelibolu) peninsula, while the Asian part is largely coterminous with the historic region of Anatolia. They are separated by the Dardanelles strait, connecting the Sea of Marmara and the Aegean Sea. This city consists of land on both sides of the Dardanelles and is naturally bordered by Koru Mountain on the Gelibolu peninsula in the north, the Marmara Sea in the north-east, the Aegean Sea in the north-west and west, and Kaz Mountain and the Aegean Sea in the south (Kılıç, 2001).

The subgenus *Microchelonus* Szépligeti, 1908 (Hymenoptera: Braconidae: Cheloninae) is cosmopolitan, koinobiont egg-larval endoparasitoid of Lepidoptera. Species of subgenus mainly exploit host groups that have concealed Lepidoptera larvae (Coleophoridae, Cosmopterygidae, Elachistidae, Gelechiidae, Momphidae, Oecophoridae, Tortricidae, Pyralidae) and Cynipidae (Hymenoptera). For this reason, members of this genus are potentially very important biological control agents against pest insects The subfamily Cheloninae is characterised as having a gastral carapace formed by the fusion of 1-3 tergites, covering the rest of the gaster (Aydogdu & Beyarslan, 2006; Tobias, 1986). This genus has as distinctive features 16 antennomeres in the female and a

foramen at the apex of the carapace, in the male. This carapace foramen is present in the female of some species as a small and circular opening (Papp, 1999).
This study area consists of forests, agricultural fields and meadows. The study was carried out in spring, summer and autumn periods between the years 2008-2016 various localities and habitats of Çanakkale Province.

MATERIAL AND METHODS

Adult *Microchelonus* subgenus were collected from various localities, habitats and altitudes using sweeping nets and were transferred into tubes containing 70% ethanol. The specimens were then pinned and labeled according to taxonomic rules and regulations. For the terminology used in this paper and for the identification of the subgenus, see Abdinbekova (1975) and Tobias (1986). Species were treated in alphabetical order in the subgenus. All records were arranged in the following order: province (alphabetically ordered)-town-local place-geographic name, habitat, altitude, date of collection, and the number of male and female individuals. References are used for known general distributions and hosts of the species in the world (Aydogdu & Beyarslan, 2006; Yu et al., 2012). The samples of the identified *Microchelonus* species are stored at the Collections of the Biology Department, Trakya University.

RESULTS

Eight *Microchelonus* species were identified within 1 genus in this study, and all species were recorded with new localities for the fauna of Çanakkale. These species are as follows:

Microchelonus SZÉPLIGETI, 1908

1. *Chelonus* (*Microchelonus*) *arnoldii* (TOBIAS, 1964)

Material examined: Çanakkale-Gelibolu-Burhanlı, mixed forests, 60m, 05.07.2012, 2♀♀, ♂.

General Distribution: Hungary, Kazakhistan, Romania, Turkey.

Hosts: So far unknown.

2. *Chelonus* (*Microchelonus*) *excavatus* TOBIAS, 1972

Material examined: Çanakkale-Gelibolu-Burhanlı, vegetable garden, 40m, 05.07.2012, ♀; Çanakkale-Gelibolu-Güneyli, pasture, crop field, 60m, 06.08.2014, ♀.

General Distribution: Russia, Mongolia, Turkey.

Hosts: So far unknown.

3. *Chelonus* (*Microchelonus*) *fenestratus* (NEES von ESENBECK, 1816)

Material examined: Çanakkale-Ayvacık, orchard, 50 m, 10.07.2008; 2♀♀; -Gelibolu-Kabatepe, pine forests, 40m, 05.07.2015, ♀.

General Distribution: England, Finland, France, Germany, Hungary, Korea, Poland, Russia, Turkey.

Hosts: So far unknown.

4. *Chelonus* (*Microchelonus*) *flavipalpis* (SZÉPLIGETI, 1896)

Material examined: Çanakkale-Kilirtbahir, pine forests, 70m, 21.06.2015, 2♀♀; -Eceabat-Bigali, orchards and vegetable garden, 80m, 20.06.2015, ♀.

General Distribution: Georgia, Hungary, Mongolia, South Russia, Ukrain, Turkey.

Hosts: Parasitoid of lepidopterans *Parametriotes theae* K. (Momphidae), *Sparganothis pilleriana* D. & S. (Tortricidae).

5. *Chelonus* (*Microchelonus*) *microphtalmus* (WESMAEL, 1838)

Material examined: Çanakkale-Bayramiç-Evciler, orchard, 400 m., 02.08.2016, 2♀♀; -Kilirtbahir, pine forests, 70m, 21.06.2015, ♀.

General Distribution: Belgium, Finland, former Yugoslavia, France, Germany, Hungary, Korea, Mongolia, Rumanian, Russia, Sweden, Turkey, Turkmenia.

Hosts: Parasitoid of lepidopteran *Colleophora hemerobiella* SCOP. (Coleophoridae).

6. *Chelonus (Microchelonus) nigritibialis* (ABDINBEKOVA, 1971)

Material examined: Çanakkale-Bayramiç-Evciler, orchard, 400 m., 02.08.2016, ♀; -Biga, crop fields, 50 m., 06.07.2011 ♀; -Dardanos, vegetable garden, 40 m., 12.07.2015, ♀; -Güzelyali, pine forests, 500 m., 02.09.2016, ♀.

General Distribution: Azerbaijan, Mongolia, Turkey.

Hosts: So far unknown.

7. *Chelonus* (*Microchelonus*) *risorius* (REINHARD, 1867)

Material examined: Çanakkale-Ayvacık, orchard, 50 m, 10.07.2008; ♀, -Kepez

General Distribution: Armenia, Croatia, England, Finland, former Czechoslavakia, Germany, Hungary, Italy, Kazakhistan, Mongolia, Russia, Turkey.

Hosts: Parasitoid of hymenopterans *Biorhiza terminalis* F., *Biorhiza pallida* O. (Cynipidae).

8. *Chelonus (Microchelonus*) *rostratus* (TOBIAS, 1966)

Material examined: Çanakkale- Bayramiç-Ayazma, 500 m., 3 , 22.7.1997, 3♀♀, ♂.; -Ezine, vegetable garden, 100 m, 11.07.2009, 2♀♀.

G e n e r a l D i s t r i b u t i o n : Armenia, Azerbaijan, Bulgaria, Hungary, Mongolia, Russia, Turkey, Turkmenia, Ukrain.

Hosts: So far unknown.

DISCUSSION

This study increases about ten times the number of localities of *Microchelonus* Szépligeti, 1908 (Hymenoptera: Braconidae: Cheloninae) previously known from the Çanakkale province of Turkey.

The majority of the 8 species described in this study are disturbed in the Palaearctic region. They can be divided into the following groups according to their zoogeographical distributions (Taglianti et al.,1999).

• **Asiatic-European:** *Chelonus* (*Microchelonus*) *arnoldii, C.* (*M.*) e*xcavatus, C.* (*M.*) *fenestratus, C.* (*M.*) *flavipalpis, C.* (*M.*) *microphtalmus, C.* (*M.*) *risorius, C.* (*M.*) rostratus.

• **Centralasiatic-European:** *C.* (*M.*) *nigritibialis*.

CONCLUSIONS

The aim of this study was to survey the Lepidoptera and Hymenoptera parsitoids from a wide range of habitats at different altitudes in the Çanakkale province of Marmara region of Turkey.

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GIANT REED GROWTH FOR NEY MANUFACTURING IN SAMANDAĞ DISTRICT OF HATAY PROVINCE

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ABSTARCT

A ney is a musical instrument made from well dried firmly fibrous giant reeds (*Arundo donax* L.) having 9 homogeneously distributed short internodes along the cane. The best quality canes for ney manufacturing naturally grown in 6.5 km long and 100-250 m width sea shore of Asi delta plain Samandağ, Turkey. A good cane for ney manufacturing must have 9 homogeneously distributed short internodes. A cane with long internodes cannot used for ney manufacturing. To increase the number of canes that can be used for ney manufacturing plants that cannot be used for ney manufacturing must be cut and removed in the giant reed growing areas. In addition to plant removal, application of growth inhibitors shortened the internode length of canes. Either plant tinning or application of plant growth inhibitor can increase the number of canes suitable for ney manufacturing.

Key words: *Arundo donax*, Asi delta plain, giant reed, ney production, Samandağ

INTRODUCTION

Giant reed *(Arundo donax* L.) is a genus of tall perennial reed-like grasses indigenous to the Mediterranean and to warmer regions of the Old World (Hickman 1993; Bell, 1997; Munz and Keck, 1959, Robbins et al. 1951). Giant reed was given several common names such as cane and common cane, but the scientific community accepted its common name as giant reed.

The plant has a long history in world culture through its influence on the creation of music, which can be traced back 5000 years (Merzouki et al. 2000, Perdue 1958). Arundo donax is a hydrophyte, growing along lakes, streams, drains and other wet sites. Ancient people of the Middle East was well known the giand reed. Woodwind musical instruments are made from giant reed stems. Therefore, commercial plantations of giant reed exist for musical instrument production.

Under optimal conditions it can grow more than 5 cm per day (Purdue 1958). Giant reed stands are among the most biologically productive of all communities. Under ideal growth conditions they can produce more than 20 tons per hectare above-ground dry mass (Perdue 1958). The world's best quality giant reeds for ney manufacturing is grown in Samandağ district in Hatay province due to suitable soil properties and environmental conditions. A ney is a musical instrument made from well dried firmly fibrous canes having 9 homogeneously distributed short internodes along the cane. The rarity of the canes that can be used for ney production increases the value of ney type canes.

It is well known that light quality influences plant growth and development. The fare red relative to red light ratio received during leaf development has been associated with plant height. Plants in higher-density receive higher fare red relative to red light ratios develop longer stems. Studies showed that the ratio of fare red relative to red light acts through the phytochrome system to

regulate stem elongation (Downs, et al., 1957). Anti-gibberellins affects plant height by suppressing gibberellin synthesis. Use of Anti-gibberellins is another way to reduce plant height. Chlormequat chloride and paclobutrazol, acts as anti-gibberellin, are recommended for height control in plants (Barret, 2001). These chemicals must be sprayed onto the plants for several times to get maximum effect. Thus, the aim of this study was to evaluate the tilling and growth regulator application efficiencies on the vegetative growth of giant reed aiming at height control.

MATERIAL AND METHODS

The giant reed growing areas that can be used for the ney production lays the coastal line between at the end of Deniz neighborhood of Samandağ and Meydan gendarme building in Asi delta plain. Asi delta plain coast in which the ney type canes grow in 6.5 km long and 100-250 m width has slightly alkaline sandy soils, slightly saline, pure in organic matter, 33.3 mg/kg phosphorous, and 511 mg/kg potassium content with having seasonal varying water level between 5 and 95 cm depth. Mean yearly temperature is 20 °C and the lowest and the highest temperatures were -2.2 and 41°C, respectively. Yearly mean precipitation, relative humidity and wind speed are 900 mm, 75% and 4.6 m/sn, respectively.

Paclobutrazol (0, 10, and 20 mg/l) and chlormequat chloride (0, 4000, and 6000 mg/l) were applied when the plant heights were about 30 cm. The plant growth regulator applications were repeated in every 10 day intervals. The plants were monitored, and the data for the plant height were collected every 10 days.

A suitable tilling work area was chosen in the natural giant reed growing plantation in Samandağ, Hatay. The giant reed plants were cut down form the soil surface in March. The plant number was tinned to 5, 10, 15, 20, 25 and 30 plant/m^2 when the plant started to regrow. During the growth period, the plant number plot was kept by the removing of the regrowth plants. The plant height, stem diameter, nod number and internode length were measured at the harvest. The experimental design consisted of a complete randomized block design with 3 replications. The data were subjected to an analysis of variance, and the means were compared using the LSD test at 5%.

RESULTS

Due to the asexual reproduction difficulties of giant reed, its genetic variability and finding new genotypes or varieties were very difficult. Therefore, selecting superior genotype for ney manufacturing is useless. Plant tilling and application of anti-gibberellins look like good solutions to reduce internode length. Paclobutrazol and Chloromequat chloride were applied as anti-gibberellins. They were used as growth retardant to prevent lodging in grain and cereal crops and for growth control of potted greenhouse crops. Plant tilling and anti-gibberellin application studies were conducted in Samandağ delta plain, Hatay.

Table 1. Effects of chloromequat chloride and paclobutrazol on plant height, stem diameter, internode length and nod number.

Application	Plant height (m)	Stem diameter (cm)	Internode length (cm)	Node number/plant
Chloromequat chloride (mg/l)				
0	3.4 A	2.3 B	23.4 A	18.6 A
4000	2.9 B	2.9 A	20.4 B	17.7 AB
6000	3.1 B	3.0 A	18.5 C	16.5 B
Paclobutrazol (mg/l)				
0	3.8 A	2.2 B	19.5 A	20.3 A
10	2.5 B	3.1 A	14.3 B	15.2 b
20	2.1 B	3.2 A	10.2 C	10.7 C
LSD 0.05	0.2	0.18	0.4	2.01

Table 1 showed that the application of chloromequat chloride and paclobutrazol reduced the mean plant height. However, chloromequat chloride was found more effective on internode length compared with paclobutrazol. The lowest internode length was obtained from 20 mg/l paclobutrazol application, and the longest was obtained from no chemical applied control plots. Stem diameter also influenced from the chemical applications. Both chloromequat chloride and paclobutrazol increased stem diameter.

Chloromequat chloride had limited effect on the mean height of plants at the concentrations applied. In contrast, paclobutrazol was found more effective in controlling plant height and node length at a concentration of 10 and 20 mg/l. Paclobutrazol offers a viable means for internode length control in giant reed.

Table 2. Effects of plant density on plant height, stem diameter and nod number.

Pant number/m^2	Plant height (m)	Stem diameter (cm)	Node length (cm)	Node number/plant
5	1.6 E	2.8 A	13.5 E	18.6 B
10	1.5 E	2.4 B	14.8 D	18.7 B
15	1.9 D	2.3 B	18.5 C	18.5 B
20	2.2 C	2.1 B	19.5 B	20.3 A
25	2.5 B	1.3 C	19.3 B	19.2 A
30	2.9 A	1.2 C	23.2 A	19.7 A
LSD 0.05	0.21	0.19	0.35	2.49

Plant density had significant effects on plant height, stem diameter, node number and node length. When plant height was considered, the lowest value was obtained from the lowest plant density with 1.6 m (Table 2). The highest plant plant heights were obtained from 30 and 25 plant/m^2 densities. Plant height increased with the increasing plant density. Internode length showed similar length response pattern. The lowest mean internode length was obtained from the lowest plant population density with 13.5 cm. Internode length increased with the increased plant number in the given area. Stem diameter decreased with increased plant population. Light plays important role on plant growth

and development. Plants grown in higher-density populations receive higher far red/red ratios and develop longer stems (Downs, et al., 1957).

CONCLUSIONS

To obtain maximum number of canes that can be used for ney production removal of all plant canopy after cutting and removal of individuals that cannot be used for ney production are required. In addition to plant removal, application of anti-gibberellin group type of chemicals helped to complete the process that necessary for production of canes with short internodes.

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ESSENTIAL OIL CONTENT AND COMPOSITION OF LEMON BALM (MELISSA OFFICINALIS L.) GENOTYPES GROWN UNDER CENTRAL ANATOLIAN REGION

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ABSTARCT

Lemon balm (*Melissa officinalis* L.) is a perennial plant from Labiatae family widely used in alternative medicine. This study was conducted at to determine herbage yield, essential oil content and essential oil components of 6 lemon balm genotypes under the continental type of climate in 2015 – 2017 growing seasons. The experimental design was randomized complete blocks with three replications. The essential oil content was determined by steam distillation and the essential oil composition was determined with gas chromatography - mass spectrometry. The herbage yield varied between 2500-3250 kg/ha. The genotype ERU02 had highest herbage yield while the genotype ERU12 had the lowest. Essential oil contents varied between 0.09 and 0.3 %. The genotype CA03 had highest essential oil content while the genotype ERU09 had the lowest. The main essential oil components were citral, citronellal, geraniol, linalool, β-caryophyllene, cermacrene-d.

Key words: *Melissa officinalis,* Lemon balm, essential oil content, essential oil composition

INTRODUCTION

Lemon Balm (*Melissa officinalis* L.), a perennial herbaceous plant distributed in the Mediterranean region and Asia, has great medicinal properties. The lemon balm leaves are used as herbal tea, fever, headache, gastrointestinal disorders, insomnia, rheumatism and calming nerves. The plant has antibacterial, antiviral, antiseptic, antispasmodic, carminative, sedative, digestive properties. Lemon balm essential oil can be used as antioxidant and also as antimicrobial. Antimicrobials are not new and have been used throughout all of recorded history (Gurib-Fakim, 2006). A report by the World Health Organization estimates that 80 percent of the world's people use plants for their healthcare (WHO 2001). Even more, the concept of pharmaceutical drugs, cosmetic, and even the food industries are founded upon the healing and beneficial effects of plant extracts (Hammer, 1999). Essential oil content of lemon balm is low (among 0.05 and 0.12 % vol.) compared with most of essential oil bearing plants. The main constituents in lemon balm oil were citral (neral, geranial), citronellal, citronellol, linalool, geranyl acetate, although, there were reports for quantitative variation in the major compounds of oil. Essential oil content, composition and herbage yield can be varying among lemon balm genotypes. In order to get higher herbage, yield the selected superior lemon balm genotypes or species must be cultivated. The objective of the current study was to determine the essential oil content, essential oil component and herbage yield of lemon balm.

MATERIAL AND METHODS

High yielding selected lemon balm genotypes were vegetatively propagated by stem cuttings. The rooted cuttings were transferred in 4-row plots, 5 m long with 5 intra-row spacing at the experimental field of Erciyes University. The planting densities were 60 x 35 cm (47.580 plants/ha),

60 x 30 cm (55.500 plants/ha), 60 x 25 cm (66.666 plants/ha), 60 x 20 cm (83.333 plants/ha) and 60 x 15 cm (110.833 plants/ha). The experimental design was a randomized complete block with three replications. The crop was fertilized with 75 kg/ha of N and 75 kg/ha of P2O5. Drip irrigation was applied during the growing period. Aerial parts of lemon balm genotypes were harvested at the flowering stage. The harvested plants were dried under the shade. Essential oil was extracted by water distillation for 3 h from air-dried leaves, by using a Clevenger-type apparatus. The obtained essential oils were dried over anhydrous sodium sulfate and stored at four degrees in a refrigerator until they were analyzed.

Essential oil analysis was done using a Hewlett-Packard 2890 GC with FID. A HP-5 MS capillary column (30 m x 0.25 mm i.d. 0.25 µm film thickness) was used. The carrier gas used was Helium (1.4 ml/ min). The column had a temperature programming of: five minutes at 45 degrees, then at three degrees per minute up until 220 degrees, and held for 10 minutes. The injector temperature was 220 degrees and the detector temperature was 250 degrees. Injection was done using the automatic settings. Samples (0.5 µl of the oil solution in hexane (1:100) were injected using the splitless technique into Helium carrier gas. Peak areas and retention times was measured by electronic integration. GC/MS analysis of the essential oil was carried out on Hewlett Packard 5970A mass selective detector (MSD), directly coupled to a HP 6890 GC. The column, temperature program and injection was performed using the same procedure outlined above. Injection was done using the automatic settings. The library search was done using "Wiley Library, WILEY275, NBS75K, NIST98, FLAVOR". EI mass spectra was measured at 70 e V ionizations voltage over the mass range 10 - 400 u. Identification of the compounds was accomplished through a comparison of retention times and mass spectra with the library standards (Stenhagen, 1974; Adams, 1995).

The statistical analysis of experimental data was determined using a general linear model procedure of SAS for Windows (Version 8.02, SAS Institute, Cary, NC, USA), applying the one-way analysis of variance (ANOVA). Differences between means were tested through LSD and values of $p < 0.05$ were considered significantly different.

RESULTS AND DISCUSSION

Dry herbage yield significantly varied among lemon balm genotypes (Table 1). The herbage yield varied between 2500-3250 kg/ha. The genotype ERU02 had highest herbage yield while the genotype ERU12 had the lowest. Essential oil contents also significantly varied among lemon balm genotype. The essential oil variation was between 0.09 and 0.3 %. The genotype CA03 had highest essential oil content while the genotype ERU09 had the lowest (Table 1). Essential oil content of lemon balm in the present work (range, 0.09 - 0.3 % v/w) was similar with studies of Rehman et al. (2013), Sarı et al. 2002, and Ayanoğlu, et al.(2008), Dukic et al. (2004) and Sadraei et al. (2003).

More than twenty-five essential oil components were detected from the essential oil of lemon balm genotype ERU02, 29 from ERU06, 28 from ERU09, 26 from ERU12, 25 from CA03 and 24 from CA07. Considerable number of essential oil components (>2%) were recorded for lemon balm genotypes. Citronellal, Neral, Geraniol, Geranial, β-Caryophyllene and Caryophyllene oxide were the major essential oil components.

Table 1. Herbage yield and essential oil content of lemon balm grown in the central Anatolia.

Lemon balm genotypes	Herbage yield (kg/ha)	Essential oil content (%)
ERU02	3100 A	0.31 A
ERU06	2980 B	0.29 A
ERU09	2870 C	0.28 A
ERU12	2740 D	0.17 B
CA03	2600 E	0.12 B
CA07		
LSD 0.05	100.2	0.083

Table 2. Essential oil components of lemon balm genotypes grown in the central Anatolia.

Components	RI*	Area (%)					
		ERU02	ERU06	ERU09	ERU12	CA03	CA07
Sabinen	973	0.91	0.84	0.37	0.91	0.91	0.91
α-Terpinene	1012	0.25	0.27	0.23	0.25	0.25	--
Limonene	1030	0.63	0.71	0.65	0.63	0.63	0.63
trans-β-Ocimene	1051	--	0.22	0.31	0.24	0.24	0.24
Linalool	1095	0.34	0.24	0.49	0.34	0.34	0.34
n-Nonanal	1102	0.21	--	0.12	0.21	--	0.21
cis- Rose oxide	1114	0.22	0.33	0.36	0.22	0.22	--
trans-Rose oxide	1123	0.28	0.22	0.16	--	0.28	--
neo-Isopulegol	1142	0.45	0.47	0.33	0.45	0.45	0.45
Citronellal	1150	**14.50**	**13.31**	**17.65**	**14.50**	**14.50**	**14.50**
Isogeranial	1167	0.20	0.22	0.20	0.20	--	0.20
Isomenthol	1180	0.38	0.34	0.31	--	0.38	0.38
Nerol	1222	1.20	1.41	2.14	1.20	1.20	1.20
Neral	1241	**25.10**	**24.15**	**27.13**	**25.10**	**25.10**	**25.10**
Geraniol	1258	**4.21**	**3.18**	**4.25**	**4.21**	**4.21**	**4.21**
Geranial	1271	**35.10**	**37.46**	**30.34**	**35.10**	**35.10**	**35.10**
Nonanoic acid	1285	0.40	0.30	0.21	0.40	0.40	0.40
Undecanal	1300	0.29	0.32	0.23	0.29	0.29	--
Dihydro citronellol acetate	1314	0.21	0.31	0.41	0.21	--	0.21
Citronellyl acetate	1351	0.30	0.30	0.36	--	0.30	--
Geranyl acetate	1365	--	0.23	0.20	0.23	0.23	0.23
α-Ylangene	1372	1.90	1.20	1.94	1.90	1.90	1.90
α-Copaene	1376	--	0.17	0.13	0.15	0.15	0.15
β-Caryophyllene	1404	**3.75**	**2.98**	**3.25**	**3.75**	**3.75**	**3.75**
β- Farnesene	1441	0.32	0.37	--	--	--	-
γ -Himachalene	1470	0.41	0.35	0.47	0.41	0.41	0.41
Valencene	1495	0.24	0.26	0.11	0.24	0.24	0.24
cis-Calamenene	1520	0.24	0.19	0.21	0.24	0.24	0.24
Caryophyllene oxide	1578	**7.50**	**6.80**	**7.48**	**7.50**	**7.50**	**7.50**
β-Eudesmol	1580	0.30	0.41	--	0.30	--	0.30

*Retantion index

The essential oil components recorded for this study was similar to the essential oil components from Serbia (Dukic et al., 2004), Slovak (Holla et al., 1997), Egypt (Shalaby El-Gengaihi and Khattab, 1995), France (Carnat et al., 1998), Greece (Basta et al., 2005) and Iran (Sadraei et al., 2003). Essential oils of lemon balm were dominated by neral (29.9 % and 39.3 %) and geranial (41.0 % and 47.3 %) respectively (Pino et al., 1999; Da Silva et al., 2005). A low citronellal content (0.2 %) was reported for lemon balm grown in Cuba (Pino et al., 1999) and zero in Brazil (Da Silva et al., 2005). Higher β-carophyllene content (14.2 %) was reported in the essential oil content of lemon balm gron in Turkey (Allahverdiyev et al., 2004).

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ALLELOPATHIC POTENTIAL OF RAPESEED (BRASSICA NAPUS L.) CULTIVARS ON WEEDS

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ABSTRACT

Rapeseed (*Brassica napus* L., spp. *oleifera*) contains water soluble allelochemicals that can be used effectively and economically to control problem weeds in cultivated areas. Four rapeseed cultivars (Westar, Bristol, ACSN3 and Eureka), three development stages (rosette, flowering and harvesting) and three extract concentrations (2, 4 and 8%) were examined for their allelopathic potential against *Amaranthus retroflexus* and *Solanum nigrum*. Cultivars, development stages and extract concentrations had significant effects on the inhibition of seed germination, and shoot and root growth of the tested weed species. Cultivars Westar and ACSN3 had the highest inhibitory effects on the germination of *A. retroflexus* (42.6%) and *S. nigrum* (41.3%) seeds. The rate of germination and seedling growth inhibitions increased with increasing extract concentrations. The highest inhibition rates were obtained from 8 g 100 mL extract concentration for both weed species. Rapeseed extracts exhibited different levels of allelopathy at rosette, flowering and harvesting stages. Shoot powder extracts of the flowering stage had the highest allelopathy followed by rosette and harvesting stages. Rapeseed cultivars having higher allelopathic potential at the vegetative stage are potentially viable green manures, while cultivars having higher allelopathic potential at harvesting stage may be useful in crop rotation to control problem weeds. However, further studies are needed to determine the amount of residue, the time interval between rapeseed harvest and the planting of the following crop, tillage practices applied after rapeseed harvest and appropriate soil conditions for maximum release of allelochemicals.

Keywords *Allelopathy; Amaranthus retroflexus; Brassica napus; germination; Solanum nigrum.*

INTRODUCTION

Allelopathy, effects of one plant on another by chemicals release or the breakdown products of their metabolites, gained great attention due to increased public and environmental concerns on extensive use of synthetic herbicides to control weeds. Herbicides are increasingly found in groundwater and surface waters due to their extensive use in agriculture, forests, pastures, lakes, parks, root-sides as well as home lawns. In cultivated lands, 3.2 billion tons of pesticides are used and $36 billion are spent to control pests in the World. Herbicides are accounted for 50% of the total pesticides used. Therefore, allelopathic suppression of weeds is receiving greater attention as a possible alternative for weed management. The genetic improvement of crops for allelopathic traits is a strategy for biological weed control in breeding programs (Wu *et al*. 1999). It has been proposed that the allelopathic potential of plants is genetically controlled (Mason-Sedun 1986; Putnam and Tang 1986). Thus, understanding of genetic control of allelopathy can enhance development of allelopathic

cultivars that can be incorporated into crop rotation systems for improved weed control. Accessions having allelopathic potential have been found in many crop species, however, no commercial cultivars carrying allelopathic properties have been developed.

Plants in Brassica species may suppress growth and development of weeds through the release of allelochemicals from plant residues incorporated into the soil (Tollsten and Bergstrom 1988; Bialy *et al*. 1990, Muehlchen *et al*. 1990; Khatib *et al*. 1997; Vaughn and Boydston 1997; Turk and Tawaha 2002). After its harvest, rapeseed (*Brassica napus* L., spp. *oleifera*) offers a long crop growing period for double cropping in Mediterranean type of climates. Another importance of rapeseed in rotation is reduction of initial weed density, so that a following crop's canopy can smother the weeds and excellent weed control can be achieved with only a few applications of herbicides. Rapeseed is an alternate crop for rotation in an agricultural system predominated by small cereal grains. Rapeseed is the third most important oilseed crop worldwide and it is also one of the major sources of edible oil for humans and residual meal for farm animals (Anonymous 2008).

Increasing allelopathic potential in combination with breeding for competitive plant types could result in crop cultivars with superior weed-suppressive ability. Therefore, allelopathic potential would be a valuable trait to breed in cultivars so they can be used for weed control in cropping systems. Allelopathic traits incorporated crop cultivars may reduce the need for weed management, particularly herbicide use. The present study was designed to evaluate the allelopathic potential of rapeseed cultivars (Westar, Bristol, ACSN3 and Eureka), harvested at various growth stages, on the germination and seedling growth of *Amaranthus retroflexus* L. and *Solanum nigrum* L.

MATERIAL AND METHODS

Crop growth

Rapeseed cultivars (Westar, Bristol, ACSN3 and Eureka) were planted in 10 m^2 plots six 5 m rows, planted 0.35 m apart in November 2005 at the experimental farm of Mustafa Kemal University (36º 15' N; 36º 30' E, 60 m altitude). The rapeseed cultivars were harvested for each species at the rosette stage in March 2006, flowering stage April 2006 and harvest stage in May 2006. The soil of the experimental site, developed from alluvial deposits of river terraces, is typical for the Eastern Mediterranean region of Turkey and is classified as Chromoxeret by USDA Soil Taxonomy (1998) and as Vertisol by FAO/UNESCO (1974) having relatively high clay content with the predominant clay minerals smectite and kaolinite. The soil of the experimental plots was a clay silt loam with a pH of 7.6, 1.7% organic matter, 0.13% total nitrogen content, and a water holding capacity of 0.34 cm^3. Based on soil analysis and local recommendations, fertilizer was applied prior to planting at a rate of 25-25-0 kg/ha NPK. Total precipitation during the plant growing period was 519 mm. The maximum and the minimum air temperature was about 27-10 °C at during the cropping period (November 2005-May 2006), while the relative humidity ranged from 50-67%.

Seed collection

The fruits of black nightshade (*Solanum nigrum* L.) were collected from infested areas in the experimental farm of Mustafa Kemal University in October 2005. The fruits were shade dried in the laboratory at ambient temperature (20-25 ºC) for 30 days and then the seeds were hand separated and floated in distilled water to remove thrashes. After rinsed with distilled water, the seeds were dried on

the filter papers at ambient temperature in the laboratory for 7 days. The panicles of redroot pigweed (*Amaranthus retroflexus* L.) were collected from the infested areas and dried at room temperatures for 7 days. Trash was removed from the seeds by floating them in distilled water. To break dormancy, imbibed seeds were stored for 21 days at 4 ºC in the dark (Faccini and Barat 1989).

Extract preparation

Rapeseed cultivars were uprooted at three different development stages (rosette, flowering and harvest maturity) and taken immediately to the laboratory where they were washed thoroughly with tap water and separated into root and shoot. After rinsing with distilled water, shoots were separately chopped into small pieces with clippers then grinded with a batch mill (Ika M 20 Universal mill with M 23 Star-shaped cutter). Grinded fresh shoot samples were separately pressed with a modified hydraulic bottle jack to have shoot extracts. The extracts were filtered through a double layer of muslin cloth and then and centrifuged (1500 g) for 4 h. The supernatant was filtered again using a 0.2 mm filterware unit to give the final shoot extracts. The extracts were divided in to two half. One half was frozen in 100 mL plastic caps at –24 ºC for the future germination test, the other half was diluted to obtain a series of solutions with different concentrations (2, 4 and 8%).

Germination bioassay

A hundred redroot pigweed and black nightshade seeds were surface sterilized with water : bleach solution (10 : 1) and placed evenly on filter paper in sterilized 90 mm Petri dishes. Ten mililiters of different concentrations of aqueous extracts (2, 4 and 8%) and distilled water was used as a control. All Petri dishes were placed in a growth chamber at 28/32 ºC for 12/12h and dark/light period for 16/8h. Treatments were arranged in a completely randomized design with four replications. An equal volume of distilled water was added to the petri dishes when moisture content of the filter paper declined. The number of germinated seeds were counted at 3, 5, 7, 14, 21 and 28 days after incubation.

Percent germination inhibition was calculated as [(CG-TG)/CG] x 100.

Where growth inhibition in %; CG, germination rate in check treatment; TG, germination rate in extract treatment. Analysis of variance was performed for all data using a general linear model procedure (SAS Inst. 1997). Data from two experiments were pooled and mean values were separated on the basis of least significant difference (LSD) at the 0.05 probability level

Growth bioassay

Seeds of *A. retroflexus* and *S. nigrum* were germinated on filter paper in growth chamber. Fifteen 2 cm long seedlings of each test weed were planted in a 90 mm Petri dishes filled with sterilized quartz sand. Ten mililiters of different concentrations of aqueous extracts (2, 4 and 8) or distilled water in the case of the control, were added. Experiments were arranged in a completely randomized design with four replications. Petri dishes were, then, incubated in an illuminated growth chamber at 30 ºC. The shoot and root length of seedlings were measured 5 days after treatment.

Percent Growth inhibition was calculated as [(LC-LT)/LC] x 100.

Where growth inhibition in %; LT, shoot or root length of treated weed seedling; LC, shoot and root length of untreated check weed seedling.

All experiments were conducted twice in a completely randomized design with four replications. Analysis of variance was performed on all data using a general linear model procedure (SAS Inst. 1997). Data from two experiments were pooled and mean values were separated on the basis of least significant difference (LSD) at the 0.05 probability level.

RESULTS

Analysis of variance showed significant differences among growth stages, application doses and cultivars for germination, seedling and root growth inhibitions of *A. retroflexus* and *S. nigrum* (Table 1). Development stage x dose interaction was significant for seedling growth of *A. retroflexus* and root growth of *S. nigrum*. Development stage x cultivar interaction was significant for all of the investigated plant parameters of *A. retroflexus* and *S. nigrum*, except for germination inhibition of *A. retroflexus*. Growth stage x dose x cultivar interaction was significant for seedling and root growth of *A. retroflexus* and *S. nigrum*, but not for seed germination of both weed species.

Table 1. Effects of rapeseed cultivars, growth stages and extract concentrations on germination, seedling and root growth of *A. retroflexus* and *S. nigrum*. Table 1. Effects of rapeseed cultivars, growth stages and extract concentrations on germination, seedling and root growth of *A. retroflexus* and *S. nigrum*.

	Germination (% of the control)		Seedling growth (% of the control)		Root growth (% of the control)	
	A. retroflexus	*S.nigrum*	*A. retroflexus*	*S. nigrum*	*A. retroflexus*	*S. nigrum*
Cultivar (C)						
Westar	42.6	35.1	25.1	20.5	53.8	40.3
Bristol	42.5	28.6	27.3	14.2	59.9	44.6
Acsn3	42.2	41.3	21.4	13.9	54.0	33.5
Eureka	34.6	36.1	25.5	20.7	57.6	41.8
LSD 0.05	5.35	4.23	1.82	1.42	2.47	2.93
Growth stage (GS)						
Rosette	41.1	33.6	26.0	17.9	56.9	38.6
Flowering	45.5	42.7	31.2	22.2	60.9	49.0
Harvest	34.8	29.6	17.2	11.8	51.2	32.6
LSD 0.05	4.66	3.66	1.58	1.23	2.14	2.53
Dose (D) g/100 ml						
2	24.6	17.3	17.9	9.1	46.9	31.9

4		40.6	32.0	23.1	15.4	56.3	37.8
8		56.2	56.5	33.4	27.6	65.8	50.4
LSD 0.05		4.63	3.66	1.58	1.23	2.14	2.59
Source of variation	d.f.						
Replication (R)	7	NS	NS	NS	NS	NS	NS
GS	2	***	***	***	***	***	***
Error 1	14	-	-	-	-	-	-
D	2	***	***	***	***	***	***
GS X D	4	NS	NS	NS	NS	NS	***
Error 2	42	-	-	-	-	-	-
C	3	**	***	***	***	***	***
GS X C	6	NS	***	***	***	***	***
D X C	6	NS	**	***	***	***	***
GS X D X C	12	NS	NS	***	***	***	***

*, **, and *** Significant at the 0.05, 0.01 and 0.001 levels of probability respectively; NS is not significant.

Water soluble extracts from Westar, Bristol, ACSN3 and Eureka showed inhibitory effects on seed germination, seedling and root growth of *A. retroflexus* and *S. nigrum* (Table 1). Inhibitory effects of extracts on germination, seedling growth and root growth of weed species varied with the rapeseed cultivar. The germination inhibition of cultivars on *A. retroflexus* and *S. nigrum* varied between 34.6 and 42.6% and between 28.6 and 41.3%, respectively. Cultivar Westar had the highest inhibitory effects on the germination of *A. retroflexus* seeds, whereas cultivar Eureka had the lowest. The inhibitory effects of Westar, Bristol and ACSN3 water soluble extracts on germination of *A. retroflexus* were similar and classified as highly allelopathic cultivars. With *S. nigrum* germination, rapeseed cultivars exerted different inhibitory effects. Bristol had the lowest (28.6%) and ACSN3 had the highest (41.3%) inhibitory effects on the germination of *S. nigrum* seeds. Among the cultivars, ACSN3 had the most consistent, while Bristol had the most inconsistent inhibitory effect on the germination of both weed species. ACSN3 and Eureka had similar inhibitory effects on the germination of both *A. retroflexus* and *S. nigrum* seeds.

Shoot and root growth of *S. nigrum* were less sensitive to water soluble extracts of rapeseed cultivars than those of *A. retroflexus*. Shoot growth inhibition of *A. retroflexus* varied between 21.4 and 27.3%, the highest and the lowest inhibition rates obtained from ACSN3 and Bristol, respectively. However, shoot growth of *S. nigrum* was differently affected by rapeseed cultivars. Cultivar Eureaka had the highest inhibition rate on the shoot growth of *S. nigrum*, while Bristol had the lowest.

Growth stages of the rapeseed had significant effect on germination, seedling and root growth of both weed species (Table 1). The highest inhibition rates for mentioned plant parameters of *A. retroflexus* and *S. nigrum* were obtained from the plant samples taken at flowering stage and the lowest were obtained from the samples taken at harvest. Based on *A. retroflexus* and *S. nigrum* germination and seedling growth, averaged across all extract concentrations, the degree of toxicity of different growth stages can be ranked in the following order of inhibition: flowering stage > rosette stage > harvest stage.

The germination of *A. retroflexus* and *S. nigrum* was influenced differently by various concentrations of extracts. To determine the most effective extract concentration on the germination of *A. retroflexus* and *S. nigrum* seeds, three different concentrations 2, 4, and 8% of shoot extract were applied. The degree of inhibition increased with the increasing extract concentration (Table 1). The highest germination inhibition rates were obtained from the 8% extract concentration with 56.2 and 56.5% for *A. retroflexus* and *S. nigrum*, respectively. Seedlings and roots showed similar patterns of inhibition. The highest and the lowest inhibition rates for both weed species were obtained from 2 and 8% extract concentrations, respectively.

Growth stage x cultivar interaction on seed germination was significant for only *S. nigrum* (Table 1). The significant interaction resulted from cultivar ACSN3 and Eureka (Fig. 1). Since ACSN3 had the highest germination inhibition rate at rosette and harvest stages while it had not highest inhibition rate at flowering stage. At the flowering stage, however, Eureka had the highest inhibition rate while it had the lowest inhibition rate at harvest.

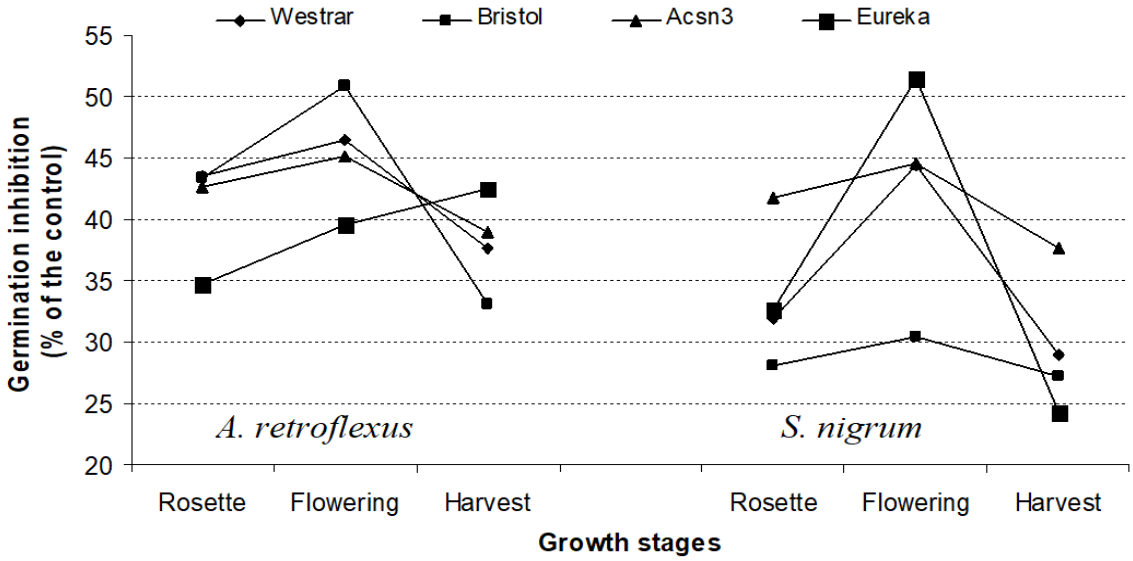

Figure 1. Growth stage x cultivar interaction on germination inhibition of *A. retroflexus* and *S. nigrum*.

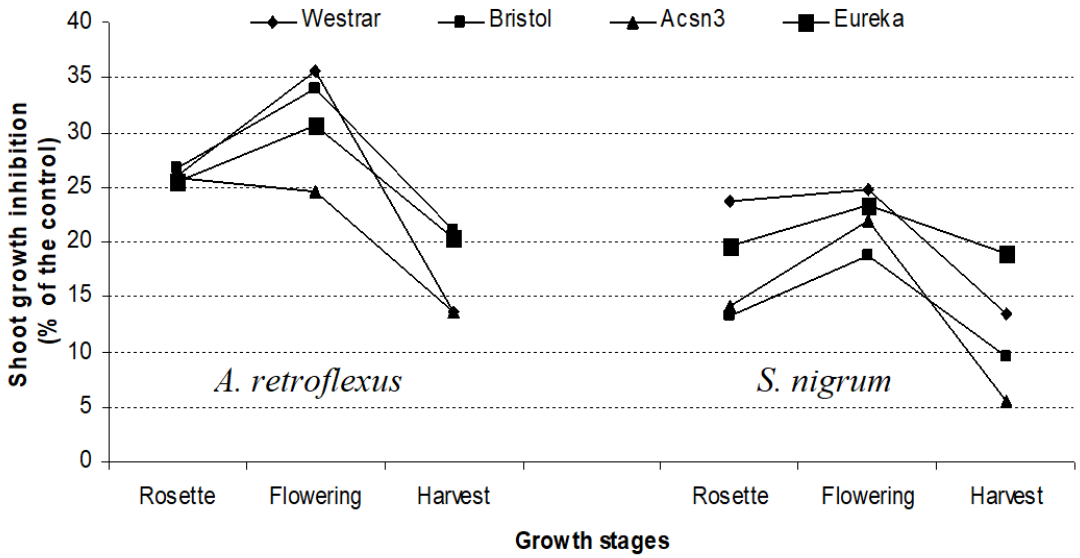

Figure 2. Growth stage x cultivar interaction on shoot growth inhibition of *A. retroflexus* and *S. nigrum*.

Growth stage x cultivar interaction on shoot and root growths of *A. retroflexus* and *S. nigrum* were significant (Table 1). Allelopathic potential of rapeseed cultivars on shoot growth of *A. retroflexus* varied with growth stages. Bristol had the highest germination inhibition rate at rosette and harvest stages (Fig. 2). At the flowering stage, Westar had the highest inhibition rate on shoot growth, but it had the lowest inhibition rate at harvest. With *S. nigrum*, rapeseed cultivars exhibited different amounts of allelopathy at rosette, flowering and harvest stages. Cultivar Westar had the highest inhibition rate on shoot growth of *S. nigrum* at rosette and flowering. At the harvest stage, however, cultivar Eureka had the highest inhibition rate on shoot growth of *S. nigrum*.

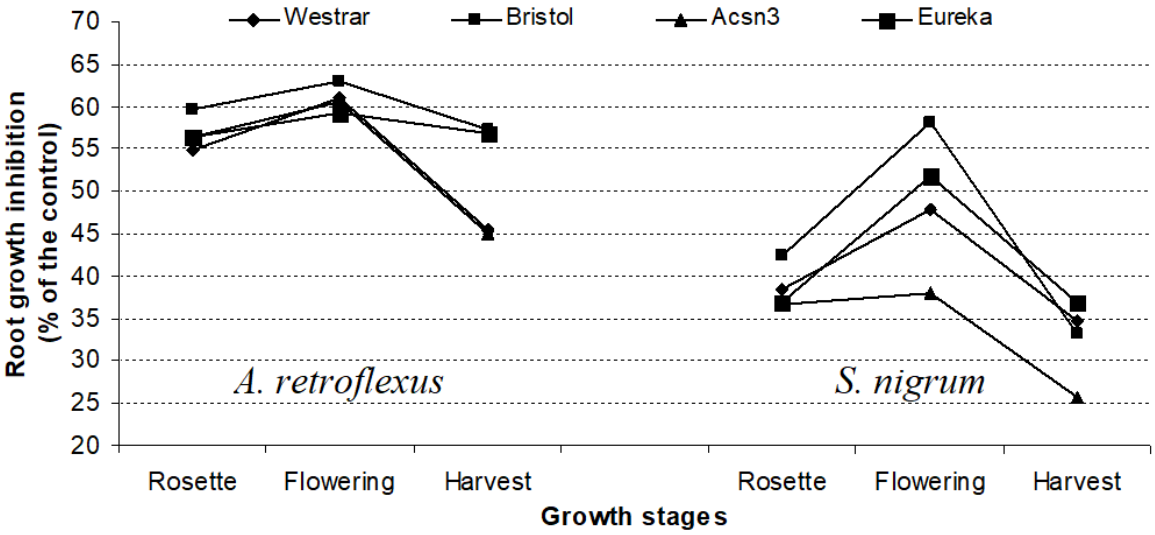

Figure 3. Growth stage x cultivar interaction on root growth inhibition of *A. retroflexus* and *S. nigrum*.

Shoot extracts of Bristol at rosette, flowering and harvest stages had the highest inhibition rate on root growth of *A. retroflexus* (Fig. 3). In the case of *S. nigrum*, however, shoot extracts of Bristol at harvest stage did not have the highest root inhibition. Of the cultivars, Eureka had the highest inhibition rate on root inhibition rate at harvest.

DISCUSSION

Germination, shoot and root growth inhibitions of *A. retroflexus* and *S. nigrum* by water soluble extracts of rapeseed reflect the allelopathic potential of individual rapeseed cultivars at rosette, flowering and harvesting stages. The degree of allelopathic suppression varied among the tested rapeseed cultivars and development stages. The allelopathic potential of rapeseed cultivars are attributed to glucosinolates that have been reported to have allelopathic activity after hydrolysis by the enzyme myrosinase and volatiles produced from glucosinolate hydrolysis products (Jimenez-Osornio and Gliessman, 1987; Brown and Morra, 1995, 1996; Eberlein *et al*. 1998; Gardiner *et al*. 1999). Plant glucosinolate content depends on genetic, environmental and husbandry factors (Fenwick et al. 1983; Milford and Evans 1991; Morra and Kirkegaard 2002). The inhibitory allelopathic impact of water soluble extracts of rapeseed at the flowering stage was more powerful than that of the other growth stages (Schumacher *et al*. 1983; Reinhardt and Bezuidenhout 2001; Zuo and Ma, 2007). Water soluble extracts of allelopathic plants generally have more pronounced effects at the flowering stage. Therefore, germination and seedling growth of small-seeded weed species are best suppressed by selecting cultivars having higher level of allelopathy at the vegetative and early generative stages. However, most growers are unwilling to apply this practice due to its cost and lack of its direct contribution to the farm budget. However, rapeseed cultivars performing higher levels of allelopathicity at harvest are more suitable for weed management in crop rotations and use of their residues has no additional cost to growers. Breeding of rapeseed cultivars that do not lose most of their allelopathic potentials at harvest may be possible by developing biotechnology techniques to incorporate the gene(s) responsible for synthesizing allelochecimals into the rapeseed. Cultivar Westar may provide important genes for breeding highly allelopathic cultivars. It has been proposed that the allelopathic potential of plants is genetically controlled (Mason-Sedun 1986; Putnam and Tang 1986). Thus, understanding the genetic control of allelopathy can enhance development of allelopathic cultivars that can be incorporated into crop rotation systems for improved weed control. An increase in the allelopathic potential of cultivars will likely have a great impact on weed control in agro-ecosystems, even though the breeding approach alone cannot overcome all weed problems.

Amount of rapeseed residues, the amount of water soluble compounds in the crop residues, and the volatile compounds produced from glucosinolates are the important factors that affect weed seed germination. The current study indicated that the degree of germination inhibition of weed seeds increased with the increasing extract concentration. The results are in agreement with the previous investigations in that the activity of extracts was directly related to the concentration of extract rates (Babu and Kandasamy 1997; Hu and Jones 1997; Kirkegaard and Sarwar 1998; Arslan *et al*. 2005; Uremis *et al*. 2005).

In addition to residue amount, applied tillage practice influences allelopathic potential of rapeseed on weed species. The highest allelopathic effects can be seen with double cropping due to

increased allelopathic substances concentration in the top layer of the soil which contains most of the annual weed seeds.

Another aspect to be considered is the time interval between rapeseed harvest and planting of the following crop. If the time interval is long, the allelopathic potential of the rapeseed residue is lost due to decomposition and leaching hence, no allelopathic effects on weeds will be seen. This situation is more pronounced in the cooler regions where a long fallow period takes place after rapeseed harvest. Rapeseed residues suppresses certain weed species especially small-seeded weed species (Khatib *et al*. 1997), while they had no effect on large seeded crop species such as corn, soybean and cotton (Uremis *et al*. 2009). Hence, rapeseed is one of the best potential allelopathic crops in crop rotations to control weeds in the regions where double cropping is possible after rapeseed harvest.

CONCLUSIONS

Rapeseed extracts had significant herbicidal effects on the germination and seedling growth of *A. retroflexus* and *S. nigrum*. Rapeseed cultivars exhibited different levels of allelopathicity on the tested weed species. Germination and seedling growth inhibitions increased with the increasing extract concentration. Cultivar Westar had the highest germination inhibition rate on *A. retroflexus,* whereas cultivar ACSN3 had the highest inhibitory effect on *S. nigrum*. Averaged across all extract concentrations, allelopathic potential of cultivars at rosette, flowering and harvest stages were highly different. The highest inhibitory effects on germination and seedling growth of the tested weed species were obtained from the shoot extracts of the plant at the flowering stage while the lowest was obtained from the shoot extracts of the plant at the harvest. Rapeseed cultivar demonstrating higher levels of allelopathy before the harvest stage can be used as green manure crops. On the other hand, cultivars having higher level of allelopathy in their harvest residues can be used in crop rotation to manage harmful weeds. However, further studies are needed to determine the amount of residue, the time interval between rapeseed harvest and the planting of the following crop, type of tillage applied after rapeseed harvest and proper soil conditions for maximum release of allelochemicals.

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DIURNAL VARIATION OF ESSENTIAL OIL CONTENT AND COMPOSITION OF THYME (THYMRA SPICATA)

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ABSTRACT

Diurnal variation in fresh and dry herbage for preparing essential oil of thyme (*Thymra spicata*) were studied. Plants were harvested hourly starting at 6 a.m. and ending at 5 p.m. Essential oils of fresh and dry plant was extracted by steam distillation and the essential oil composition was determined with gas chromatography - mass spectrometry. Essential oil content slightly varied among harvesting hours. The highest essential oil content was obtained from 6 a.m. harvest, the lowest was obtained from 2 p.m. harvest. Twenty eight essential oil components were determined. Thymol, carvacrol, a-terpinen and cymol were the major essential oil components in both fresh and dry plant. Small variation in essential oil components were detected among harvest hours. The results indicated that the cooler hours of the day was the best harvesting time for essential oil yield with the highest active ingredients.

Key words: diurnal variation, essential oil, thyme, *Thymra spicata,*

INTRODUCTION

Thyme (*Thymus vulgaris*), is an evergreen sub-shrub native to the southern Europe and the Mediterranean, is a perennial medicinal and aromatic plant belonging to the Lamiaceae family (Davis, 1982, Morales, 2002; Zarzuelo and Crespo, 2002). Tyme essential oil of is among the top 10 essential oils (Letchamo and Gosselin, 1996; Zarzuelo and Crespo, 2002). Tyme essential oil can be used as antioxidant and also as antimicrobial. The major constituents of commercial thymol are p-cymene, -terpinene and carvacrol (Arslan and Derviş, 2011 Hudaib et al. 2002, Tian and Lai, 2006). Tyme has multiple biological activities including anti-inflammatory [Braga et al, 2006], immunomodulatory (Amirghofran et al.,2012), antispasmodic (Babaei et al. 2008), antioxidant (Aeschbach et al. 1994), antibacterial (Didry, 1994), antifungal (Arslan and Derviş, 2011), antiviral (Bukovska, 2007), antihelminthic (Rasooli et al.., 2006), carminative (Dapkevicius et al. 2002), and free radical scavenging properties (Fujisawa and Y. Kadoma). Thymol is the main phenolic components that is mainly responsible for antioxidative activity [5]. The leaves of thyme and its essential oil are used in foods for the flavour, aroma and preservation. Currently, thyme has changed from a traditional herb to a serious drug in rational phytotherapy due to its broad biological activities.

Although biosynthesis essential oil components is controlled genetically, it is strongly affected by temperature, humidity, wind velocity, light intensity and photoperiod. Seasonal variation of thyme essential oil content and composition has been widely studied. However, there are a few study on diurnal fluctuation of thyme essential oil. The purpose the current study was determine diurnal essential oil fluctuation of thyme.

MATERIALS AND METHODS

Thyme plants, grown in the research area of Erciyes University, Kayseri were harvested at two-hour intervals starting from 6:00 to 16:00 in July, 10 and 15 2016. Harvested plant samples were divided in two groups. Group one was freshly studied immediately after harvest and group two was dried in the shade at 26 °C for one week to determine diurnal essential oil content and component variations of fresh and dry herbage of thyme. In each group, approximately 100 g fresh material was sampled for analysis.

The fresh and dried samples were subjected to steam distillation for 3 h using a Clevenger type apparatus. Essential oils obtained were dried over anhydrous sodium sulphate and stored at –20ºC until gas chromatography-mass spectrometry (GC-MS) analysis. The oil percentage was expressed as w/v with respect to dry matter of the initial material.

GC-MS analysis

Analysis of the essential oil carried out by using Thermo Scientific Focus Gas Choromatograph equipped with MS, auto sampler and TR-5MS (5% Phenyl Polysilphenylene-siloxane, 0.25 mm x 60 m i.d, film thickness 0.25).The carrier gas was helium (99.9%) at a flow rate of 1 mL/min; ionization energy was 70 eV. Mass range m/z 50-650 amu. Data acquisition was scan mode. MS transfer line temperature was 250 ºC, MS Ionization source temperature was 220 ºC, the injection port temperature was 220 ºC. The samples were injected with 250 split ratio. The injection volume was 1µl. Oven temperature was programmed to from 50 ºC to 220 ºC at 3 ºC /min. The structure of each compound was identified by comparison of their mass spectrum (Wiley) a data was handled through using of Xcalibur software program. The retention indices (RIs) were calculated for all volatile constituents using a homologous series of *n*-alkane standard solutions C8-C20 (Fluka, product no. 04070) and C21-C40 (Fluka, product no. 04071).

Statistical Analysis

All experiments were conducted in a completely randomized design with three replicates. The statistical analysis of experimental data was determined using a general linear model procedure of SAS for Windows (Version 8.02, SAS Institute, Cary, NC, USA), applying the one way analysis of variance (ANOVA). Differences between means were tested through LSD and values of $p < 0.05$ were considered significantly different.

RESULTS AND DISCUSSION

There were no significant differences among harvesting hours in terms of essential oils content (Table 1). Essential oil content of fresh thyme harvested hourly, varied between 0.75 and 1.20 %. The lowest fresh plant essential oil content was obtained from 14:00 harvest with 0.72 % while the highest was obtained from 18:00 harvests with 1.25 %. When dry plant essential oil contents were considered essential oil contents did not significantly vary among harvesting hours. The highest dry plant essential oil content was obtained from 10:00 harvest with 2.95 %, while the lowest was obtained from 14:00 harvest with 2.45 % fallowed by 16:00 harvesting hours. In the current study, essential oil contents were similar to the essential oil contents reported by Rumińska (1983) Letchamo et al. (1995), Shalaby and Razin (1992), Jackson and Hay (2002), Asllani and Toska (2003), Hudaib et al. (2002), and Badi et al. (2004).

Table 1. Diurnal essential oil content variations of fresh and dry thyme.

Harvesting hour	Temperature (°C)	Humidity (%)	Essential oil content (%)	
			Fresh herbage	Dry herbage
6:00 AM	23	46	1.23	2.80
8:00 AM	26	44	1.15	2.88
10:00 AM	27	33	1.18	2.95
12:00PM	33	42	0.90	2.48
14:00 PM	31	46	0.72	2.45
16:00 PM	27	50	0.85	2.58
18:00 PM	25	54	1.25	2.76
LSD 0.05			NS	NS

NS: Non significant

Both fresh and dry herbage essential oil content of thyme was affected from harvesting hours (Table 1). The lowest fresh and dry essential oil contents were obtained when the harvest was done at the hottest hours of the day (Table 1). Ramezani et al. (2009) reported that essential oil content of coriander (*Coriandrum sativum*) diurnally varied.

Table 2. Diurnal essential oil component variations of fresh thyme.

Essential oil Compounds	RI	Fresh Plant Essential oil content (%)						
		6:00	8:00	10:00	12:00	14:00	16:00	18:00
α-Pinene	1027	0.85	0.74	0.90	0.62	0.65	0.68	0.69
α-Thujene	1031	1.17	0.96	1.34	0.92	0.88	0.97	0.77
Camphene	1072	0.68	0.65	0.67	0.42	0.51	0.46	0.78
β-Pinene	1111	0.25	0.23	0.25	0.20	0.18	0.22	0.19
Sabinene	1126	0.09	0.09	0.12	0.08	0.14	0.50	0.05
β-Myrcene	1169	1.43	1.31	1.52	1.06	1.08	1.22	1.09
δ-4-Carene	1183	1.05	1.06	1.20	0.86	0.91	1.20	0.93
Bornylene	1200	0.41	0.41	0.41	0.29	0.32	0.37	0.32
Eucalyptol	1208	0.72	0.78	0.83	0.90	0.57	0.72	0.63
β-Phellandrene	1211	0.07	0.11	0.14	0.67	0.11	0.16	0.11
γ-Terpinene	1250	10.51	11.17	11.72	7.91	7.38	8.85	8.71
p-Cymene	1275	21.80	21.20	21.37	15.19	15.37	14.70	14.74
1 Octen 3 ol	1453	0.51	0.45	0.45	0.42	0.42	0.44	0.42
Cis-Sabinene hydrate	1464	1.04	0.94	1.03	0.87	0.88	0.86	0.80
Camphor	1508	0.42	0.36	0.44	0.40	0.34	0.39	0.71
trans-Sabinene hydrate	1546	0.31	0.31	0.31	0.89	1.30	1.79	0.61
Linalool	1550	2.12	1.92	1.70	1.30	1.44	1.12	1.19
Caryophyllene	1588	1.32	1.55	1.40	1.98	2.41	4.04	2.47
Thymyl methyl ether	1594	0.77	0.63	0.20	0.25	0.22	0.25	0.62
Terpinene 4-ol	1597	0.50	0.68	0.54	1.44	1.81	1.75	1.06
Carvacrol methyl ether	1604	0.15	0.23	0.21	0.18	0.17	0.25	0.36
Borneol	1694	1.51	1.79	1.42	1.00	1.50	1.36	1.96
Caryophyllene oxide	1966	0.74	0.76	0.62	0.58	0.33	0.50	0.60
Thymol	2216	44.95	44.12	43.72	41.02	34.21	30.42	27.51
Carvacrol	2237	3.52	4.08	4.84	8.58	17.08	22.80	29.49

The relative amounts of each component were determined by the GC-MS analysis (Table 2 and 3). More than 25 components, representing about 95.8% of the oil, were identified in both fresh and dry plant essential oil, but most of them constituted less than 1 %.

Table 3. Diurnal essential oil component variations of dry thyme.

Essential oil Compounts	RI	Fresh Plant Essential oil content (%)						
		6:00	8:00	10:00	12:00	14:00	16:00	18:00
α-Pinene	1027	0.54	0.70	0.14	0.46	0.65	0.67	0.59
α-Thujene	1031	0.97	0.93	0.18	0.71	0.96	1.18	1.66
Camphene	1072	0.34	0.56	0.05	0.27	0.41	0.44	0.52
β-Myrcene	1169	1.28	1.23	0.36	0.94	1.15	1.31	1.03
δ-4-Carene	1183	1.77	1.04	0.41	1.12	1.26	1.92	0.94
Bornylene	1200	0.44	0.36	0.11	0.30	0.38	0.44	0.29
Eucalyptol	1208	0.43	0.80	0.28	0.40	0.68	0.47	0.56
β-Phellandrene	1211	0.25	0.11	0.00	0.16	0.16	0.25	0.11
γ-Terpinene	1250	10.41	8.38	12.51	7.33	9.61	10.50	11.25
p-Cymene	1275	18.27	20.14	14.79	13.80	17.71	13.67	14.22
α-Terpinolene	1285	0.32	0.07	0.06	0.15	0.00	0.29	0.05
1 Octen 3 ol	1453	0.40	0.51	0.21	0.37	0.47	0.43	0.30
Cis-Sabinene hydrate	1464	0.96	0.89	0.51	0.85	0.96	0.97	0.57
Camphor	1508	0.22	0.25	0.05	0.25	0.25	0.28	0.50
trans-Sabinene hydrate	1546	1.67	0.60	0.88	1.95	1.04	1.97	1.53
Linalool	1550	1.42	1.65	0.46	1.24	1.46	1.57	1.12
Linalyl acetate	1560	0.31	0.12	0.22	0.61	0.32	0.33	0.11
Endobornyl acetate	1575	0.20	0.19	0.13	0.18	0.15	0.18	0.16
Caryophyllene	1588	1.99	1.60	2.69	3.36	2.50	2.78	2.72
Thymyl methyl ether	1594	0.56	0.95	0.00	0.18	0.14	0.07	0.33
Terpinene 4-ol	1597	2.89	0.89	1.35	2.81	2.16	2.94	2.99
Carvacrol methyl ether	1604	0.31	0.39	0.11	0.20	0.08	0.47	0.14
Borneol	1694	0.68	1.51	1.06	1.31	1.23	1.60	1.30
Farnesol	1722	0.31	0.16	0.32	1.83	0.13	0.43	0.95
Thymol	2216	30.56	37.01	39.97	32.29	30.03	31.02	33.61
Carvacrol	2237	15.38	13.68	14.69	22.21	20.84	19.34	15.29

Only 5 or 6 components detected in the fresh herbage essential oil of thyme in concentrations greater than 2 % were γ-terpinene, p-cymene, linalool, thymol and carvacrol. More than 15 components were present in amounts less than 1%. (Table 2 and 3). Among the essential oil components, thymol was the major component (>30%) while p-cymene was the second major component (>9%) of the essential oil of thyme harvested hourly between 6:00 and 17.00. Carvacrole was the third major component (>3%). Among major essential oil component, γ-terpinen and p-cymene were not greatly varied among harvesting hours.

Essential oil compositions in the fresh and dry herbage of thyme were within the limits reported by Kluczyńska, (2001) and Lis (2003). Seasonal essential oil component variations of thyme were well documented (McGimpsey et al., 2006, Jordan et al. 2006). At the flowering stage, carovacrol and thymol were higher in summer. However, Nejad-Ebrahimi et al. (2008) obtained the highest carvacrol content when the harvest was done at the vegetative stage.The percentage of major essential oil components of thyme flactuated hourly.

CONCLUSIONS

Thyme *is* cultivated in small areas in Turkey for multiple purposes. Diurnal flactuation of thyme essential oil content and composition in the central Anatolia has not been studied. This study was conducted to determine diurnal essential oil content and composition fluctuations.

Greater essential oil content in fresh and dry plant were obtained in the early morning and late afternoon harvest hours than the noon harvest hours. The main essential oil compounds of the thyme essential oils in the fresh and dry plants were thymol, γ-terpinene, p-cymene and carvacrol. This study showed that the best harvesting hours of thyme for essential oil were between 6:00 and 10:00.

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SOIL PHOSPHATASE ACTIVITY IN DIFFERENT BUFFER PH AND STOCK CULTURE

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ABSTRACT

The aim of this study is to determine the phosphatase enzyme activities in different buffer pH, sterile and non-sterile soils and to compare phosphatase enzyme activities in different stock culture methods (bacteria and Freeze dry culture). Wooster and New Mexico soils, varying in organic matter content and pH were selected for the study. For culture preparation, added 4.5 ml extraction solution to 0.5 g soil, shaken 30 minute, waited 1h. 0.5 ml top solution was taken, and put in 1 lt LB Medium. Solutions were shaken at 37 ^{o}C until turbidity reached A600 of 0.8.

30 ml **bacteria cells culture** or 10 mg **freeze dry cell culture ()** were harvested with centrifugation and added 4 ml MUB Buffer pH=6 (AcdP: acid phosphatase) or pH=11 (AlkP: alkaline phosphatase), 0.2 ml Toluene, 1 ml, 0.025 M p-nitrophenol phosphate solution. Samples were incubated 1 h at 37 ^{o}C, and then added 1 ml 0.5 M $CaCl_2$ and 4 ml 0.5 M NaOH, read 405 nm wave length with spectrophotometer and calculated p-nitrophenol (PNP) content from calibration curve standards. This study focused on the changes of phosphatase enzyme activity of **sterile (with toluene)** and **non-sterile (without toluene)** soil at different buffer pH application rates of MUB (pH 4, 5, 6, 7, 8, 9, 10, 11 and 12) and at different stock culture (bacteria culture and freeze dry culture) methods.

According to the results; AcdP enzyme activity increased with increasing buffer pH up to pH 6 in sterile and non-sterile acidic Wooster (pH:6.45), but decreased over buffer pH 6. AlkP enzyme activity increased with increasing buffer pH up to pH 11 in sterile and non-sterile alkaline New Mexico soils (pH:7.80), but decreased over buffer pH 11.

The investigation show that soil AcdP and AlkP enzyme activities have been affected with sterile and non-sterile soil condition and these enzyme activity values were in parallel with different stock culture isolation methods.

Keywords **:** Alkaline phosphatase, bacteria cells culture, freeze dry cell culture.

INTRODUCTION

Soil enzymes are sensitive indicators of various specific and general soil processes and functions. The activity of soil enzymes often correlates well with nutrient mineralization and cycling, decomposition and formation of soil organic matter, and the decomposition of xenobiotics (e.g., pesticides) (Chen et al., 2003; Nannipieri et al., 2003). They can also integrate information concerning overall microbial activity (Frankenberger and Dick, 1983; Trasar-Cepeda et al., 2000; Aon and Colaneri, 2001; Baum et al., 2003). Enzyme activities in soil are dependent on the number of microorganisms present, the presence of substrate, the presence or absence of inhibitors and the environmental conditions of the soil (Tabatabai, 1994).

Phosphatase activity is important because phosphate is often the most limiting nutrient in both natural and agricultural soil systems. Once the phosphate is cleaved away from the organic moiety, it becomes more available for plant and microbial uptake (Tabatabai and Dick, 2002).

Phosphatase enzymes can serve as indicators of the organic phosphorus mineralization potential and biological activity of soils (Dick and Tabatabai, 1992). Their activity is related to soil and vegetation conditions (Herbien and Neal, 1990), and responds to changes in management (Adams, 1992; Clarholm, 1993) and seasonal changes in soil temperature and moisture (Dormaar et al., 1984; Speir and Cowling, 1991). The phosphatases are significantly affected by soil pH and acid phosphatase activity is found predominantly in acid soils and alkaline phosphatase activity in neutral or alkaline soils (Eivazi and Tabatabai, 1977; Dick and Tabatabai, 1984). The ratio of alkaline phosphatase to acid phosphatase has been proposed as a way to assess the optimum pH of a soil for crop production (Dick et al, 2000).

Alkaline phosphatase activity is thought to be primarily derived from microbial sources and not from higher plant material (Tabatabai, 1994). Like phosphatases, in general, its activity is affected by soil properties such as pH, texture, and organic matter content (Haanstra and Doelman, 1991; Speir et al., 1999; Moreno et al., 2001).
The effect of MUB buffer pH on soil alkaline phosphatase is attributed to its presence in the active site of the enzyme and its overall ability to effect the composition of soil microbial communities (Chen et al, 2000; Kieliszewska, 2001; Nannipieri et all., 2003).

The specific objectives were; (1) to determine the rate effect of different MUB tampon pH on alkaline phosphatase activity in sterile and non-sterile soils and (2) to determine the rate effect of different culture method on alkaline phosphatase activity.

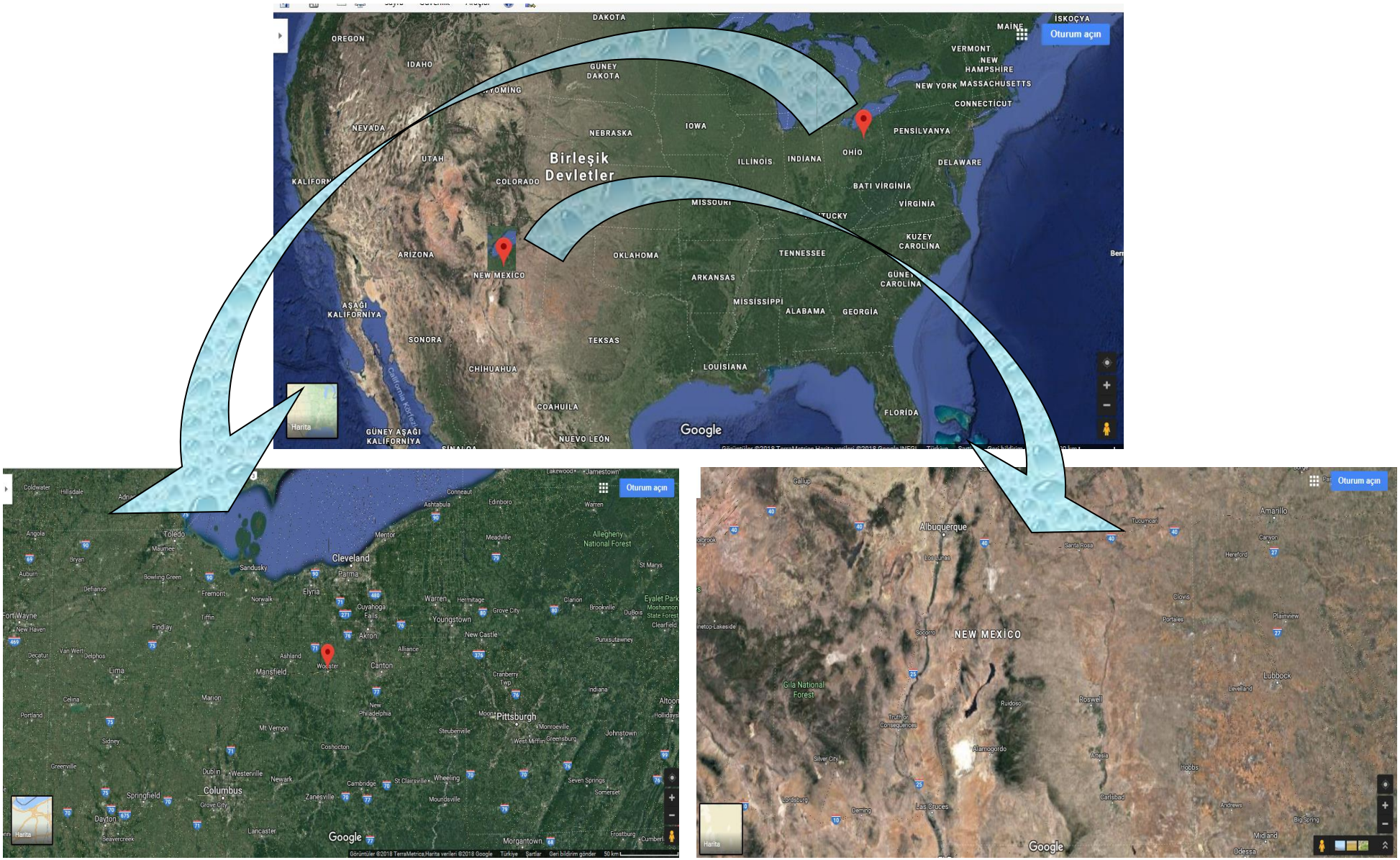
MATERIALS AND METHODS

Soil samples

Four Ap-horizon soils obtained from various states in USA were used in this research. Wooster soil was from Ohio State and New Mexico soil from New Mexico State areas (Figure 1). The soil samples

were dried air indoors, crushed and sieved 2-mm and stored at 4 ºC for phosphatase enzyme activity measurements. Selected properties of the two soils are provided in Table 1.

Total organic matter (SOM) content was determined by following the standard loss-on-ignition (LOI) method (Nelson and Sommers, 1996), total N content of the soil was measured by the Dumas method (Anonymous, 1989), soil pH by using a glass electrode pH meter (1:2.5, soil:water), cation exchange capacity by the method of Sumner and Miller (1996), and exchangeable cations were determined as prescribed by Warncke and Brown (1998).The available P in soil was determined using the Bray PI extraction procedure (Kuo, 1996), water holding capacity by the tensiometer method (Topp et al., 1993), soil particle size distribution was determined by the Bouyoucus hydrometer method (Gee and Bauder, 1986), and electrical conductivity (EC) by using an EC meter according to the method of Rhoades (1996).


Birleşik Devletler
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Culture Preperation

Add 4.5 ml Extraction Solution to 0.5 g soil. 30 minute shaken, 1 hours wait. Taken 0.5 ml top solution, and put in 1 lt LB Medium. At 37 ºC shaken and untill turbidity reached A600 of 0.8.

30 ml **bacteria cells culture** or 10 mg **freeze dry cell culture** were harvested with centrifugation and added 4 ml MUB Buffer pH=6 (AcdP: acid phosphatase) or pH=11 (AlkP: alkaline phosphatase), 0.2 ml Toluene, 1 ml, 0.025 M p-nitrophenol phosphate solution. Samples were incubated 1 h at 37 ºC, and then added 1 ml 0.5 M $CaCl_2$ and 4 ml 0.5 M NaOH, read 405 nm wave length with spectrophotometer and calculated PNP content from calibration curve standards. This study focused on the changes of phosphatase enzyme activity of **sterile (with toluene)** and **non-sterile (without toluene)** soil at different buffer pH application rates of MUB (pH 4, 5, 6, 7, 8, 9, 10, 11 and 12) and at different stock culture (**bacteria culture** and **freeze dry culture**) methods (Figure 2, 3).

0.5 g soil (Natural Soil)
Add 4.5 ml Extraction Solution,
25 ml Minimal salt,
30 minute shaken, 1 hours wait
Take 0.5 ml top solution, and
put solution into the 1 lt LB Medium
Added 30 µg ml-1 cycloheximide
At 37 ºC shaken and untill turbidity reached A600 of 0.8
Take 30 ml Cells culture
Harvested with Centrifugation, Remove Supernatant

Sterile Soil	**Non-Sterile Soil**
Phosphotase enzyme activity	**Phosphotase enzyme activity**
	0.2 ml Toluene
4 ml MUB Buffer pH=4-11 (for breaks cells)	4 ml MUB Buffer pH=4-11 (for breaks cells)
1 ml, 0.025 M p-nitrophenol phosphate solution	1 ml, 0.025 M p-nitrophenol phosphate solution
Swirled for a few seconds	Swirled for a few seconds
Incubated 1 hours at 37 ºC	Incubated 1 hours at 37 ºC
Add 1 ml 0.5 M $CaCl_2$	Add 1 ml 0.5 M $CaCl_2$
Add 4 ml 0.5 M NaOH	Add 4 ml 0.5 M NaOH
Swirled for a few seconds	Swirled for a few seconds
Filtered the samples	Filtered the samples
Read 405 nm wave length with spectrophotometer	Read 405 nm wave length with spectrophotometer
Calculate p-nitrophenol content from calibration curve	Calculate p-nitrophenol content from calibration curve

Figure 2. Culture preperation and **p**hosphatase enzymes activity assay in sterile and non-sterile soils.

Bacteria Culture Method	Freeze Dry Culture Method
0.5 g soil (Natural Soil)	0.5 g soil (Natural Soil)
Add 4.5 ml Extraction Solution,	Add 4.5 ml Extraction Solution,
25 ml Minimal salt,	25 ml Minimal salt,
30 minute shaken, 1 hours wait	30 minute shaken, 1 hours wait
Take 0.5 ml top solution, and	Take 0.5 ml top solution, and
put solution into the 1 lt LB Medium	put solution into the 1 lt LB Medium
Added 30 µg ml-1 cycloheximide	Added 30 µg ml-1 cycloheximide
At 37 ºC shaken and untill turbidity reached A600 of 0.8	At 37 ºC shaken and untill turbidity reached A600 of 0.8
Take 30 ml Cells culture	Take 30 ml Cells culture
Harvested with Centrifugation, Remove Supernatant	Harvested with Centrifugation, Remove Supernatant
Bacteria Culture	**Freeze Dry**

Phosphotase enzyme activity
0.2 ml Toluene
4 ml MUB Buffer pH=4-11 (for breaks cells)
1 ml, 0.025 M p-nitrophenol phosphate solution
Swirled for a few seconds
Incubated 1 hours at 37 ºC
Add 1 ml 0.5 M $CaCl_2$
Add 4 ml 0.5 M NaOH
Swirled for a few seconds
Filtered the samples
Read 405 nm wave length with spectrophotometer
Calculate p-nitrophenol content from calibration curve standards

Figure 3. Culture Preperation and Phosphatase Enzymes Activity assay in bacteria culture method and freeze dry method.

Culture preperation

Each soil was maintained at field moisture condition and the remainder was left on the laboratory bench for 48 h at laboratory temperature (25–28 ºC) to air-dry and passed through a 2 mm screen. Soil samples were stored in glass jars at 4 ºC for subsequent phosphatase enzyme activity measurements.

Added 4.5 ml extraction solution to 0.5 g soil, shaken 30 minute, waited 1h. 0.5 ml top solution was taken, and put in 1 lt LB Medium. Solutions were shaken at 37 ºC untill turbidity reached A600 of 0.8. Thirty mL of the spectinomycin-treated culture was transferred into a sterile centrifuge tube. The contents in each tube were incubated with gentle shaking for 4 hours at 37°C. The purpose of this incubation was to activate alkaline phosphatase activity of the bacterial culture. The cells were then harvested by centrifugation and washed with 10 mL of 20 mM phosphate buffer (pH 11) containing 0.5 mM EDTA (to remove extra Zn).

The washed bacterial cells were dispersed in 5 mL THAM buffer (pH 11) and disrupted by sonication using a 350 watt capacity Model S-185 Sonicator set at power level 5. Cells were broken using 8 short bursts of 5 seconds each (Mulrooney, 2005).

Alkaline phosphatase assay

30 ml cells culture or 10 mg cells (freeze dry) were harvested with centrifugation and added 4 ml MUB Buffer pH=11, 0.2 ml Toluene, 1 ml, 0.025 M p-nitrophenol phosphate solution. Samples were incubated 1 h at 37 ºC, and then added 1 ml 0.5 M $CaCl_2$ and 4 ml 0.5 M NaOH, read 405

nm wave length with spectrophotometer and calculated p-nitrophenol content from calibration curve standards (Tabatabai (1994).

2.5 Statistical analysis

Each treatment of soil was replicated three times resulting in a total of 192 experimental units (i.e. two soils, two sterilizations, two culture methods, eight MUB pH, and three replications). Analysis of variance (ANOVA) was used to determine the significance of each treatment on soil and bacterial culture alkaline phosphatase activity.

RESULTS

The main soil characteristics were as follows; soil pH ranged in 6,45–7.80; EC (dS/m), 0.64–2.13; clay (%), 36.0–46.0; organic matter content (%), 5,47–2.57; total N (%), 0.27–0.13; available p (mg kg^{-1}), 85.0-95.0. The main characteristics of these soils are outlined in Table 1.

Table 1. Some chemical and physical properties of experimental soil.

Soil properties	**Wooster**	**New Mexico**
pH (1:2.5)	6.45	7.8
Organic matter, %	5.47	2.57
Total N, %	0.27	0.135
Plant Available P mg kg^{-1}	95	85
Exchangeable cations, cmol kg^{-1} soil		
Ca	9.62	20.81
Mg	2.53	4.73
K	0.51	2.68
Na	1.07	0.67
Cation exchange capacity, cmol kg^{-1}		28.21
Electrical conductivity, dS m^{-1}	0.64	2.13
Salt, %	0.019	0.042
Water holding capacity at 1/3 Bars, %	30.22	28.25
Particle size distribution, %		
Sand	35.5	33.0
Silt	18.5	31.0
Clay	46.0	36.0

Soil phosphatase enzyme activities in different sterilization condition

The phosphatase enzyme activity values in sterile Wooster soil ranged from 1.26 µg PNP g^{-1} soil h^{-1} in sterile soil to 22.51 µg PNP g^{-1} soil h^{-1} in non-sterile soil. This activity values in sterile New Mexico soil ranged from 1.01 µg PNP g^{-1} soil h^{-1} in sterile soil to 23.33 µg PNP g^{-1} soil h^{-1} in non-sterile soil. The highest phosphatase enzymes activities were observed in buffer pH 6 in Wooster soil, and in buffer pH 11 in New Mexico soil (Table 1).

According to the results; AcdP enzyme activity increased with increasing buffer pH up to pH 6 in sterile and non-sterile acidic Wooster (pH:6.45), but decreased over buffer pH 6. AlkP enzyme activity increased with increasing buffer pH up to pH 11 in sterile and non-sterile alkaline New Mexico soils (pH:7.80), but decreased over buffer pH 11 (Table 2, Figure 4).

Table 2. Phosphatase Enzymes Activity in sterile and non-sterile Wooster and New Mexico soils in Different Buffer pH.

	Phosphatase Enzymes Activity, µg PNP g^{-1} soil h^{-1}			
	Wooster soil		**New Mexico Soil**	
Buffer pH	**Steril Soil**	**Non Steril**	**Steril Soil**	**Non Steril**
4	1.26 d	11.53 c	1,01 e	5.93 g
5	1.56 c	14.09 b	1,47 d	9.33 f
6	1.90 b	21.96 a	1,60 c	14.38 e
7	2.77 a	22.51 a	1,86 b	20.31 d
8	2.65 a	18.12 ab	2,11 ab	24.71 c
9	2.22 ab	14.08 b	2,24 ab	30.52 b
10	2.24 ab	12.94 bc	2,46 a	34.37 ab
11	2.01 b	11.35 c	2,76 a	38.32 a
12	2.29 ab	10.05 d	2,55 a	23.33 c

†When comparing phosphatase activities as affected by sterilization, different letters indicate differences at the $P < 0.05$ level of significance.

The investigation shows that soil AcdP and AlkP enzyme activities have been affected with sterile and non-sterile soil condition. The enzyme activity values of AcdP (at buffer pH=6) and AlkP (at buffer pH=11) of bacterial cultures obtained by different culture methods.

Although New Mexico soil had the lowest organic matter concentrations of the two soils, it also had the highest native pH. It is well known that alkaline phosphatase activity is higher in alkaline soils than in acid soils (Eivazi and Tabatabai, 1977).

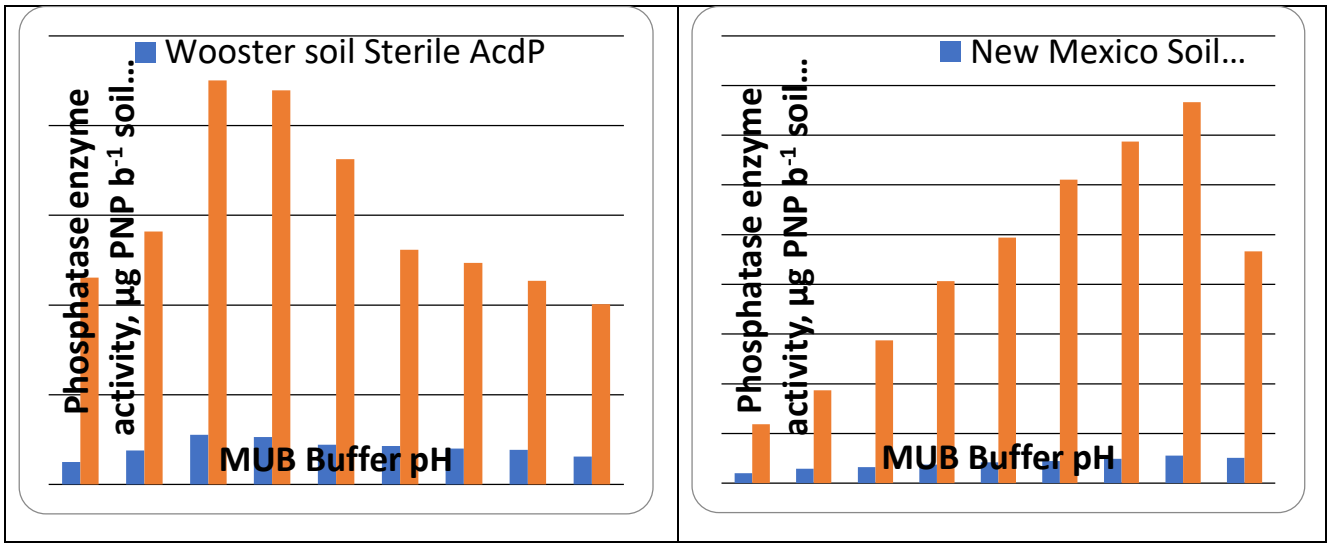

Figure 4. Phosphatase Enzymes Activity in sterile and non-sterile Wooster and New Mexico soils in Different Buffer pH

Soil phosphatase enzyme activities in different culture methods

The phosphatase enzyme activity values in sterile Wooster soil ranged from 6.54 µg PNP g^{-1} soil h^{-1} to 52.26 µg PNP g^{-1} soil h^{-1} in bacteria culture and freeze dry method. This activity values in New Mexico soil ranged from 1.95 µg PNP g^{-1} soil h^{-1} to 56.18 µg PNP g^{-1} soil h^{-1} in culture and freeze method. The highest phosphatase enzyme activities were observed in buffer pH 6 in Wooster and in buffer pH 11 in New Mexico soil (Table 3).

Table 3. Phosphatase Enzymes Activity in Wooster and New Mexico soils in bacteria culture and freeze dry culture methods in different buffer pH.

	Phosphatase Enzymes Activity, µg PNP g^{-1} soil h^{-1}			
	Wooster soil pH=6.45		New Mexico Soil pH=7.80	
Buffer pH	Culture Method	Freeze Dry Method	Culture Mehod	Freeze Dry Method
4	9.98 d	8.44 e	2,25 e	1,95 g
5	20.97 b	23.02 bc	13,62 d	12,06 e
6	52.26 a	49.11 a	16,22 d	14,36 e
7	25.87 b	28.47 b	22,13 c	19,71 d
8	22.18 b	21.51 bc	28,14 c	25,26 c
9	13.87 c	16.08 c	34,63 b	31,10 b
10	10.68 dc	11.37 d	38,15 b	34,10 b
11	9.50 d	8.74 e	56,18 a	50,16 a
12	6.59 e	3.74 f	5,38 e	4,72 f

†When comparing phosphatase activities as affected by culture method, different letters indicate differences at the $P < 0.05$ level of significance.

According to the results; AcdP enzyme activity increased with increasing buffer pH up to pH 7 in culture method and Freeze culture method in both soils, but decreased over buffer pH 7. AlkP enzyme activity increased with increasing buffer pH up to pH 11 in culture method and Freeze culture method in New Mexico soils (pH:7.80), but decreased over buffer pH 11 (Table 3, Fig. 5).

The investigation shows that soil phosphatase enzyme activities have been affected with culture method and Freeze culture method condition. The soil alkaline phosphatase activity by different MUB pH and culture method interaction effects were also statistically significant (P<0.05).

The investigation show that soil AcdP and AlkP enzyme activities have not been affected with sterile and non-sterile soil condition and these enzyme activity values were in parallel with different stock culture isolation methods.

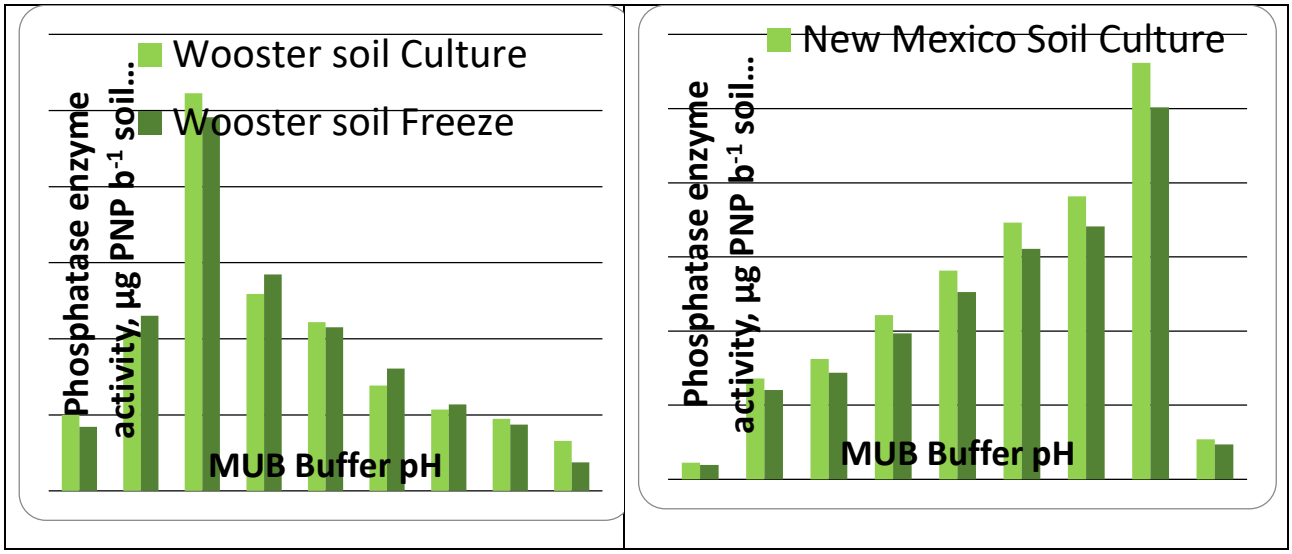

Figure 5. Phosphatase Enzymes Activity in Wooster and New Mexico soils in in bacteria culture and freeze dry culture methods in different buffer pH.

DISCUSSION

The soils selected for this study contained higher levels of alkaline phosphatase activity as compared to the activity levels reported by others (Frankerberger and Dick, 1983; Dick et al., 2000; Dick, 1983). Soils with high pH would generally have higher activity, and the relationship between alkaline phosphatase activity and soil pH suggests that the rate of synthesis and release of this enzyme by soil microorganisms or the stability of this enzyme once it is introduced to the soil is related to soil pH (Dick et al., 1983; Juma and Tabatabai, 1988). However, the higher soil pH would also create a condition where one could predict the potential for alkaline phosphatase activity (Impellitteri et al., 2002).

Phosphatase can be enriched by extracting cells from soils using minimal salts medium and growing cells in LB medium. LB medium is an excellent general purpose medium because it is very efficient in stimulating growth and is suitable for a wide variety of bacteria. It is categorized as a rich medium meaning it contains high quantity of all the nutrients (such as peptides and peptones, vitamins and trace elements) needed for bacteria to proliferate (Eitinger et al., 1997).

CONCLUSION

An assay procedure was developed to measure phosphatase enzyme activity in soil. The optimum assay condition for phosphatase enzyme activity method in soils was achieved at pH 6.0 for acid soils and at pH 11.0 for alkaline soils an incubation temperature of 37°C.

Phosphatase enzyme activity method reported here does not require any specialized equipment, is rapid, inexpensive and can be easily adopted for use in most soil biochemistry laboratories.

Phosphotase enzyme activity was more sensitive to the soil pH, and effected pH level and organic matter content of the soils.

The investigation show that soil AcdP and AlkP enzyme activities have been affected with sterile and non-sterile soil condition and these enzyme activity values were in parallel with different stock culture isolation methods. The investigation of soils together could give more reliable and accurate information about effects of buffer pH on soil alkaline phosphatase enzyme activity.

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EFFECTS OF SOIL TILLAGE ON SOIL PROPERTIES

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ABSTRACT

Improved soil quality is related to agricultural enhancement and sustainability. Soil quality has been affected by management practices Non-tillage (NT) and conventional tillage (CT). To evaluate the effects of management practices on some soil properties, composite soils from 48 conventionally-tilled (CT) and non-tilled (NT) farmer's fields under sugar beet (*Beta vulgaris*) were sampled, processed, and analyzed for microbial populations, basal respiration (BR), enzyme activity, and some chemical properties. Averaged across fields, NT had observed less bacterial and total microbial populations, but 2 times more fungal populations than CT. The NT had less BR than CT. Moreover, NT had higher acid phosphatase, more alkaline phosphatase, and greater dehydrogenase activity than CT. Urease activity was lower in NT over CT. NT had higher TC, TOC, TN, and AP than CT. Among the SQ indices, soil biological quality (SBQ) was higher and chemical quality (SCQ) was higher in NT over CT. Likewise, the overall SQ was higher in NT than in CT. The SBQ significantly accounted for of the variability in the overall SQ. Significantly higher values of biological and chemical properties and soil quality in NT than in CT are due to the surface placement of crop residues, dominance of energy efficient fungal food webs, and cooler, moist, undisturbed soil environment.

Key words: Bacterial population, fungal population, basal respiration, soil enzyme activity

INTRODUCTION

Soil quality is responsive to management practices (Islam and Weil 2000a). Although high input production agriculture produces greater amounts of food, feed, and fiber, frequent use of conventional tillage is identified as one of the contributors of soil erosion, agricultural CO_2 emissions to the atmosphere, net loss of organic matter, and soil quality degradation (Dick 1997, Islam and Weil 2000a, Lobell et al. 2006).

The negative impacts of CT on farm economics and soil quality have led to adaptation of sustainable agricultural management practices. No-till, as an important component of sustainable management practices, reduces fuel, labor, and equipment cost and minimizes soil erosion (Crovetto 2006). Moreover, continuous NT is expected to improve soil quality by providing suitable habitats for soil organisms, increasing microbial diversity and efficiency, enhancing nutrient recycling and carbon sequestration, and greater physical resilience (Dick 1984; Islam and Weil, 2000a, Crovetto 2006). Therefore, replacing CT with NT could improve functional stability of the soil.

Improved soil quality is related to agricultural enhancement and sustainability. However, appropriate information is lacking regarding the impact of tillage management practices on farmer's field soil quality. The objectives of the study were to compare the effects of CT and transitional NT on soil quality by measuring chemical properties, microbial populations, and enzyme activities in selected farmer's field.

MATERIALS AND METHODS

Experimental site and soil sampling

The study was conducted at Askale Plain ($39^0$54' N latitude and $41^0$13' E longitude at 1880-m above mean sea level), Erzurum, Eastern Turkey. The Askale Plain is one of the rapidly adopting NT farming regions of the Turkey. Based on area distribution within a similar soil-mapping unit, 36-paired CT and NT (~7 - 8 years) farms were selected. The dominant soil series is Askale loam (ustorthents) and contains 31.7$\pm$4.5 g clay, 26.1$\pm$5.8 g silt, and 40.2$\pm$7.1 g sand, respectively (Soil Survey Staff, 1999. The area is under semiarid climate with an average annual rainfall of 4270$\pm$30 mm. Average annual temperature is 15.4ºC. Major crops grown in this region are wheat, barley, secale, maize, cabbage, cotton, sugar beat, and potatoes.

At each CT or NT farm, three composite soil samples were randomly collected from 15-m x 5-m subplots at 0 – 30-cm depth in October 2008. Soil samples were 2-mm sieved to remove stones, roots, and large organic residues. After sieving, a portion of the soil was incubated at room temperature (25^0C) for 7-d to stabilize microbial activity followed by analysis of biological activities. Another portion of the soil spread on a dark colored polyethylene sheet and air-dried at room temperature for 7-d and analyzed for selected chemical and physical properties.

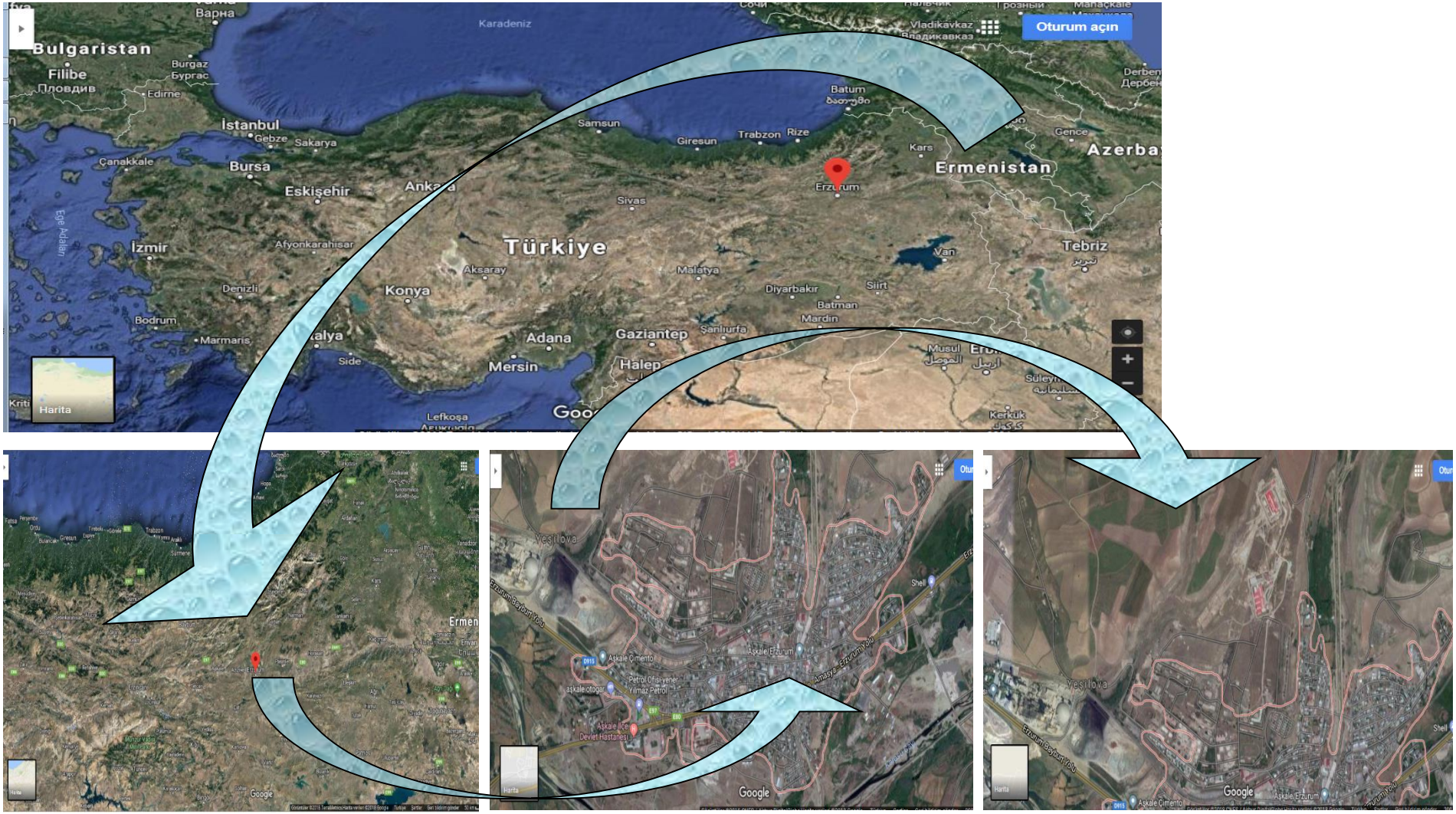
Analysis of soil microbial populations and enzyme activity

Culturable bacterial and fungal cells were enumerated using spread soil dilution plate method (Cynathia 2003). For bacteria and fungi, each 10-g ODE soil was homogenized by diluting the series (10^6–10^7) in 100 ml phosphate-buffered saline solution (PBS, 0.15-M potassium phosphate, 0.85% NaCl, pH 7.2) and placed onto petridishes (Zuberer 1994). Soil extract agar (SEA) was used for bacterial incubation at 30ºC for 7-d (Zuberer 1994) and dextrose-peptone agar (DPA) was used for fungal incubation at 25ºC for 7-d (Parkinson 1994). After the incubation, the colony forming units (cfu) per gram of oven-dried equivalent (ODE) of field-moist soil were determined using an automated colony counter (Canbolat at al., 2006, Madigon et al. 2006).

Basal respiration (BR) was determined using *in vitro* static incubation of unamended field-moist soil (Islam and Weil 2000a). A 20-g ODE of field-moist soil adjusted to 70% water-filled porosity in 25-ml glass beaker and placed in a 1-L mason jar along with a glass vial containing distilled water and a plastic vial containing 10-mL of 0.5-M NaOH. The mason jars were incubated in the dark at 25$\pm1^0$C for 20-d. The CO_2 evolved from the soil was absorbed in the 0.5-M NaOH followed by precipitation as $BaCO_3$ by the addition of excess 1-M $BaCl_2$. The remaining NaOH in each vial was then titrated to the phenolphthalein endpoint with a standardized 0.5-M HCl to calculate BR.

BR (mg CO_2 kg^{-1} soil d^{-1}) = (CO_2soil - CO_2air)/20 d

1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Where CO_2soil is the evolution of CO_2 during 20-d incubation of field-moist soil and CO_2air is the ambient air CO_2 in a blank mason jar.

Soil enzyme activities such as acid (AcdP) and alkaline phosphatase (AlkP) were assayed using p-nitrophenyl phosphate (pNPP) substrate and expressed as μg pNPP g^{-1} soil h^{-1}. Urease activity was assayed using urea solution and expressed as μg NH_4-N g^{-1} soil $2h^{-1}$. Dehydrogenase (DH) activity was assayed using triphenyl tetrazolium chloride and expressed as μg TPF g^{-1} soil $24h^{-1}$. Soil enzyme activities were determined by following Tabatabai (1994).

Analysis of soil chemical and physical properties

Soil organic matter (SOM) content was determined by following the standard loss-on-ignition method. A factor of 1.724 was used to convert SOM into total carbon (TC) content. The $CaCO_3$ content was determined using the pressure calcimeter method. Total organic C (TOC) was calculated after substrcating $CaCO_3$ from the TC content. Total N content was determined by the micro-Kjeldahl method. Soil pH was determined using a glass electrode meter in 1:2.5 soil:water ratio. Melich-I solution (0.125M H_2SO_4+0.5M HCL) was used to extract soil for exchnageable cations and the exchnageable cations were determined by using atomic absorption spectrophotometer. Effective cation exchange capacity (ECEC) was calculated as the sum of the exchnageable cations. Available P (AP) was determined by following ammonium molybdate-ascorbic acid method, after extracting the soil with 0.5-M Na_2CO_3. Soil microelements were determined by DTPA extraction method (Lindsay and Norvell, 1978). Mechanical analysis of soil was conducted using the standard hydrometer method and the soil textural class was determined by following the USDA textural triangle.

Calculation of soil quality index

The inductive additive approach based on normalization, summation, and average of selected biological and chemical properties into integrators of biology quality (SBQ), chemical quality (SCQ), and an overall soil quality (SQ) were calculated (Islam and Weil 2000b). The datum of each individual soil property (X_0) measured or calculated was transformed on a [> 0 to ≤ 100] scale relative to the maximum value (X_{max}) of that X_0 in the data-set ($X_i = X_0/X_{max}$). Equal weight was assigned to X_i's such that each X_i was in [> 0 to ≤ 100] scale and the SQ indices were calculated on a relative scale [> 0 to ≤ 100] by dividing with the total number of soil properties used (n).

Statistical analysis

All the statistical analyses were carried out using analysis of variance procedure of the SAS [SAS Institute 2008]. Treatment means were separated by the least significant difference (LSD) test at $p \leq 0.05$ unless otherwise mentioned. Relationship between soil pH and enzyme activity was fitted to linear and non-linear models and the best fit was graphically represented using SigmaPlot®.

RESULTS AND DISCUSSION

Microbial populations, basal respiration, and enzyme activity

Bacterial, fungal, and total microbial populations and basal respiration rates (BR) significantly varied in response to the effects of tillage systems (Table 1). Non-tillage (NT) had a lower number of bacterial and total microbial populations (11%) than CT. However, the fungal populations in NT were more than 2 times higher than in CT. The NT had 46% less BR than CT. The acid- (AcdP) and alkaline phosphatase (AlkP) activity was significantly higher (13 and 26%, respectively) in NT over CT (Table 2). Likewise, dehydrogenase activity in NT has increased by 64% than in CT. However, urease activity did not vary significant between NT and CT. When plotted, the enzyme activity showed a close relationship with soil pH (Fig. 1). The AcdP activity decreased non-linearly with increasing soil pH in both CT and NT (Fig. 1a). In contrast, the AlkP activity increased linearly with rise of soil pH (Fig. 1b). The rate of increase of AlkP activity as influenced by soil pH was higher in CT than in NT. Urease and DH activity decreased linearly with rise of soil pH. The rate of decrease of DH activity was more obvious in CT than in NT (Fig. 1c). However, the rate of decrease of urease activity was more detectable in NT than in CT (Fig. 1d).

Soil microbial populations and their activities respond quickly to changes in inputs, management practices by changing in their species composition, dominance, and activities (Hendrix *et al.,* 1986; Beare *et al.,* 1997, Lupwayi *et al.,* 1998, Islam and Weil 2000a). A significantly higher number of bacterial and total microbial populations and BR with lower number of fungal populations in CT than NT are due to greater physical disturbance from annual plowing (Islam and Weil 2000a). Plowing affects soil biology by fragmenting crop residues, roots, fungal hyphae, and mycorrhizal associations (Hendrix *et al.,* 1986; Beare *et al.,* 1997). An intense competition among the heterotrophic microbes for the available C favored a dominance of bacterial food webs because of their greater adaptability, generalist feeding habits, short generation time, smaller size, and rapid dispersal under disturbed environments like CT (Adu and Oades 1978, Kassim et al. 1981, Hendrix *et al.,* 1986). The low C-assimilation efficiency of the bacteria (20 - 40%) is most probably responsible for higher BR in CT (Adu and Oades 1978, Kassim et al. 1981). Frequent plowing mixes crop residues, degrades soil structures and exposes protected C and nutrients to microbes, and subsequently causes an increase in catabolism (loss of C as CO_2) from accelerated decomposition of SOM (Beare *et al.,* 1997; Islam and Weil, 2000a). A higher BR in CT is more related to stressed or a higher number of bacterial populations. Moreover, increasing aeration and rising soil temperature after plowing accelerate chemical oxidation of SOM.

In contrast, a higher number of fungal populations with lower BR in NT are associated with surface placement of crop residues in cooler, moist, partially anaerobic, and undisturbed soil ecosystems like NT (Beare *et al.,* 1997). Moreover, fungi have a higher C-assimilation efficiency (40 – 70%) than bacteria, and so they release less CO_2 as BR and accumulate more C over time (Adu and Oades 1978, Kassim et al. 1981). A difference in microbial populations as influenced by tillage

operations may reflect in their differences to regulate soil enzyme activities. A significantly lower enzyme activity in CT as compared with NT is possibly due to greater survival strategy of microbes especially bacteria from greater physical disturbance exerted by annual plowing and a labile C stress (Acosta-Martinez *et al.,* 2007, Islam and Weil 2000a). Higher enzyme activity in NT over CT is associated with greater substrate-assimilation efficiency of fungus than bacteria.

It is reported that pH influence enzyme activity by influencing the concentration of inhibitors or activators and substrates in the soil (Deng and Tabatabai, 1997; Dick et al., 2000). The inverse relationship of the AcdP activity with increasing pH suggested that the rate of synthesis and release of the AcdP by soil microbes or the functional stability of the AcdP affected by soil pH (May and Douglas, 1976). Others reported that optimum soil pH for urease activity is ranged between 6 - 6.8 (Singh and Nye, 1984). Similarly, the maximum DH activity is reported at soil pH ranged from 6.6 - 7.2 (Batra, 2004). Since urease and DH activity has greatly influnced by subtrates avaialability, a lower amount of C and N in CT than NT consequently resulted lower levels of urease and DH activity (Rao and Ghai, 1985). However, AlkP activity turned out to be more functional at high soil pH (Deng and Tabatabai, 1997).

Soil carbon, nitrogen, exchangeable nutrients, and pH

Total C (TC), organic C (TOC), total N (TN), and available P (AP) concentration significantly varied between CT and NT (Table 3). The TC (15%), TOC (16%), TN (16%), and AP (25%) concentrations were higher in NT than in CT. Soil pH was lower in NT than in CT but did not vary significantly between them. The exchangeable cations except K concentration did not vary significantly by tillage systems (Table 4). NT had 8% higher K than CT. Soil ECEC did not vary significantly.

Significantly higher TC, TOC, TN, and AP concentrations in NT than in CT are due to the effects of surface placement of crop residues, dominance of fungi food webs, cooler, moist, and partially anaerobic undisturbed soil environment (Islam and Weil, 2000a). Since carbon is stoichiometrically link with nitrogen in SOM, a higher TOC may have invariably increased TN content or vice-versa in NT than in CT. A lack of difference in concentration of exchangeable cations between NT and CT is possibly due to less solubility of the metals in high pH soil. Kabata-Pendias (2001) reported that Mn, Cu, Fe, and Zn solubility decreased with increased soil pH. A higher pH in both CT and NT is most probably associated with greater content of $CaCO_3$, Ca, and Mg. Moreover, a higher soil pH is associated with the effects of higher ECEC and base saturation.

Soil quality indices

The values of soil biological quality index (SBQ), soil chemical quality index (SCQ), and overall soil quality index (SQ) significantly influenced by tillage systems (Table 2). Among the SQ indices, the SBQ was higher by 13% and SCQ was higher by 10% in NT over CT. Likewise, the overall SQ was higher by 11% in NT than in CT. When plotted, the SBQ significantly accounted for 90% of the variability in the SQ. In contrast, the SCQ accounted for 56% of the variability in

the SQ. Greater metabolic efficiency of fungal food webs with higher enzyme activity, organic C, and N contents is likely associated with higher soil quality. A significantly greater accountability of the SQ variations by the SBQ over SCQ suggested that the biological properties are more sensitive as indicators for early detection of SQ changes in response to management practices.

CONCLUSIONS

Non-tillage had higher fungal populations, enzyme activity, total organic C and N contents, and soil quality than CT. The greater amount of C and N and a simultaneous decrease in BR in NT is attributed to the dominance of metabolically efficient fungal food webs. Reduced numbers of fungus with a simultaneous increase in bacterial populations and BR in CT are due to grater physical disturbance from annual plowing and labile-C stress over time.

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Table 2: Tillage effects on acid- and alkaline phosphatases, urease, and dehydrogenase activities in soil

Tillage System	Acid-P	Alkaline-P	Urease	Dehydrogenase
	_____ ($\mu g\ g^{-1}\ h^{-1}$) _____		($\mu g\ g^{-1}\ 2\text{-}h^{-1}$)	($\mu g\ g^{-1}\ 24\text{-}h^{-1}$)
CT	29.9b	57.4b	19.0a	72.9b
NT	33.8a*	72.4a	17.1a	119.8a

CT=Conventional tillage, NT=Non-tillage, Acid-P=Acid phosphatase, and Alkaline-P=Alkaline phosphatase, *Means separated by same lower case letter within each column are non-significant at $p \leq 0.05$.

Table 3: Tillage effects on soil pH, total and organic carbon, calcium carbonate, total nitrogen, and available phosphorus contents

Tillage System	pH (1:2)	TC	$CaCO_3$	TOC	TN	CN ratio	AP
		_____ g kg^{-1} _____					mg kg^{-1}
CT	8.3a*	16.2b	4.1a	12.1b	1.9b	8.5a	13.3b
NT	8.1a	18.6a	4.2a	14.4a	2.2a	8.5a	16.6a

CT=Conventional-tillage, NT=Non-tillage, TC=Total carbon, TOC=Total organic carbon, TN=Total nitrogen, and AP=Available phosphorus. *Means separated by upper case letter within each column are non-significant at $p \leq 0.05$.

Table 4: Tillage effects on extractable cations and effective cation exchange capacity in soil

Tillage System	Ca	Mg	K	Na	Fe	Cu	Zn	Mn	ECEC
	_____ g kg^{-1} _____			_____ mg kg^{-1} _____				_____ cmol kg^{-1}	
CT	4.2a*	1.0a	1.2b	78.2a	23.2a	3.6a	2.3a	10.6a	35.1a
NT	4.4a	1.0a	1.3a	64.4b	22.3a	3.4a	2.1a	10.6a	34.6a

CT=Conventional tillage, NT=Non-tillage, Ca=Calcium, Mg=Magnesium, K=Potassium, Fe=Iron, Na=Sodium, Cu=Copper, Zn=Zinc, Mn=Manganese, and ECEC=Effective cation exchange capacity. *Means separated by same lower case letter within each column are non-significant at $p \leq 0.05$.

Table 5: Tillage effects on biological, chemical, and overall soil quality indices

Tillage System	SBQ	SCQ	SQ
	_____ (%) _____		
CT	61.7b	65.4b	63.5b
NT	69.8a	71.9a	70.8a

CT=Conventional tillage, NT=Non-tillage, SBQ=Soil biological quality, SCQ=Soil chemical quality, SQ=Overall soil quality. *Means separated by same lower case letter within each column are non-significant at $p \leq 0.05$.

Figure Captions

Figure 1: Effects of pH on (a) acid- and (b) alkaline phosphatase, (c) dehydrogenase, and (d) urease activity in conventional and non-tillage soil.

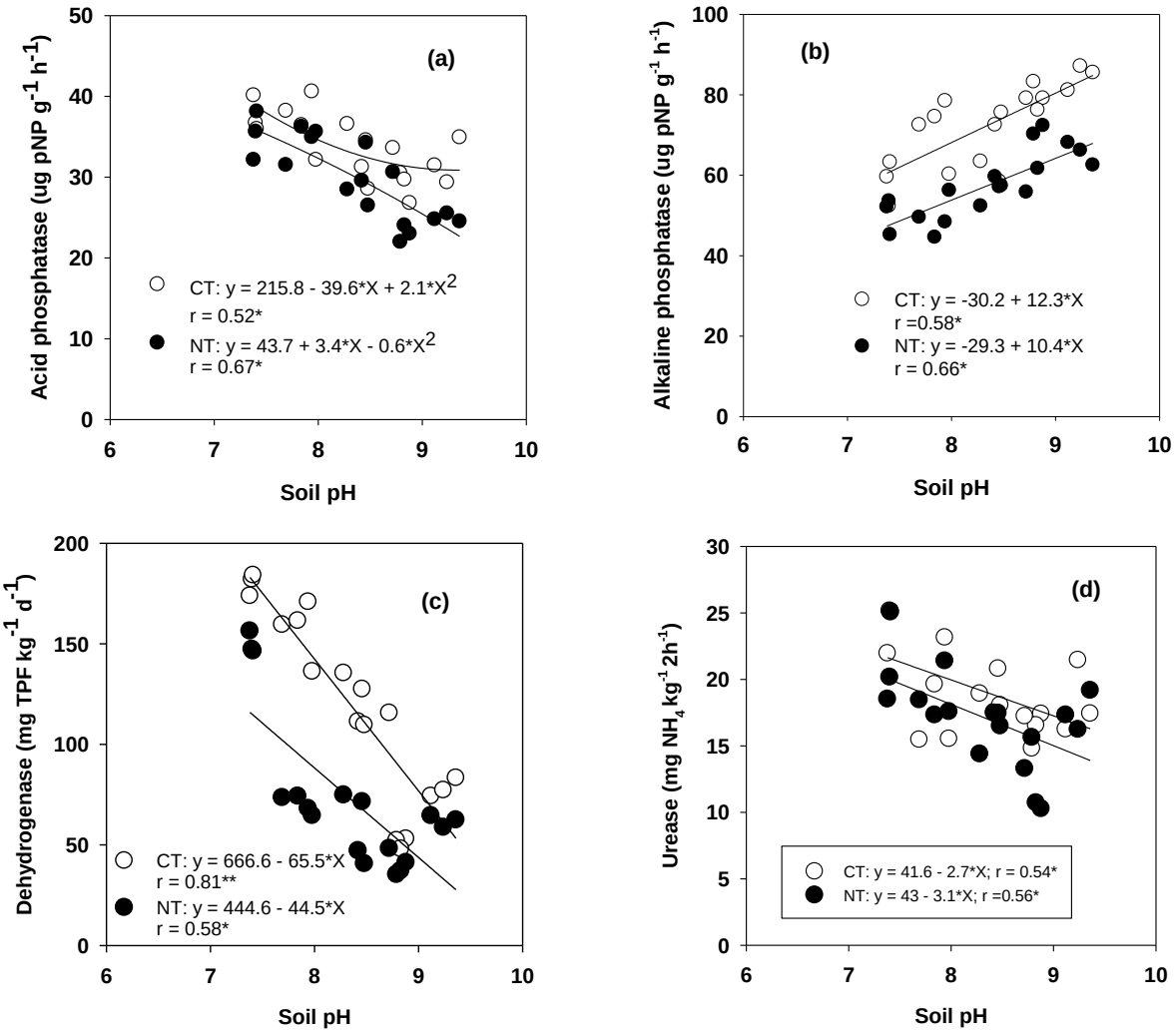
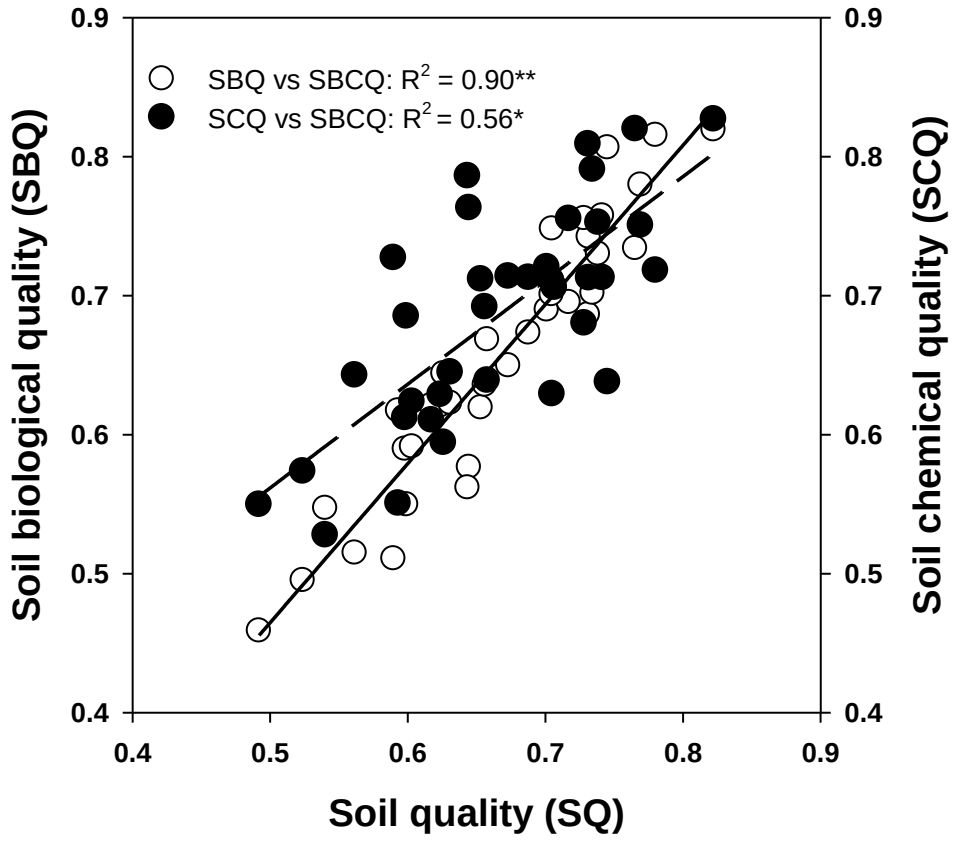


Figure 2: Relationship among biological, chemical, and overall soil quality indices in conventional and non-tillage.



THE RELATIONSHIP BETWEEN EMOTIONAL INTELLIGENCE AND SOCIAL MEDIA ATTITUDES OF UNIVERSITY STUDENTS

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ABSTRACT

High emotional intelligence is related with academic success, and good skills in interpersonal communication. In our study, emotional intelligence (EI) levels of university students, the factors affecting EI and the relationship of emotional intelligence with social media attitudes were evaluated. Our study was performed with Trakya University undergraduate students between April 2017-August 2018. Age, gender, demographic characteristics, socioeconomic status, parental education, and the longest place of residence of the volunteers were recorded to evaluate students. Emotional Intelligence was evaluated with Bar-On Emotional Quotient inventory (Bar-On EQ-I). To evaluate social media attitudes of the university students Social Media Attitudes Scale (SMAS) was used. SMAS scores and Bar-On EQ-i scores were significantly higher in participants who were using social media for a longer period of time than new users. SMAS scores were significantly higher in participants who used social media more frequently. Also, Bar-On EQ-i scores tended to be higher with increasing frequency of social media use. Appropriate use of social media by young people has positive effects in terms of communication with the environment, socio-cultural development, sharing feelings and thoughts in a clear and understandable way, communication and mental relaxation; its inappropriate use may cause losing too much time, decreasing efficiency and performance at work, distracting attention, and adverse effects on socialization. Social media may be helpful to support learning, but caution should be exercised to avoid its negative consequences.

Education programs should be established to improve younger generations' emotional intelligence in order to improve their abilities to cope with social problems and catching up with their times. Sociodemographic features that affect emotional intelligence should be identified, its relationship with social media use to which young people spend a great deal of time should be determined, and both should be included in education programs to contribute to young people's personal development.

Keywords: Social media, nursing student, university student, emotional intelligence

INTRODUCTION

Emotional intelligence has recently attracted an increasing interest in the whole world and scientists add dimensions such as adaptation to and relationship with the environment to the

definition of intelligence. Previous research has shown that although intelligence quotient (IQ) gives a quantitative measurement for cognitive intelligence, emotional quotient determines success in life and maintains quality of life [1, 2]. High emotional intelligence is related with academic success, and good skills in sociocultural, interpersonal communication and stress management areas; low emotional intelligence is related with unusual behaviors, alcohol and substance dependence and insufficient interpersonal relations [3]. Emotional intelligence is the sum of all non-cognitive skills and abilities that affect coping of the individual with external influences. Studies have shown associations between emotional intelligence and achievements such as creativity and communication skills [4, 5].

Using empathy and interview skills developed since the education period in professional life may help to reduce the anxiety encountered and increase the harmony in the working environment. Studies that evaluated the relationship between emotional intelligence and sociodemographic features of individuals demonstrated that the lessons they have learned during their education life and their parents' attitudes may support the development of emotional intelligence. In the studies, there were also positive correlations between personal skills, interpersonal skills, compatibility, coping with stress, and general mood subscales of emotional intelligence, understanding people and establishing relationships [6, 7].

Research has shown that use of internet and especially social media is getting more and more important place in lives of young people. In recent years 95% of young people aged 18-33 reported active use of social media. Social sharing sites help to share experiences and social relations and the use of social media programs among young people seems to be widespread. The use of social media is rapidly becoming a central part of young people's lives, and more than 90% of them now use social media almost every hour of the day [8, 9].

It is important to determine the sociodemographic characteristics and lifestyle features which affect emotional intelligence in order to determine the needs of young people and to develop education programs for them. As the communication skills are very important nowadays, it is important to develop curriculums in order to promote emotional intelligence of young people while they are attending to school and before they enter to professional life.

In our study, emotional intelligence levels of university students, the factors affecting emotional intelligence and the relationship of emotional intelligence with social media attitudes were evaluated.

MATERIAL AND METHOD

Emotional Intelligence was evaluated with Bar-On Emotional Quotient inventory (Bar-On EQ-i) which was validated in Turkish **(13-15).** Bar-On EQ-i is a Likert type measure consisting of 87 items. During rating the items 5 means fully agree, 4: agree, 3: undecided, 2: disagree, and 1: strongly disagree**.** This scale measures 5 subscales of emotional intelligence (personal skills, interpersonal skills, compatibility, stress management, and general mood). Construct validity and criterion validity studies of Bar-On EQ-i were conducted during Turkish validity and reliability

study. Construct validity studies included comparison of extreme groups and item-total score correlations and demonstrated that both discriminative features and correlations with relevant dimensions were statistically significant; criterion validity studies demonstrated that emotional intelligence was a theoretical construct apart from intelligence quotient but related with personality features. According to these results it was concluded that Turkish version of Bar-On EQ-i can be used in scientific studies [10-12].

Personal information form includes questions about age, gender, school, socioeconomic status, parental education, and the longest place of residence of the volunteers.

To evaluate social media attitudes of the university students Social Media Attitudes Scale (SMAS) which was developed by Otrar and Argın was used. This scale includes a total of 23 items (6 of which are positive and 17 of which are negative) and 4 factors (need to share, social competence, social isolation, and relationship with authorities) [13].

This study evaluated the levels of emotional IQ and social media use in university students and the relationship between them. The data were summarized using appropriate descriptive statistics. Mean and standard deviation were used for numerical variables and frequency and percentage were used for categorical variables.

Approval was obtained from Trakya University Scientific Research Ethics Committee and informed consents were obtained from all participants. All statistical analyses were performed with SPSS 20.0 Package Program. The data were summarized with appropriate descriptive statistics. Mean and standard deviation were measured for numerical variables and frequency and percentage were measured for categorical variables. Normal distribution of data was controlled with Shapiro-Wilk test. Student t test was used for comparison of two groups. One-way ANOVA was used for comparison of more than two groups. Multiple comparisons after one-way analysis of variance were evaluated with Bonferroni test. Chi-square test was used for the relationships between categorical variables. Significance level for all statistical analyses was defined as 5%.

RESULTS

Our study included 208 university students between 18-24 years of age. This study included voluntary students from Trakya University Health Vocational School and Applied Sciences High School. Distribution of the students to classes showed that 58 were at grade 1, 91 were at grade 2, 34 were at grade 3 and 25 were at grade 4 (Table 1).

Table 1. Distribution of participants according to grades

Grade	N
1	58
2	91
3	34
4	25

Among the participants 135 were females and 73 were males. The mean SMAS score of the female students was 64.3 and the male students was 68.6. The mean score of the male students was statistically significantly higher. Although no significant difference could be found in emotional intelligence the mean Bar-On scale score of the females was higher than the males.

Health Vocational High School Students formed 38% of all students and Applied Sciences High School students formed 62%. The mean SMAS and Bar-On scale scores of Applied Sciences High School Students were higher than Health Vocational High School students. Also, Bar-On emotional quotient increased with increasing SMAS score (Table 2).

No difference was found in SMAS and Bar-On EQ-i scores of the participants according to education level of the parents of the participants. However, scores from both scales tended to increase as the level of mother's education increased. With increasing father's education only SMAS scores tended to increase.

Although there weren't some statistically significant difference students with a better socioeconomic status tended to have a better score in Bar-On emotional intelligence scale. However, there was not a trend for increasing SMAS scores. Also, familial attitudes were asked and the mean scores from both scales did not change according to familial attitudes.

There was not a difference in scores from the scales according to mothers' occupations however, children of housewives tended to get lower scores from both scales. Also there was not a statistically significant difference according to fathers' occupations (Table 2).

Table 2. Sociodemographic features and the mean SMAS and Bar-On EQ-i scores of the study participants.

	N	%	SMAS	Standard Deviation	P	Bar-On EQ-i	Standard Deviation	P
Gender								
Male	73	35.1	68.68	14.6	.032*	295.9	33.6	.361
Female	135	64.9	64.33	13.4		299.7	25.5	
School								
Health Vocational High School	79	38	62.73	12.6	.012*	291.9	22.1	.035*
Applied Sciences High School	129	62	67.77	14.5		300.5	31.6	
Mother's Education								
Primary school or lower	146	70.2	64.8	14.1	.254	296.9	27.3	.834
Secondary School	59	28.4	68.0	13.8		297.5	32.3	
University or higher	3	1.4	72.0	8.0		307.0	10.3	

Father's Education								
Primary school or lower	92	44.2	64.36	14.4	.365	298.02	27.1	.944
Secondary school	99	47.6	66.84	12.7		296.61	28.9	
University or higher	17	8.2	68.24	18.1		297.18	35.9	
Socioeconomic status								
Moderate	165	79.3	66.1	13.7	.628	297.05	27.0	.821
Good	43	20.7	64.9	15.0		298.16	34.6	
Family attitudes								
Protective	119	57.2	65.92	13.617	.991	297.66	27.8	.668
Authoritarian	28	13.5	66.0	15.171		300.68	32.0	
Democratic	61	29.3	65.66	14.490		294.97	28.9	
Father's Occupation								
Public Servant	23	11.1	65.9	17.2	.880	298.4	28.9	.973
Worker	56	26.9	66.0	12.8		295.7	25.1	
Self Employed	72	34.6	64.8	12.8		297.8	32.8	
Other	57	27.4	66.8	15.3		297.5	26.7	
Mother's job								
Public Servant	2	1.0	65.5	2.1	.653	296.0	7.0	.178
Worker	23	11.1	69.9	15.2		300.3	40.3	
Self Employed	8	3.8	69.5	6.1		310.2	43.0	
Housewife	165	79.3	64.6	14.1		295.9	26.3	
Other	10	4.8	73.1	12.3		301.6	24.2	

*****indicates statistically significant difference.

Young people were connecting to social media sites more commonly by mobile devices. They were using social media not only during their leisure times at home but also during when they are outdoors (Table 3).

Table 3. Preferences for social media connection device and place of the participants

	N	%
The most common place to connect to social media		
Home	60	28.8
Outside	148	71.2
The most common device to connect to social media		
Mobile device	204	98.1
Computer	4	1.9

Table 4, shows social media use and scores from both scales of the participants. SMAS scores and Bar-On EQ-i scores were significantly higher in participants who were using social media for a longer period of time than new users. No significant difference could be found according to the time spent at each entry to social media. However, there was a tendency for higher SMAS and Bar-On EQ-i scores in participants who stayed longer at each entry. SMAS scores were significantly higher in participants who used social media more frequently. Also, Bar-On EQ-i scores tended to be higher with increasing frequency of social media use but the difference was not statistically significant.

Table 4. Social media use, SMAS and Bar-On Scale scores of the participants.

	N	%	SMAS	Standard Deviation	P	Bar-On Scale	Standard Deviation	P
Duration of social media use								
Less than 1 year	5	2.4	44.4	8.5	.002*	271.4	15.6	.041*
1-2 years	8	3.8	61.3	10.9		285.5	17.5	
2-3 years	31	14.9	63.6	11.4		291.0	28.3	
More than 4 years	164	78.8	67.1	14.1		299.8	28.8	
Time spent at each entry to social media								
5-10 min	29	13.9	62.3	12.5	.391	295.2	31.5	.543
11-30 min	54	26	64.9	13.9		297.3	21.7	
31-60 min	53	25.5	65.8	13.5		292.8	28.9	
61-120 min	31	14.9	66.7	16.1		299.1	27.5	
More than 121 min	41	19.7	68.9	13.8		302.8	34.6	
Frequency of social media use								
More than once every day	184	88.4	67.1	13.4	.006*	299.2	29.2	.086
Once or less every day	24	11.6	56.4	12.3		283.3	19.9	

*indicates statistically significant difference.

DISCUSSION

In our study the mean Bar-On EQ-i scores of the participants were at moderate level. Previous studies also reported moderate level emotional intelligence scores in students attending health related schools [14, 15].

Although there was not a statistically significant difference in emotional intelligence according to gender, the mean score of females from Bar-On EQ-i was higher than males. Some studies detected that emotional intelligence differ according to gender with a 90% confidence interval and females have higher emotional intelligence than males [16]. The mean SMAS score was higher in males.

SMAS and Bar-On EQ-i scores were significantly higher in those who started to use social media earlier in our study. Also, participants who used social media for a longer duration each time had higher mean scores in both scales although the difference was not statistically significant. Those who used social media more frequently in 24 hours had higher SMAS scores than others. With increasing social media use Bar-On EQ-i also increased but the difference was not statistically significant. Other studies showed that young people who used social media a few times a month had lower social media attitude scores than those who use everyday or at least once a week. This shows that increasing frequency of social media use also increases attitudes towards social media [17].

Studies on use of internet and social media show that most young people spend at least half an hour every day. With the rapid progress in technology, people tend to meet the internet and social media at an earlier age and tend to use it more often and for a longer period of time than older adults. Due to their age, it is normal for young people to be in an effort to establish close relationships with their surroundings. The use of social media is a tool that enables young people to communicate with each other and introduce themselves to other people [9, 18]. In our study 88.4% of the participants used social media more frequently and their SMAS scores were higher than those who used social media less frequently. Although it was not statistically significant Bar-On EQ-i scores were higher as frequency of social media use increased.

The nature of mankind is not suitable for living alone and this leads him to communicate in various forms. The increasing use of social media also provides new communication environments for people. Thus, individuals who do not want to be separated from the society they belong use the possibilities of technology in this area in order to keep communication alive [19, 20].

Studies that evaluated mother's working status, its effect on emotional intelligence scores, and academic achievement found a positive relationship between emotional intelligence scores and mother's working status [21]. In our study, no difference could be found both in SMAS and in emotional intelligence scales according to mother's occupation but children of mothers who were not working had higher scores in both scales than children of working mothers.

In our study no significant difference could be found in scores from the scales according to father's occupation. Previous studies also couldn't prove a relationship between father's occupation and emotional intelligence. Under normal conditions emotional connection between the mother and the child begins as soon as the baby has first formed in the mother's womb and continues as the child grows, and develops, which is always more prominent than the connection with the father. Although the relationship with the father increases in some stages of life it is not easy to replace

the relationship with the mother. Father's occupation may affect the quality and duration of time he spends with his child; but no evidence could be detected to prove that father's occupation has a strong influence to affect emotional intelligence score of his child.

Studies that investigated the correlation between emotional intelligence and education level of mother found a positive correlation. Mother's love to her child starts when she first feels it and this love enables to provide the most emotional support possible throughout her life as long as it is not adversely affected by some external factors [22]. In our study no statistically significant difference could be found in SMAS or Bar-On EQ-i scores according to mother's education. Regardless of their education level mothers try to support the development of their children with their endless love and affection. However, mothers with higher education find better opportunities which facilitate their efforts. Our study also showed a trend of increase in both scales as mother's education level increases.

Previous studies couldn't find a relation between fathers' education level and emotional intelligence of the participants [22]. In our study only SMAS scores tended to increase with increasing paternal education. Increased education level decreases financial concerns of the father. This increases the time he spends with his child and facilitates sharing mother's responsibilities. But with increasing education level father's expectations on academic and social grounds increase, leading to a pressure on the child and affecting emotional development adversely. Considering all these factors together, low level of education does not always mean low emotional support from the father. Development of emotional intelligence will be better when father enjoys a good time with his child, tries to form strong bonds and shows timely support. Increased paternal education level also decreases socioeconomic concerns and increases access to social media.

There wasn't a statistically significant difference according to socioeconomic status of the patients however, Bar-On EQ-i scores tended to be better in participants with a better income status. Some previous studies have found a positive relationship between emotional intelligence level and socioeconomic status of the family [23]. As families' financial concerns decrease they respond more easily to needs of their children. This increases sociocultural development of individuals but can't support emotional development by itself. Emotional intelligence strengthens with family bonds and their affection, respect and support towards each other.

General attitudes of families as protective, authoritarian, or democratic were not associated with scores on SMAS or Bar-On scales. There are other studies which investigated the association between family attitudes and emotional intelligence. Some studies have found significant relations between emotional intelligence and family attitudes of individuals [24]. We didn't question familial problems of the participants. Considering that they won't want to share their familial problems they were only asked to choose from the options as authoritarian, protective, or democratic. Inability to find an association between social media use, emotional intelligence and attitudes of families may be due to the wide spectrum of family attitudes.

Emotional intelligence was first defined by scientists as "the ability of individuals to evaluate their own and others' feelings, to distinguish between these feelings, and to use them in shaping their

thoughts and behaviors". Research has shown that emotional intelligence is indispensable to be successful in social, cultural and academic grounds [25, 26].

Some studies have indicated that cognitive intelligence measured by tests is not an important indicator of success in life; instead those who recognize and control feelings of themselves and others are more successful in academic and sociocultural grounds. Emotional intelligence may be developed with personal effort. Research has shown that cognitive intelligence alone can give young people the profession they desire but it can't provide pleasure from the life [26-28].

Appropriate use of social media by young people has positive effects in terms of communication with the environment, socio-cultural development, sharing feelings and thoughts in a clear and understandable way, communication and mental relaxation; its inappropriate use may cause losing too much time, decreasing efficiency and performance at work, distracting attention, and adverse effects on socialization. Social media may be helpful to support learning, but caution should be exercised to avoid its negative consequences.

Education programs should be established to improve younger generations' emotional intelligence in order to improve their abilities to cope with social problems and catching up with their times. Sociodemographic features that affect emotional intelligence should be identified, its relationship with social media use to which young people spend a great deal of time should be determined, and both should be included in education programs to contribute to young people's personal development.

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SORGHUM CROP, AN ALTERNATIVE FOR DOBROGEA FARMERS IN THE CONTEXT OF CLIMATE CHANGES

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ABSTRACT

Dobrogea is the most drought area of Romania (average 1961-2016 :464 mm rainfall precipitation). Climate change in recent years has accentuated this phenomenon. For farmers from this area sorghum crop is a solution. At Sport Agra in Amzacea, in the last few years there have been experimented new sorghum crop technologies designed to face the current climate changes. These technologies include the following elements: changing the sowing epoch with one month before the usual period recommended by classical technologies; (beginning of April in order to benefit from the soil's humidity la 4-5 cm depth boosting the germination process); choosing early hybrids in order to avoid the drought season which starts in June; applying adequate crop protection treatments, with pre-emergent and post-emergent herbicides and last generation insecticides. The agricultural crops in this area are not irrigated, so dr.ing Dumitru Manole proposed a new technology, with the sowing of the crops earlier. This way the plants will benefit from the moisture from the soil accumulated in the winter. The obtained production from sorghum crop was over 10t/ha for most of the varieties tested.

Keywords: Sorghums, Climate changes, Technologies

INTRODUCTION

The crop taking into study is sorghum, which is recommended for these arid areas; called the camel of crops due to its drought resistance (Amsalu Ayana and col.1998), sorghum requires the following technological elements: Selecting early hybrids to overcome the drought periods that occur between the 5-10th of June until the 20-25th of August. There are recommended hybrids with shorter vegetation period (Poschiscanu et al., 1015).

Sorghum sowing is recommended between 20th April and 10th May (Trotus et al. 2015) ensuring a minimum of 120-140 kg / ha of nitrogen (Owen, 1967), treatment of seeds before sowing with chemicals containing thiamethoxam, pre-emergence herbicide with Buctril Universal 0,8 l/ha (bromoxinil+2,4D) and post-emergence with Dual Gold (metalaclor) 1,5 l/ha. The results from comparative crops in a 2-year dynamics have demonstrated sorghum crops with outstanding yields of over 10 t/ha.

The agricultural crops in this area are not irrigated, so the dr.ing Dumitru Manole proposed a new technology, with the sowing of the two crops earlier by about a month. This way the plants will benefit from the moisture from the soil accumulated in the winter.

MATERIALS AND METHODS

Experimental plots were placed at S.C. SPORT AGRA S.R.L. Amzacea, Constanța. The experience was situated on a land belonging to the South Dobroudja plateau, represented by cambic chernoziom, with a profile deeper than other chernozioms, a blackish-brown soil of 40-50 cm thickness, medium texture (Demeter, 2009). The content of nutrients was: mobile P index - 72; N index - 4; Humus - 3.11; K index - 200; Neutral pH - 7.2. The climate is deeply temperate continental, with an average annual temperature of 10.7-12.12°C, with a high temperature between June and August. Meteorological data are presented in Tables 1 and 2. Sowing was carried out on April.

Table 1. Precipitation and temperature during 2016 growing vegetation season.
(Valul lui Traian Station,Constanta)

	Month								
	Jan.	Febr.	March	Apr	May	June	July	Aug.	
Periods	The growing season 2016: Precipitation (mm) for 10-day periods								Sum
1-10	0	12.0	10.0	0	60.0	3.5	56.0	4.0	145.5
11-20	95.0	18.5	19.0	0	21.0	20.0	0	0	173.5
21-30	15.0	0	15.0	20.0	16.0	0	0	0	66.0
Sum	110.0	30.5	44.0	20.0	97.0	23.5	56.0	4.0	385.0
	Average 1961-2010 : monthly values of precipitation (mm)								Sum
	27,7	24,0	29,1	31,8	37,7	47,1	38,9	37,4	273,7
	The growing season 2016: Mean air (^{o}C) for 10-day periods								Mean
1-10	2.5	4.1	6.8	10.3	13.9	19.8	22.6	23.2	12.9
11-20	4.8	5.2	7.9	12.9	16.8	21.4	24.2	22.6	14.57
21-30	4.3	5.4	10.2	13.5	18.7	22.1	23.8	21.4	14.92
Mean	3.9	4.9	8.3	12.2	16.5	21.1	23.5	22.4	14.1
	Average 1961-2010 : monthly values of mean air temperature (^{o}C)								Mean
	0,4	0,9	4,4	9,7	15,3	19,4	21,9	16,9	12.12

Table 2. Precipitation during 2017 growing vegetation season.
(Valul lui Traian Station,Constanta)

	Month								
	Jan.	Febr.	March	Apr	May	June	July	Aug.	
Periods	The growing season 2016: Precipitation (mm) for 10-day periods								Sum
1-10	60	5.0	4.0	0	13.0	18.0	9.0	0	109.0
11-20	10	13.5	31.0	35.0	12.0	6.0	0	0	107.5
21-30	0	2.0	5.0	6.0	2.0	4.0	92.0	6.0	117.0
Sum	70.0	20.5	40.0	41.0	27.0	28.5	101.0	6.0	333.5
	Average 1961-2010 : monthly values of precipitation (mm)								Sum
	27,7	24,0	29,1	31,8	37,7	47,1	38,9	37,4	273,7

RESULTS AND DISCUSSIONS

As written in Table 1, year 2016, provided higher amount of rainfall between May and June, 100 mm higher than the multiannual average. These precipitations favored the development of sorghum crops. Regarding the sorghum crop, the main technological links pursued by the research team consisted of the following: Choosing early hybrids to overcome the burning periods that occur between June 5 through August 20-25, Recommendation of shorter vegetation hybrids, Sowing the sorghum between April 20th-May 10th according to classical technology (Trotus et col 2015.), Provide a minimum of 120-140 kg / ha of nitrogen,
Treatment of seeds before sowing with chemicals containing thiamethoxam to combat tanymecus sp. in the early stages of vegetation, Pre-emergence herbicide with Dual Gold (metalaclor) 1,5 l/ha and post-emergence with Buctril Universal 0,8 l/ha (bromoxinil+2,4D)

The experiments were carried out in 2016 on 6 hybrids, as shown in Table 3. Most of the hybrids were sown one month earlier (9 April) compared to the classic technology recommended by specialists (Trotus et al. 2015) and EURALIS. Hybrid Arkanciel was sown and in recommended (May 14). Table 10 shows data regarding sorghum productivity consisting in very high yields of about 10-11 tons / ha for most hybrids, due to the change of the sowing date which the plants benefit from the moisture accumulated in the soil during the winter and also avoid the drought

crashes begin in June. It can be seen in the Arkanciel hybrid a production increase of 2212 kg/ha, obtained by its earlier sowing.
The data obtained in the experimental year 2017 are presented in Table 4. The sowing took place this year on April 4, and the hybrid Arkanciel was sown on 4 May. From the data presented, it can be seen that this year, through earlier sowing, large production increases of over 10000 kg / ha were obtained. This year, with the Arkanciel hybrid, an increase of 3436 kg/ha was obtained. Tables 3 present the data on the technical sheet of sorghum culture on the two plots. Experiments in plots 1 were made on 2195 sqm. Treatment of seed prior to sowing was performed with chemicals containing thiamethoxam. Pre-emergence herbicide was carried out with Dual Gold (metalaclor) 1,5 l/ha and post-emergence with Buctril Universal 0,8 l/ha (bromoxinil+2,4D). Figures 3 are referred to: - Experimental field-sorghum. All of these data show that sorghum is a valuable alternative crop for this dry area.

Table 3. Demonstrative plots for sorghum crop - Amzacea 2016

Hybrid	Pre-emergent plant	Surface sqm	Seeds/ha	Sowing date	Emergence date	Yields kg/ha
ES Arfrio	Wheat	2195	230000	9 April	18 April	10013
ES Aqulion	Wheat	2195	230000	9 April	18 April	12340
ES Alize	Wheat	2195	230000	9 April	18 April	11785
Arack	Wheat	2195	230000	9 April	18 April	11919
Arkanciel	Wheat	2195	230000	9 April	18 April	10022
Arkanciel	Wheat	2195	230000	2 May	14 May	7810
ES Foehn	Wheat	2195	230000	9 April	18 April	8601

Table 4. Demonstrative plots for sorghum crop - Amzacea 2017

Hybrid	Pre-emergent plant	Surface sqm	Seeds/ha	Sowing date	Emergence date	Yields kg/ha
Alize	Wheat	2195	220000	4 April	14 April	10439
Foehn	Wheat	2195	220000	4 April	14 April	11504
Arkanciel	Wheat	2195	220000	4 April	14 April	10336
Arkanciel	Wheat	2195	220000	4 May	16 May	6900
Albanus	Wheat	2195	220000	4 April	14 April	10130
Typhon	Wheat	2195	220000	4 April	14 April	8859
Armorik	Wheat	2195	220000	4 April	14 April	10645

Figure 2 Sorghum harvest

Figure 1. Experimental field-sorghum

CONCLUSIONS

At Sport Agra Amzacea, there have been experimented in the last few years new and improved sorghum crop technologies in order to adapt to the new climate changes. These technologies comprise the following technological elements: Selecting early hybrids to overcome the drought periods that occur between the 5-10th of June until the 20-25th of August. There are recommended hybrids with shorter vegetation period. Changing the sowing age - the hybrids were

sown one month earlier (4 and 9 April). The results from comparative crops in a 2-year dynamics have demonstrated sorghum crops with outstanding yields of over 10 t/ha.

The agricultural crops in this area are not irrigated, so the farmer proposed a new technology, with the sowing of the two crops earlier by about a month. This way the plants will benefit from the moisture from the soil accumulated in the winter.

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INVESTIGATION OF EXTRACELLULAR ALPHA-AMYLASE ACTIVITY OF *PENICILLIUM CYCLOPIUM*

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ABSTRACT

A variety of fungus species have been screened for α-amylase activity by a starch plate culture method. *Penicillium cyclopium* was found to be 2.25±0.06 having the highest starch degrading activity in which starch degrading activity is defined as the ratio of the diameter of clear zone to the diameter of fungus colony (DCZ/DFC). Extracellular amylolytic activities of *P. cyclopium* is evaluated under varying pH and temperature reaction conditions. The optimum pH and temperature for reaction were found to be 5 and 30°C, with the enzyme activity values of 2.94±1.01 and 2.06±1.04 U/ml, respectively. The starch hydrolysis percentage of *P. cyclopium* was also investigated with starches from different sources. The highest hydrolysis percentages were found for corn starch in 5, 10 and 15 min, however rice starch was ranked the highest hydrolysis percentage in 30 minutes.

Keywords: *Penicillium cyclopium*, α-amylase, hydrolysis percentage, thermal stability, pH stability

INTRODUCTION

Alpha-amylase, (α-amylase) is a protein enzyme (EC 3.2.1.1) that belongs to a family of endo-amylases that catalyzes the initial hydrolysis of starch into shorter oligosaccharides through the cleavage of α -D-(1–4) glycosidic bonds (Kandra, 2003).The end products of α -amylase action are oligosaccharides with varying length with an α -configuration and α -limit dextrins, which constitute a mixture of maltose, maltotriose, and branched oligosaccharides of 6–8 glucose units that contain both α -1,4 and α - 1,6 linkages (Van der Maarel et al., 2012 ; Whitcomb and Lowe, 2007). Although other amylolytic enzymes contributes the breakdown process of starch, but the participation of α-amylase is the most critical step for the initiation of the degredation process (Tangphatsornruang, 2005). Starch conversion, detergent industry, ethanol production, biofuel production are the industrial uses of α-amylases. Not only do food, textile and paper industry use

α-amylases, but also medical industry uses the potential of α-amylases for the degradation of biofilms (De Souza and De Oliveira Magalhães, 2010; Chi et al., 2009).

Although plants and animals are the sources of amylases, microbial amylase production dominates the commercial industry (Gupta et al., 2003). As it is shown that the stability of microbial amylases are higher than amylases from other sources, the huge production capacity and manipulation convenience are the other benefits of the microbial amylase usage (Tanyildizi et al., 2005). A limited species of mesophilic fungi are the most studied group and many studies focus on the selection of the outperforming species and description of cultural conditions on a commercial scale (Gupta et al., 2003).

Aspergillus and *Penicillium* are commonly studied species as they are suitable for solidstate fermentation and their amylase products are generally accepted as GRAS (Generally Recognized As Safe) (Kathiresan and Manivannan, 2006; Gupta et al., 2003). Although a number of *Penicillium* species are screened for starch degrading activity, α-amylase activity of *Penicillium cyclopium* remains unstudied (Saranraj and Stella, 2013).

The aim of the study was the determination of α-amylase activity of *Penicillium cyclopium* by a starch plate culture method and the evaluation of extracellular amylolytic activities of *P. cyclopium* under varying pH and temperature reaction conditions. In addition, thermal and pH stability of extracellular α-amylase from *P. cyclopium* and its hydrolysis potential on starches from different plant sources were investigated.

MATERIAL AND METHODS

Five species of fungal cultures (*Penicillium glabrum*, *Penicillium expansum*, *P. cyclopium*, *Talaromyces radicus*, *Fusarium solani*) fungal cultures originally obtained from University of Valencia; Spain and Westerdijk Institute of Utrecht, Netherlands; stored in Trakya University Science Faculty Department of Biology were assessed for amylase activity. CDB (Czapek-Dox Broth) medium containing 2.5% agar and 2.0% starch (pH 6) was used to prepare screening plates. Incubation was performed via three-point inoculation method. After 7 days in an incubator at 30˚C, the plates were treated with 1% iodine solution as described by Abou-Zeid in 1997. The diameter of clear zone (DCZ) and the diameter of fungal colony (DFC) were measured. The measurements used to calculate DCZ to DFC ratios for each species.

Growth medium was used as described by Abou-Zeid[13] containing 3 g $NaNO_3$, 1 g KH_2PO_4, 0.5 g $MgSO_4.7H_2O$, 0.5 g KCl, 0.01 g $FeSO_4.7H_2O$, 0.01 g yeast extract, 20 g maltose in 1 l distilled water. The flasks containing fungal cultures were incubated in 30˚C water bath with a shaking frequency of 200 rpm. After 7 days, the culture containing media was filtered through Whatman No:1 paper filter. The filtrate was used as crude enzyme source.

In order to determine the biochemical properties of α -amylase from *P. cyclopium* varying incubation pH and temperature of reaction and the thermal stability and pH stability of the crude

enzyme was tested. The starch degrading ability of α -amylase from *P. cyclopium* on starch samples from different plant sources was also investigated with varying incubation periods. α-amylase activity was measure according to methods described by Abou-Zeid in 1997. Blank, control and three replicates of sample tubes were prepared and their absorbance values at 620 nm were measured. The difference between control and samples were used to calculate the amount of starch degraded by crude enzyme via formula obtained from starch standard curve graph.

0.8% soluble starch was dissolved in boiling 0.05 M KH_2PO_4-NaOH buffer (pH 5.0) and then cooled. For the assay 0.1 ml of crude enzyme solution was placed in a test tube after 5 min following the addition of 0.2 ml of starch substrate. The reaction was stopped by the addition of 5 ml of iodine solution. The absorbance was measured at 620 nm against blank containing enzyme, buffer and iodine solution (Abou-Zeid, 1997). One unit of α-amylase is defined as the amount of enzyme which hydrolyses 0.1 mg of starch at 30˚C when 8 mg of starch is present (Balkan and Ertan, 2004).

The effect of temperature on the activity of α-amylase was measured by incubating 0.1 ml enzyme and 0.2 ml of starch solution at 30˚C, 40˚C, 50˚C and 60˚C for 5 minutes. The effect of pH on the activity of α-amylase was measured by incubating 0.1 ml enzyme and 0.2 ml of buffers, adjusted to pH from 4 to 8, containing soluble starch (For pH 4 and 5 sodium acetate buffer; for pH 6 to 8 phosphate buffer were used.). Thermostability was determined by incubating crude enzyme at temperatures ranging from 30˚C to 60˚C for 20 min prior to substrate addition. Stability of the enzyme at different pH values was examined by the incubation of the enzyme at various pH values varying from 4.0 to 8.0 for 1 hr at 4˚C. Hydrolysis percentage potential on starches from different plant sources were determined by using standard, wheat, corn, potato and rice starch as substrate.

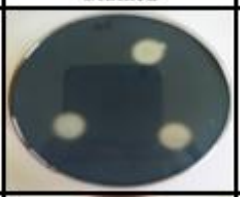
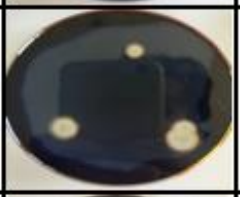
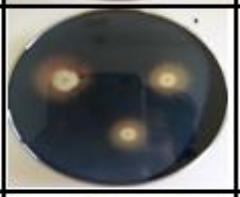
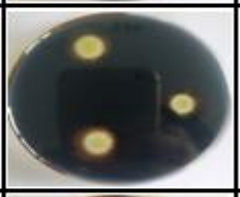
RESULTS AND DISCUSSION

Penicillium glabrum, P. expansum, P. cyclopium, T. radicus, F. solani species were assessed for amylase activity by starch plate method and the diameter of clear zone (DCZ) and the diameter of fungal colony (DFC) were measured and their DCZ to DFC ratios are calculated (Table 1). *P. cyclopium* was found to be 2.25 ± 0.06 having the highest starch degrading activity in which starch degrading activity is defined as the ratio of the diameter of clear zone to the diameter of fungus colony (DCZ/DFC). As there is no prior studies on the characterization of biochemical properties of extracellular amylases from *P. cyclopium*, further biochemical studies performed by crude extracellular enzyme extracts of *P. cyclopium.*

Alpha-amylase activity was measure according to methods described by Abou-Zeid in 1997. The difference between control and samples were used to calculate the amount of starch degraded by crude enzyme via formula obtained from starch standard curve graph (Fig. 1).

The effect of temperature and pH on the activity of α-amylase were evaluated by incubating enzyme and substrate in varying temperatures and varying pH buffers. The results in Figure 2 and Figure 3 show that the optimum pH and temperature for reaction were found to be 5 and 30°C, with the enzyme activity values of 2.94±1.01 and 2.06±1.04 U/ml, respectively. The optimum temperature of α-amylases is generally related to natural habitat and growth of microorganism and the optimum pH of α-amylases varies from 2 to 12 but most bacterial and fungal α-amylases prefers conditions of acidic to neutral range (Saranraj and Stella, 2013). In accordance with that, α-amylase from *P. cyclopium* performed best at slightly acidic conditions.

Table 1. DCZ/DFC ratios of fungus species

Fungus	Unstained	Stained	DCZ/DFC
Penicillium glabrum			0.3
Penicillium expansum			1.3
Penicillium cyclopium			2.25
Talaromyces radicus			0.7
Fusarium solani			0.2

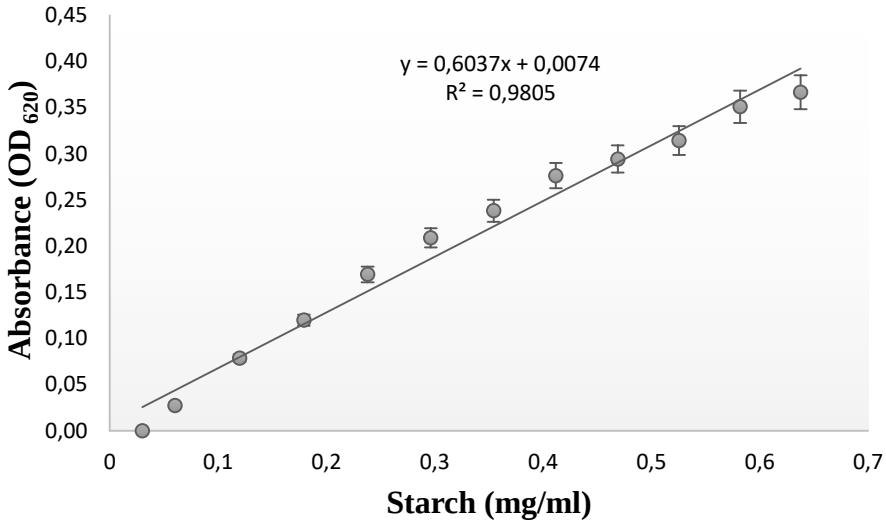


Figure 1. Calibration curve of starch

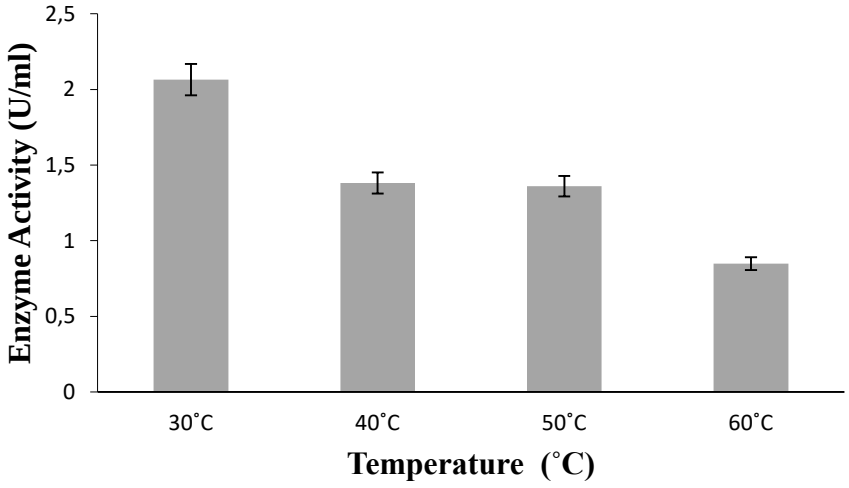


Figure 2. Alpha-amylase activity of *P. cyclopium* under different temperatures

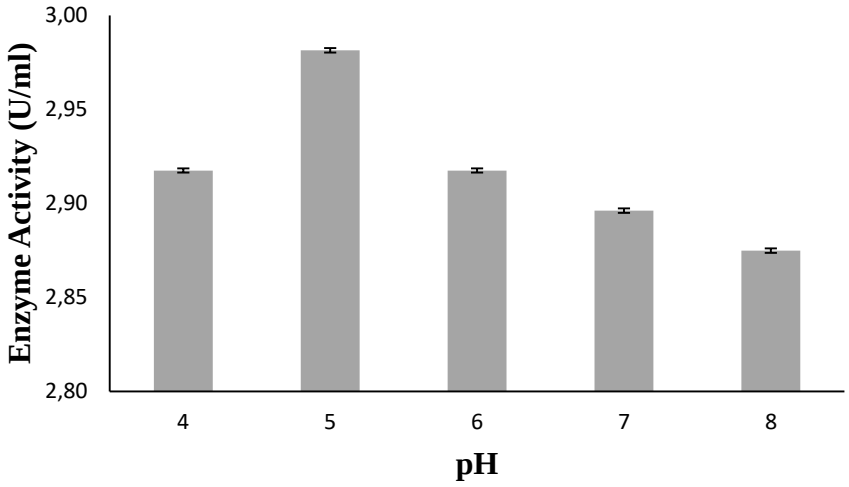


Figure 3. Alpha-amylase activity of *P. cyclopium* under varying pH

Thermostability was determined by incubating crude enzyme at varying temperatures and stability of the enzyme at different pH values was examined by the incubation of the enzyme at various pH values. Thermal stability of the crude enzyme was kept for 30 °C and 40 °C up to 97 % but as temperature increases above 40 °C the thermal stability of the enzyme was lost (Fig. 4). pH stability of the enzyme was performed best at pH 5 but tested pH values cause only up to 3% decrease in enzyme activity (Fig. 5). According to study performed by Balkan and Ertan, α-Amylase from *Penicillium chrysogenum* was found to be stable at a pH range from 5.0–6.0 and at 30°C for 20 min (Balkan and Ertan, 2004).

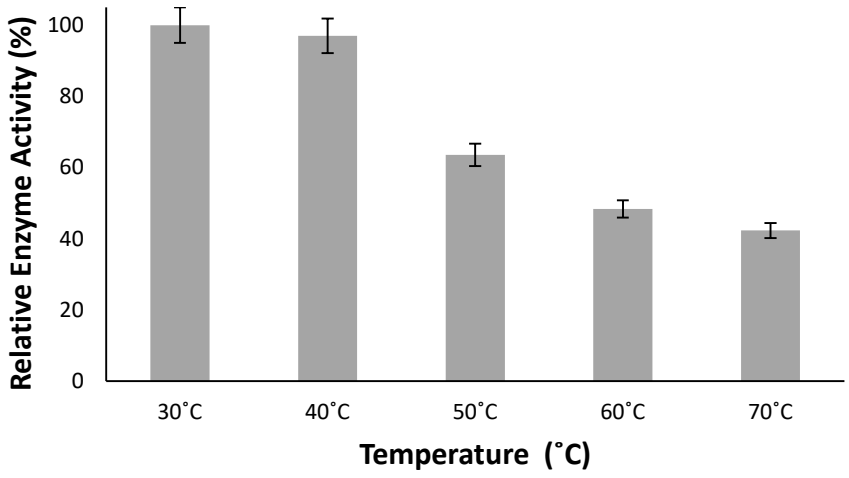


Figure 4. Thermal stability of α-amylase activity from P. cyclopium

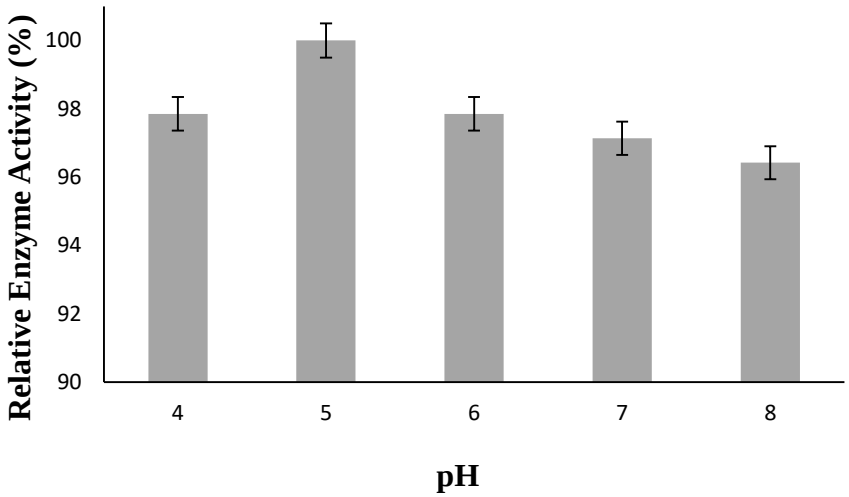


Figure 5. pH stability of α-amylase activity from P. cyclopium

The starch hydrolysis percentage of *P. cyclopium* was also investigated with starches from different sources. The highest hydrolysis percentages were found for corn starch in 5, 10 and 15 min, however rice starch was ranked the highest hydrolysis percentage in 30 minutes (Table 2).

Table 2. Hydrolysis percentage of starches from different sources by α-amylase from *P. cyclopium* under varying incubation durations

Time	**Hydrolysis percentage of different starch (%)**				
min	**Soluble Starch**	**Wheat Starch**	**Corn Starch**	**Potato Starch**	**Rice Starch**
5	8.6	17.4	25.9	16.2	14.7
10	18.0	25.7	31.4	26.0	24.0
15	23.5	32.0	36.8	27.8	35.5
30	41.8	49.9	42.9	37.7	50.7

CONCLUSION

The results of this study is the first information on extracellular amylolytic activity of *P. cyclopium*. In addition, characteristics of biochemical properties for the enzyme are investigated for the first time in literature. Optimum temperature and optimum pH of the enzyme were determined and found to be 5 and 30 °C, respectively. Thermostability and pH stability studies also showed that the crude enzyme was stable for 30 °C and 40 °C up to 97 % and varying pH values from 4 to 8 causes only up to 3% decrease in enzyme activity. Further studies are being made to evaluate the potential of α-amylase from *P. cyclopium* in industrial use.

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GENETIC MARKERS ASSOCIATED WITH CANINE *HIP DYSPLASIA*

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ABSTRACT

Dogs were domesticated from its wolf ancestors about 15,000 years ago. Beginning with the wolf, mankind has created dog breeds that are hunters or herders, big or small, and lean or squat. Thus, dogs became the most diverse mammal species morphologically. On the other hand, the selection of some morphological traits made some dog breeds more susceptible to orthopedic diseases. Hip dysplasia, one of these diseases, is the most common orthopedic disease in dogs. But still, the etiology, mechanobiology, and pathology underlying this disease are not well understood. Canine hip dysplasia (CHD) tends to be more common in some breeds (German Shepherds, Rottweilers, Great Danes atc.) than others and in some lines than others, which indicates that there is a genetic component to the disorder. Various attempts have been made to identify genetic loci underlying CHD development with the goal to develop a DNA test that would outperform, and eventually obviate, phenotype-based selection for CHD. Several initiatives have been made to develop a DNA test to determine the genetic loci underlying CHD. As a result of the investigations, many genetic variations have been reported to be associated with CHD. This review focuses on these markers of dogs.

INTRODUCTION

Mankind has bred a large number of dog breeds, each with its own characteristics, with the artificial selection that it has applied for thousands of years. The dogs whose smell, hearing and sight properties are sharpened have been used for safety and hunting for a very long time. However, during the selection studies, besides the desired characteristics, it has been preserved by being selected in the undesirable properties, and even it has become the fixed characteristic of some races (André et al. 2017). One of these genetic defects is CHD, which is very common in some breeds, while it is rarely seen in some breeds. Hip dysplasia (HD) is an inherited, non-congenital disease that is particularly prevalent in large and giant breeds of dog and the expression of HD genes may be influenced by a number of environmental factors (Ginja et al. 2010). It causes pain and movement restriction, genetic origin and can be detected by radiographic techniques (Figure 1) in dogs that have reached a size of 6-8 months(King 2017). In studies up to now, many genes have been proposed as marker candidates associated with CHD; CHST3 (carbohydrate chondroitin 6

sulfotransferase) gene (Bartolomé et al. 2015), FBN2 (fibrillin 2) gene (Friedenberg. 2011, Lavrijsen et al. 2014), OSTF1 (Osteoclast Stimulating Factor 1), PGM2 (Phosphoglucomutase-2), MYPN (Myopalladin), OLFM1 (Olfactomedin 1), IBSP (Integrin Binding Sialoprotein), FGF12 (Fibroblast Growth Factor 12) genes (Distl et al. 2013), *KSR2 gene* is (Kinase suppressor of Ras 2) gene (Fels and Distl 2014).

Today, security forces use service dogs that specialize in different categories (mine, patrol, bomb, search and rescue, etc.). The education of these dogs is started with births and is completed by the end of one year. Detection of CHD disease at 6-8 months of age in dogs that have a long, laborious and costly training process results in the removal of the CHD dog from education. This can lead to economic losses as well as time and motivation losses. In our research, genetic markers associated with CHD will be investigated in dogs used as breeding centers in centers where breeding and training of service dogs are conducted. It is aimed to reduce the frequency of the disease and contribute to the training and breeding of more effective healthy dogs by removing the dogs carrying the disease-related markers from the population in the dogs in the dog training centers.

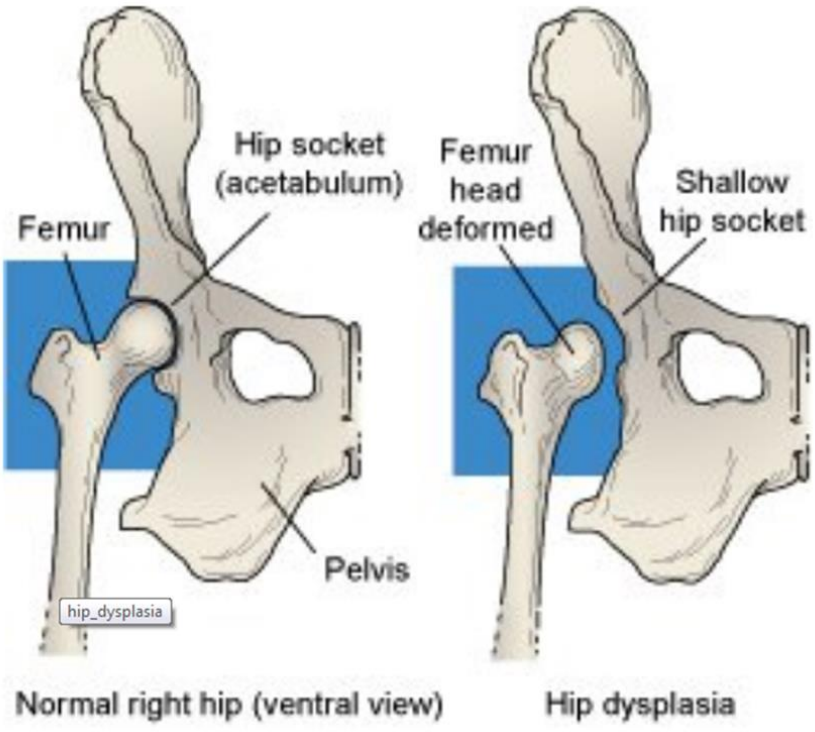

Figure1. Normal right hip and hip dysplasia (Anonymous, 2018)

MATERIAL AND METHODS

German Shepherd, Malinois, and Labrador Retriever dogs 20 blood tissue samples were collected which were raised in the Nevşehir Horse and Dog Training Center and stored at -20 °C in a deep freezer as far as molecular genetic studies are performed. The primer pair was designed using the Primer 3 program in the NCBI (Table 1).

Table 1. We designed Primer pairs for use in this research.

Gene Name/Gene region	Primer sequences	Product size (bp)	Reference
OSTF1/Intron 3	F: AACCTACCTGCCCCTAAGGA	349	This study
	R: GTGAAAGCTGAGGTCTGGGA		
IBSP/Exon 4 and Intron 5	F: TGGCCTGTGCTTTCTCAGTA	555	This study
	R: TTCTAGACTGGGCCCAAATGC		
FGF12/Exon 2and Intron 2	F: CAAACCCTGGGATGGGAGAA	368	This study
	R: CCCCAGCTGAAGGGAATTGT		

PCR amplifications were performed with the PCR master mix (Thermo, K0171) in accordance with the manufacturer's instructions. The PCRs were carried out in volumes of 25 µl using; 12,5 µl PCR Master Mix, 50 ng (5 µl) genomic DNA, 1 µl (5 pmol) each primer, and the rest was ddH2O. The amplification was performed at 95 °C for 3 min, followed by 35 cycles at 95 °C for 30 sec, annealing for OSTF1, IBSP, FGF12 genes at 62, 60, 64 °Crespectively 30 sec, 72 °C for 45 secand a final extension of 72 °C for 10 min on T100 Thermal Cycler (Biorad).

The PCR products were subjected to electrophoresis on 2 % agarose/ethidium bromide gel (Aga003R, Bioshop, Canada) in 1× TBE buffer (TBE-001, New Bioscience). Gels were visualized under UV light and documented in WGD30S Molecular Imager apparatus (Wisd) (Figure 1). PCR products were sequenced by automated fluorescent sequencing using Quick Start Kit (Beckman Coulter, RN608120) and GenomeLab GeXP DNA analyzer (Beckman Coulter). SNPs were detected by BioEdit Sequence Alignment Editor software (Hall, 1999) (Figure 2).

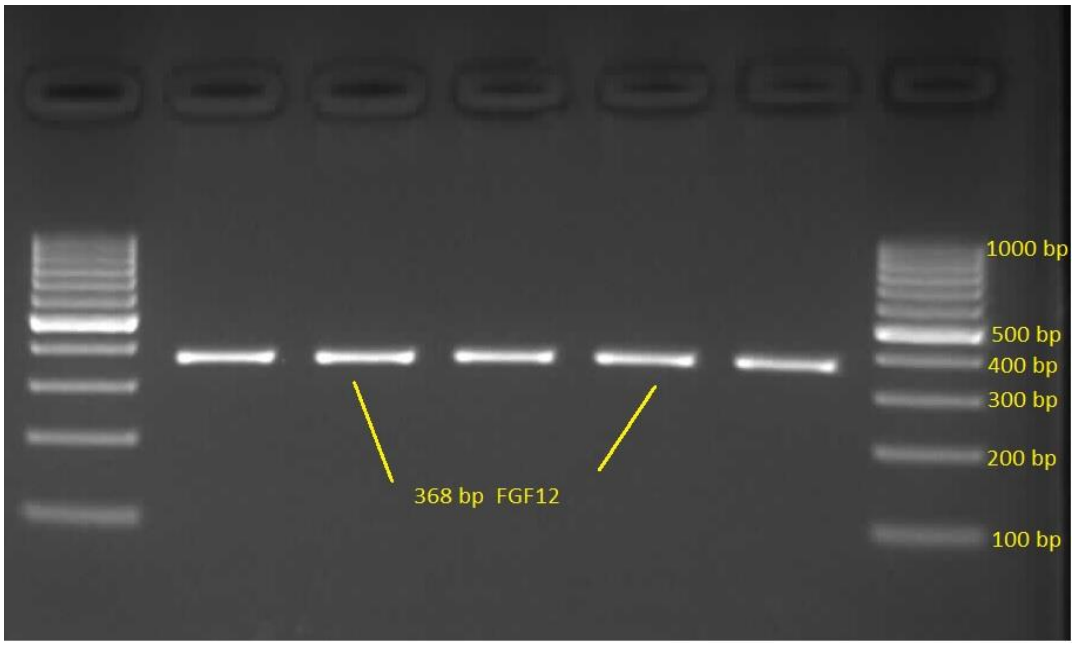

Figure 2. FGF12 gene product on agarose gel electrophoresis

RESULTS

In our studies, we researched 20 example dogs up to now, we could not detect variation in the exon 2-intron 2 region of the FGF12 gene. The FGF12 gene region (part of exon 2-intron 2) was found to be monomorphic in German Shepherd, Malinois, Labrador Retriever breed dogs. However, was found A>G SNP in the exon 4-intron 4 region of the IBSP gene (Figure 2) and A>G SNPin the intron 3 region of the OSTF1 gene (Figure 3). The variations detected in the samples will be investigated in relation to CHD by studying more samples.

Our research goal is to study with 5 different SNPs and in total 210 dogs of which at least 10 percent in morphological trait dogs which have canine hip dysplasia. These will be clinically controlled dogs about healthy or unhealthy. We continue to search with existing Candidate Marker Genes for variations in the genes and collect blood samples from the dogs. Also, we will continue to search additional 2 genes (Carbohydrate Sulfotransferase 3-CHST3 and CX3C Chemokine Receptor 1-CX3CR1).

It hope that there are positive outcomes from this case-control study with Canine hip dysplasia in dogs.

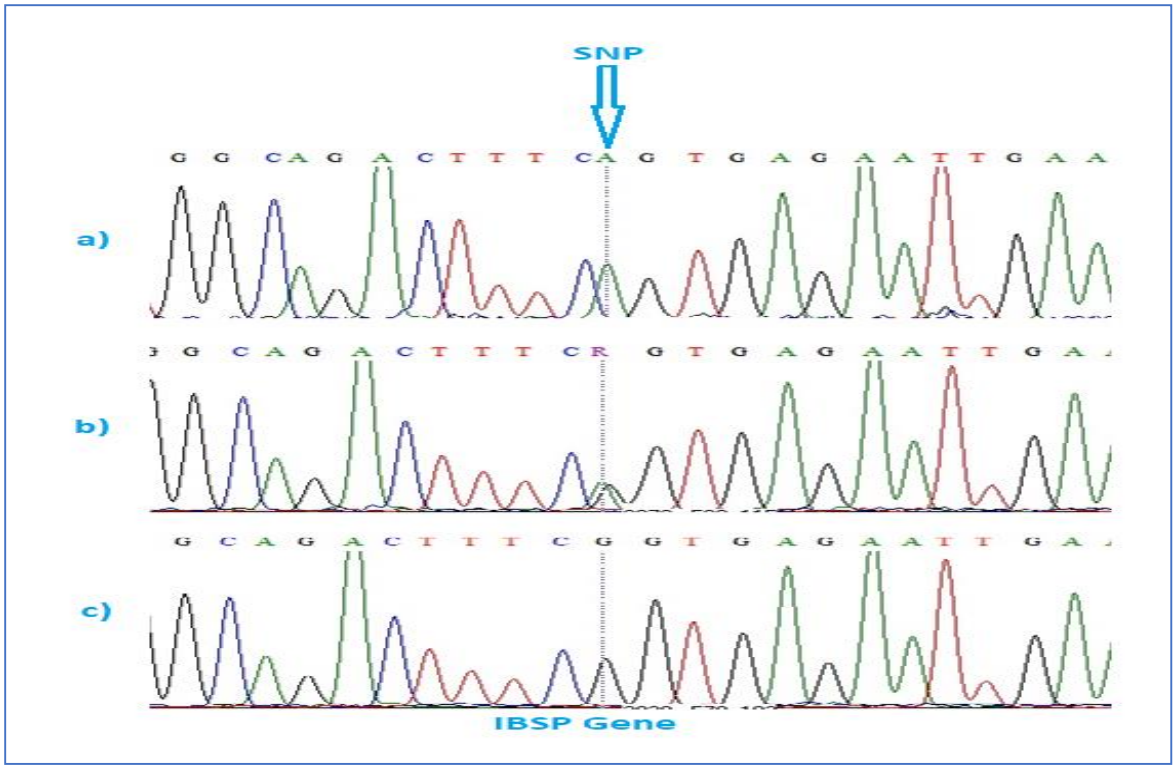

Figure 2. IBSP gene; a) AA genotype, b) AG genotype, c) GG genotype

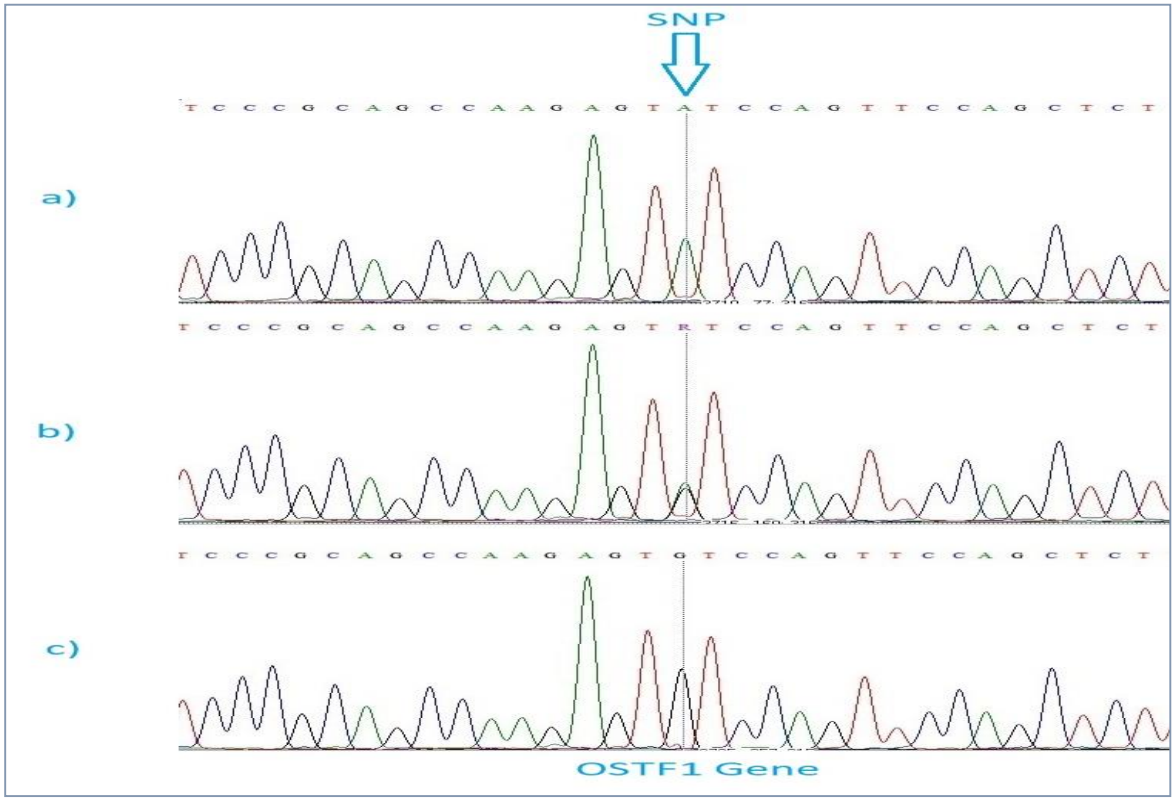

Figure 3. OSTF1 gene; a) AA genotype, b) AG genotype, c) GG genotype

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DNA FINGERPRINTING OF REGISTERED TURKISH RICE (Oryza sativa L) CULTIVARS

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ABSTRACT

Rice is one of the most important crops in the world. It is also existing as one of the most profitable crop in Turkey. The sstu Turkey with 10 SSR markers of registered rice varieties to determine the finger izleirn by identifying fragments of these varieties they created were made in 2016 and 2017. Studies in production in Turkey with registration or have received permission assortment of some 60 local and foreign rice varieties were used as materials. Characterization of 60 varieties with 10 SSR markers, genotyping of varieties combined with 10 SSR fingerprints have been created in the study.

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Key words: Rice (*Oryza sativa* L), fingerprinting, moleculer chracterization, SSR

INTRODUCTION

A number of DNA molecular markers such as RFLP, RAPD, SSR, AFLP and SNP have been increasingly used to investigate germplasm diversity and genetic relationships by plant breeders since 1990 (Melchinger, 1999). Since these markers do not affect the environmental conditions. DNA markers in microsatellite (SSRs) are more advantageous for these studies than other markers and SSRs markers are well distributed within the genome in eukaryotic organisms and they are mostly existing in large amounts and show polymorphism (Morgante and Olivieri, 1993). SSR markers are especially very useful and suitable markers in the identification and purity of varieties (Weising et al. 1997; Ni et al. 2002; Kostova 2006). It is reported in some studies that SSR markers is so appropriate to investigate genetic differences in rice (Powell et al. 1996) indicating that markers are suitable for the investigation of genetic variation for both aromatic and non-aromatic paddy varieties and their relatives (Ghneim et al. 2008). For this purpose, some researchers have developed SSR markers for rice (Zhao and Kochert 1993) and Pervaiz et al. (2009) reported that they developed more than 20 000 SSR markers and were mapped for paddy genome-specific regions.

The genetic diversity and characterization studies with SSR markers in rice were carried out by different researchers. For instance, Hossain et al. (2007) used 30 SSR markers to determine the characterization and differences of the 21 paddy variety in their study. They indicated that number of alleles in the loci were changed between three (RM165, RM219, RM248, RM463, RM470, AVE RM 517) and nine (RM223), and 4.53 alleles in 30 loci and also PIC (polymorphic

information content) values for 30 loci ranged from 0.30 (RM219) to 0.84 (RM223). The most common allele frequency for each locus were ranged from 24% (RM223 and RM334) to 81% (RM219). In another study in Pakistan, Pervaiz et al. (2010) found in 32 polymorphic SSR loci and 3 loci were found to be monoformic in 142 alleles utilizing 75 SSD markers in the local rice genotype. The number of alleles for each marker were ranged from 2 to 13 with an average of 4.4 and allele sizes were ranged from 11bp to 71 bp. Rahman et al. (2012) studied 34 microsatelite molecular markers to determine their characterization and their differentiation in 21 paddy cultivars. The number of alleles per locus were ranged from 2 to 11 and 14 paddy varieties were different from each other. These alleles have been found to be useful in recognizing these varieties for molecular characterization and DNA fingerprinting. According to the PIC (polymorphic information content) values, RM401 was the most appropriate marker in the variety recognition and difference analysis followed by RM566, RM3428, RM463, and RM8094. Based on the study results, five clusters were observed in the UPGMA dendogram and eight SSR markers (RM10713, RM279, RM424, RM6266, RM1155, RM289, RM20224 and RM5371) were produced different alleles in aromatic rice varieties. Then they suggested that these markers could be used for variety recognition and DNA fingerprinting studies.

Choudhary et al. (2013) analyzed the genetic width by using 64 SSR markers in high yield 100 paddy varieties registered in India in between 1970 and 2010. Based on their study results, 52 markers showed polymorphism from these 64 SSR markers and a total of 184 alleles were identified and an average of 3.63 alleles were determined per locus. In another study, Worede et al. (2013) investigated genetic differences using 24 SSR markers in 24 paddy cultivars and found 144 alleles in total. Kumar et al. (2014) studied the local varieties collected from different villages in the Chhattisgarh province with the SSR markers. A total of 44 different alleles were detected from 15 polymorphic SSR markers while the number of alleles per locus ranged from 1 to 4 with the average allele number being 2.93 in their study. Li et al. (2014) used 29 SSR markers to determine polymorphism in 394 Korean rices. They considered total 381 alleles as raw data to understand genetic differences and population structure. Based their studies, the number of alleles per locus was 3 to 44 (mean 13.14) and mean genetic differences (GD) were waxy, low amylose, medium amylose, and high amylose brass, respectively, 0.6014, 0.5922, 0.5858 and 0.7232, respectively. The varieties with high amylose content gave the highest values in terms of both GD and PIC values in their study.

There are some studies also in Turkey on determining on the genetic diversity of Turkish rice varieties. Cömertpay et al. (2013) conducted a study using SSR to determine kinship relationships in commercial varieties. In another study, Bay (2009) used seed storage proteins and 20 RAPD primers in his study and observed that the settlement of varieties was differently from each other but basically similar dendrograms and the two main groups were formed among registered some varieties in Turkey. While Sürek-95, Serhat-92 and Rocca varieties were in a main group, all other varieties were included in another main group in this study. Yüzbaşıoğlu et al (2016) worked polymorphisms using markers for this purpose in the registered 37 rice varieties in Turkey IRAP (inter-retrotransposon amplified polymorphism) and they designed specific primers for most abundant Houba (Tos5 / Osr13) RIRE1 Hopi (Osr27) and Osr30 retrotranposons rice genome. In this study, the highest polymorphism was observed as Hopi (Osr27) with 75%, followed by Osr30 with 57%, Houba with 52% and RIRE1 with 45%.

The aim of this study is to settle of the DNA fingerprints used to determine the identities by combining the fragment lengths and peak images of 60 paddy species utilizing from 10 SSR markers.

MATERIAL AND METHOD

Material

In Turkey, there was 48 rice registered or production permitted varieties in 2016 (when the project has started). The material used in this study was obtained from Trakya Agricultural Research Institute and Trakya Genetik ARGE Consultancy Production Import Export and Marketing Ltd. However, some of the varieties are not in currently in production and also some of local varieties were obtained USDA or Turkish Gene Bank collected from Turkey or Turkish Gene Bank. The list of varieties used in the research is given in Table 1.

Table 1. The list of characterized rice materials in the study (registered or production permitted and local varieties in Turkey until 2016).

#	The name of variety
1	Altınyazı
2	Bafra Yıldızı
3	Demir
4	Durağan
5	Ergene
6	İpsala
7	Kargı
8	Kıral
9	Koral
10	Meriç
11	N1-41T-1T-0T
12	Sürek-95
13	Şumnu
14	Yavuz

METHOD

SSR markers used in the study

There were used 50 suggested SSR markers representing all rice chromosoms obtained from Gramene database in the study (Table 2).

Table 2. SSR markers used in molecular analysis in the study (http://archive.gramene.org/db/markers/ssrtool**)**

Marker	Marker	Ch #	Loc. (cM)	Primer (Forward)	Primer (Revers)	Tm (°C)
SSR1	RM495*	1	2,8	aatccaaggtgcagagatgg	Caacgatgacgaacacaacc	55
SSR2	RM1	1	29,7	gcgaaaacacaatgcaaaaa	Gcgttggttggacctgac	55
SSR3	RM283*	1	31,4	gtctacatgtacccttgttggg	Cggcatgagagtctgtgatg	61
SSR4	RM259	1	54,2	tggagtttgagaggaggg	Cttgttgcatggtgccatgt	55
SSR5	RM312	1	71,6	gtatgcatatttgataagag	Aagtcaccgagtttaccttc	55
SSR6	RM5	1	94,9	tgcaacttctagctgctcga	Gcatccgatcttgatggg	57
SSR7	RM237*	1	115,2	caaatcccgactgctgtcc	Tgggaagagagcactacagc	55
SSR8	RM431*	1	178,3	tcctgcgaactgaagagttg	Agagcaaaaccctggttcac	55
SSR9	RM154*	2	4,8	accctctccgcctcgcctcctc	Ctcctcctcctgcgaccgctcc	61
SSR10	RM452*	2	58,4	ctgatcgagagcgttaaggg	Gggatcaaaccacgtttctg	61
SSR11	RM489	3	29,2	acttgagacgatcggacacc	Tcacccatggatgttgtcag	55
SSR12	OSR13*	3	53,1	catttgtgcgtcacggagta	Agccacagcgcccatctctc	53
SSR13	RM338*	3	108,4	cacaggagcaggagaagagc	Ggcaaaccgatcactcagtc	55
SSR14	RM55	3	168,2	ccgtcgccgtagtagagaag	Tcccggttattttaaggcg	55
SSR15	RM514*	3	216,4	agattgatctcccattcccc	Cacgagcatattactagtgg	55
SSR16	RM307	4	0	gtactaccgacctaccgttcac	Ctgctatgcatgaactgctc	55
SSR17	RM124*	4	150,1	atcgtctgcgttgcggctgctg	Catggatcaccgagctcccccc	67
SSR18	RM507*	5	0	cttaagctccagccgaaatg	Ctcaccctcatcatcgcc	55
SSR19	RM413*	5	26,7	ggcgattcttggatgaagag	Tccccaccaatcttgtcttc	53
SSR20	RM161*	5	96,9	tgcagatgagaagcggcgcctc	Tgtgtcatcagacggcgctccg	61
SSR21	RM178	5	118,8	tcgcgtgaaagataagcggcgc	Gatcaccgttccctccgcctgc	69
SSR22	RM334	5	141,8	gttcagtgttcagtgccacc	Gactttgatctttggtggacg	55
SSR23	RM133*	6	0	ttggattgttttgctggctcgc	Ggaacacggggtcggaagcgac	63
SSR24	RM510	6	20,8	aaccggattagtttctcgcc	Tgaggacgacgagcagattc	57
SSR25	RM454	6	99,3	ctcaagcttagctgctgctg	Gtgatcagtgcaccatagcg	55
SSR26	RM162*	6	108,3	gccagcaaaaccagggatccgg	Caaggtcttgtgcggcttgcgg	61
SSR27	RM125*	7	24,8	atcagcagccatggcagcgacc	Aggggatcatgtgccgaaggcc	63
SSR28	RM11	7	47	Tctcctcttcccccgatc	Atagcgggcgaggcttag	55
SSR29	RM455*	7	65,7	aacaacccaccacctgtctc	Agaaggaaaagggctcgatc	57
SSR30	RM118*	7	96,9	ccaatcggagccaccggagagc	Cacatcctccagcgacgccgag	67
SSR31	RM408*	8	0	caacgagctaacttccgtcc	Actgctacttgggtagctgacc	55
SSR32	RM152*	8	9,4	gaaaccaccacacctcaccg	Ccgtagaccttcttgaagtag	53
SSR33	RM25	8	52,2	ggaaagaatgatcttttcatgg	Ctaccatcaaaaccaatgttc	53
SSR34	RM44*	8	60,9	acgggcaatccgaacaacc	Tcgggaaaacctaccctacc	53
SSR35	RM284*	8	83,7	atctctgatactccatccatcc	Cctgtacgttgatccgaagc	55
SSR36	RM433*	8	116	tgcgctgaactaaacacagc	Agacaaacctggccattcac	53

SSR37	RM447*	8	124,6	Cccttgtgctgtctcctctc	Acgggcttcttctccttctc	55
SSR38	RM316*	9	1,8	ctagttgggcatacgatggc	Acgcttatatgttacgtcaac	55
SSR39	RM105	9	32,1	gtcgtcgacccatcggagccac	Tggtcgaggtggggatcgggtc	63
SSR40	RM215*	9	99,4	caaaatggagcagcaagagc	Tgagcacctccttctctgtag	55
SSR41	RM474	10	0	aagatgtacgggtggcattc	Tatgagctggtgagcaatgg	55
SSR42	RM271*	10	59,4	tcagatctacaattccatcc	Tcggtgagacctagagagcc	55
SSR43	RM171	10	73	aacgcgaggacacgtacttac	Acgagatacgtacgcctttg	55
SSR44	RM484*	10	97,3	tctccctcctcaccattgtc	Tgctgccctctctctctctc	55
SSR45	RM552	11	40,6	cgcagttgtggatttcagtg	Tgctcaacgtttgactgtcc	55
SSR46	RM536*	11	55,1	Tctctcctcttgtttggctc	Acacaccaacacgaccacac	55
SSR47	RM287	11	68,6	Ttccctgttaagagagaaatc	Gtgtatttggtgaaagcaac	55
SSR48	RM144	11	123,2	Tgccctggcgcaaatttgatcc	Gctagaggagatcagatggtagtgc	57
SSR49	RM19	12	20,9	Caaaaacagagcagatgac	Ctcaagatggacgccaaga	55
SSR50	RM277*	12	112.2	Cggtcaaatcatcacctgac	Caaggcttgcaagggaag	55

DNA isolation from one rice grain

Healthy and appropriate amount of DNA was obtained from the single rice grains whose embryo was removed and polished based on determined method in the study of Rajendra Kumar et al. (2007). The obtained gDNAs were used in SSR marking studies. Detailed steps of the method were given below;

• A 1ml of polished grain was placed in 2ml ependorf tubes with the endosperm. 600 lendl extraction buffer (1.25 M NaCl, 0.1 M Tris, 0.25 M EDTA, 2% CTAB, 3% PVP) was added.
• incubated at 370C for 45-60 minutes.
• Two 3mm metal balls were added into each sample tube.
• Samples were ruptured at a speed of 30 rpm for 5 minutes in the tissue shredder (RETSCH MM400) to allow the tissues to break apart completely.
• 600 ilavel of chloroform was added.
• Samples were mixed gently and centrifuged at 12,000 rpm for 10 minutes at room temperature.
• The supernatant was transferred to a new 1.5 ml tube and equal volume of isoproponol was added.
• They were centrifuged at 12,000 rpm for 10 minutes at room temperature.
• The supernatant was discarded and washed twice with 70% ETOH.
• Pellets were allowed to dry at room temperature under a fume hood.
• Dried DNA pellets were dissolved with sterile water to 50-75 50l depending on the desired DNA concentration.

The measurement of DNA contents and quality analysis

OPTIZEN NanoQ Spectrophotometer was used for the DNA quantification of the samples, and the absorbance value of the samples at 260 nm wavelength was measured and the amount of gDNA was determined in ng / µl. In addition, ODD260 / OD280 (for nucleic acid purity) wavelength values were observed to determine whether protein contamination was present in gDNA. Samples completed process having been assayed on an agarose gel at a concentration of 0.8% and run at

120V 80mA for one hour. After electrophoresis, the DNA quality of the samples was examined under gel UV light.

Multiplying SSR locus with PCR

SSR loci were amplified by PCR using 50 primers given in Table 2 in gDNAs isolated from rice grains. The components and concentrations used for this process are given in Table 3.
PCR reactions were prepared in 25 del volume. In addition to the PCR reaction prepared for each primer in 64 subjects, a negative control without DNA was added. The prepared reaction mixtures were incubated at the temperature conditions presented in Table 3 to amplify the SSR loci.
The primary sequences of these SSR markers were synthesized and made ready for use by dilution at a concentration of 10 tirilM. In the characterized genotypes, each SSR marker locus was amplified by PCR and analyzed. The components given in Table 3 were used for all PCRs and the reaction temperatures using for each marker using the appropriate binding temperatures.
The DNA fragments amplified by the reaction were analyzed in the capillary electrophoresis system (AATI Fragment Analyzer).

Table 3. The protocol PCR and its used components

Used PCR Components		PCR Protocols			
PCR Components	Concentration	Cycle	Temperature (°C)	Duration	Cycle Number
Gdna	50 ng	1	94	2 dk	1
PCR Buffer	1X	2	94	45 sn	35
$MgCl_2$	2.5 µM		53 - 61*	1 dk	
DNTPs	0,2 mM)		72	1 dk	
Primer (forward)	1 µM	3	72	10 dk	1
Primer (revers)	1 µM				
Taq DNA polimeraz	1 U				
dH_2O	13,8 µl				

*Each SSR marker was changed based on primer Tm values.

Electrophoresis Analysis

Capillary electrophoresis was used in the study. Thus, high resolution separation and analysis of each SSR locus was carried out for each sample. The DNA fragments of the PCR-amplified SSR loci were in the range of about 100-300 bp and for the analysis of PCR products, 1 to 5000 bp lar DNA ladder SS was preferred.
PCR products were diluted 1/5 by using Dilution Buffer and the final volume to 96 1/ plate was 24 1/l. As the capillary electrophoresis device used contains 12 capillaries in parallel, 1-500b of DNA ladder was added at the end of each row of PCR products. After the samples were placed in the wells in the plates, mineral oil was added to the samples to avoid evaporation. The

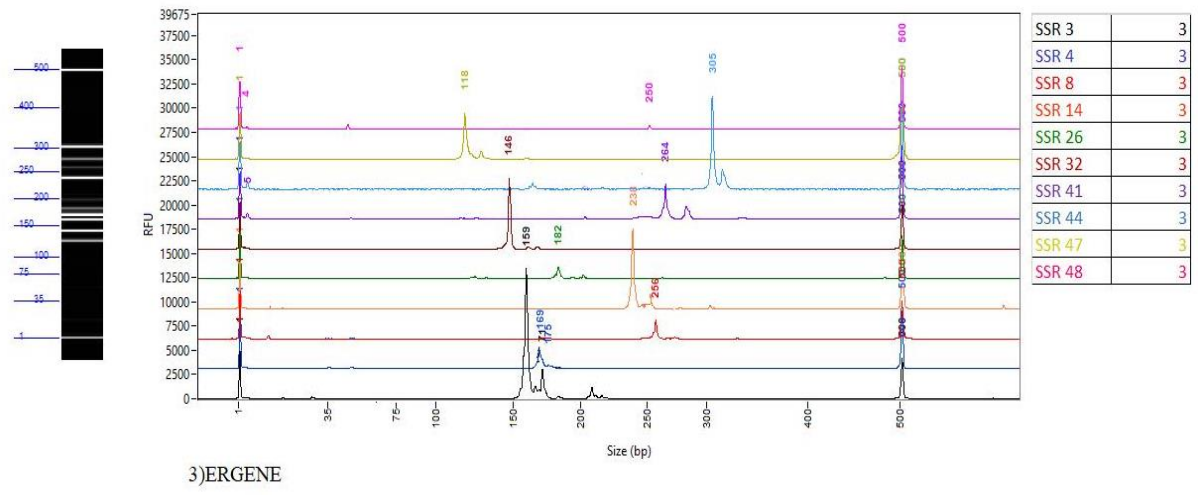
electrophoresis procedure was carried out at a rate of 9.0 kV, 80.0 min. Capillary electrophoresis results were analyzed using the ProSize sistem software program developed specifically for the system.

RESULTS

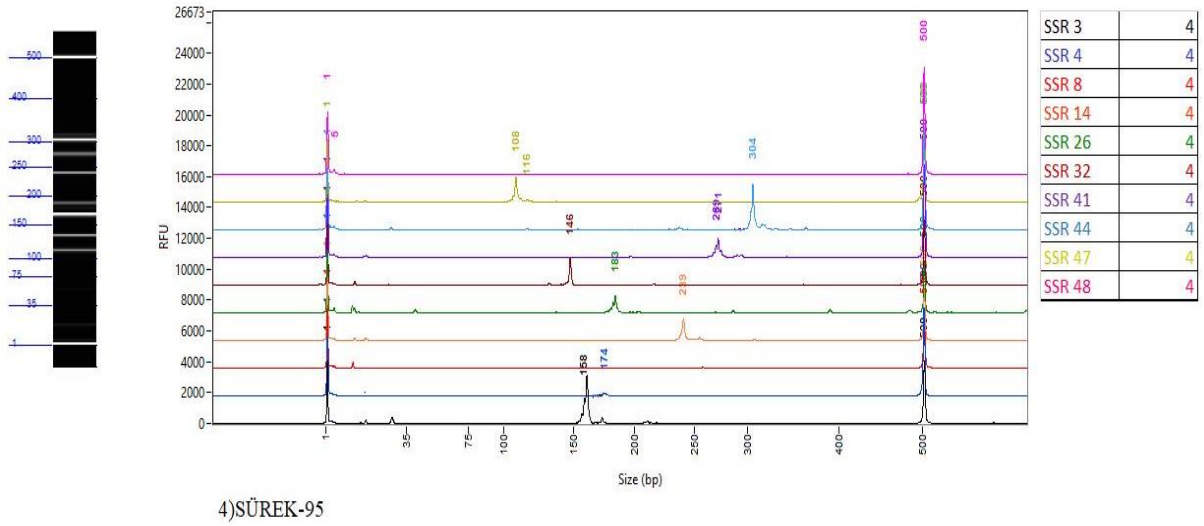
Initially, 50 SSR markers were proposed in the Gramenegen database to demonstrate genetic diversity in rice. Then, 10 SSR markers which show polymorphism in these markers were selected and their peaks were combined by removing the fingerprints of the varieties with these 10 SSR markers.

Based on study results, Capillary electrophoresis and classified ProSize sistem software program results were given in Figure 1. The 10 selected SSR markers were given as barcode pictures for each variety respectively in the figures.

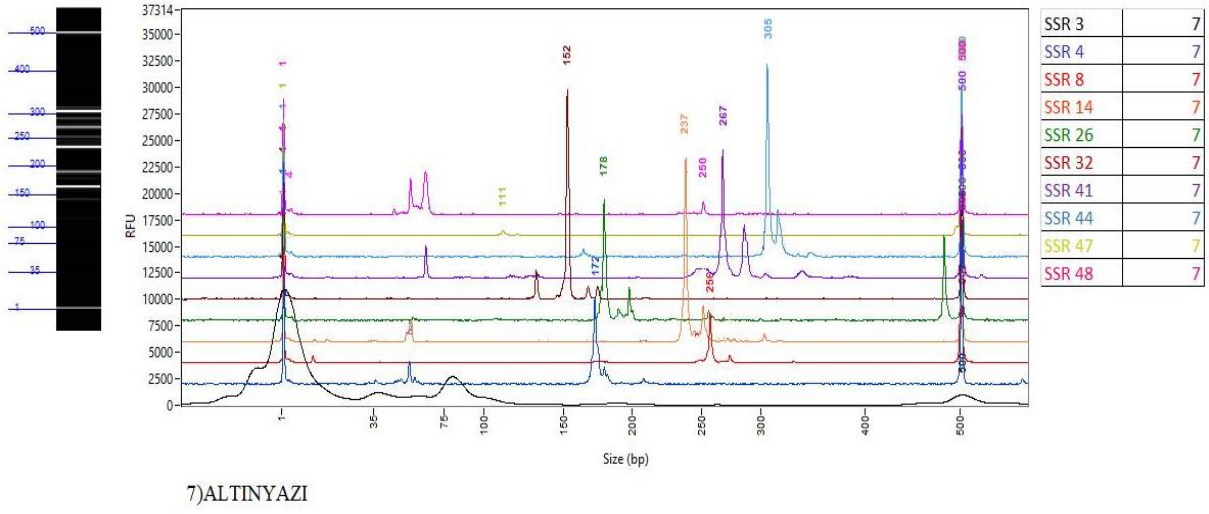
Figure 1. Some registered and production permitted rice variety barcodes utilizing SSR markers



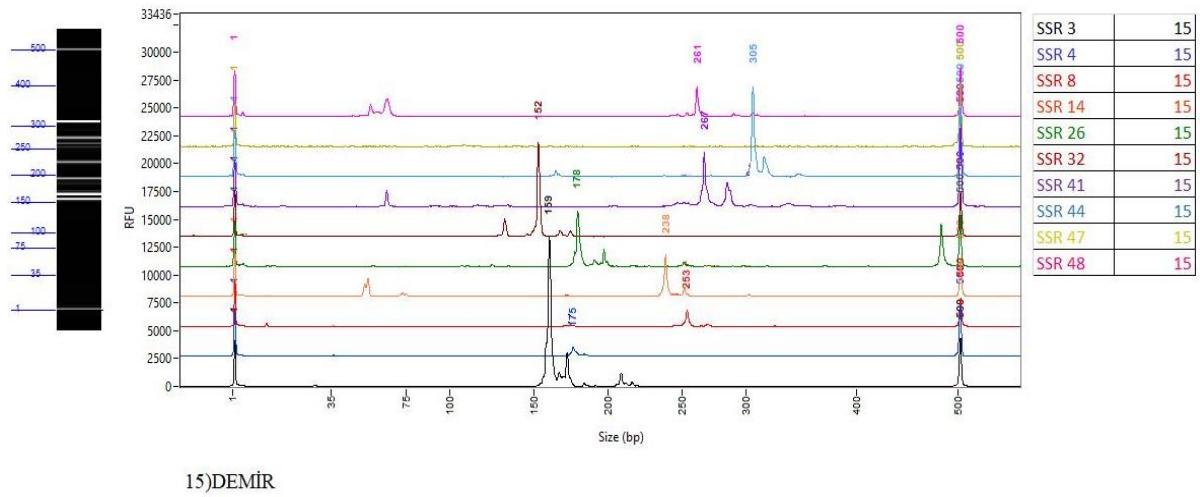
1- Ergene rice variety molecular barcode with 10 SSR markers



2- Sürek-95 rice variety molecular barcode with 10 SSR markers

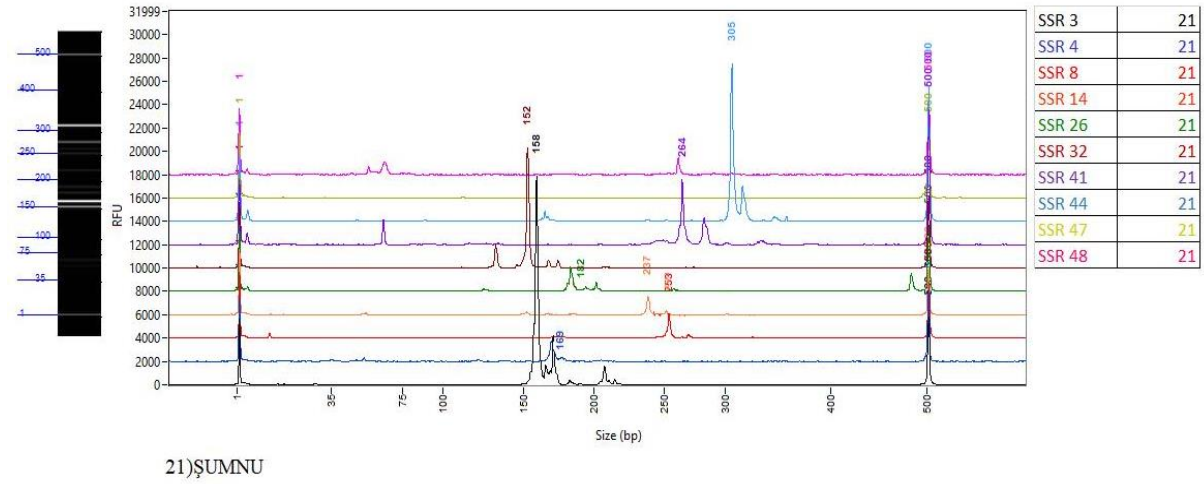


3- Altınyazı rice variety molecular barcode with 10 SSR markers



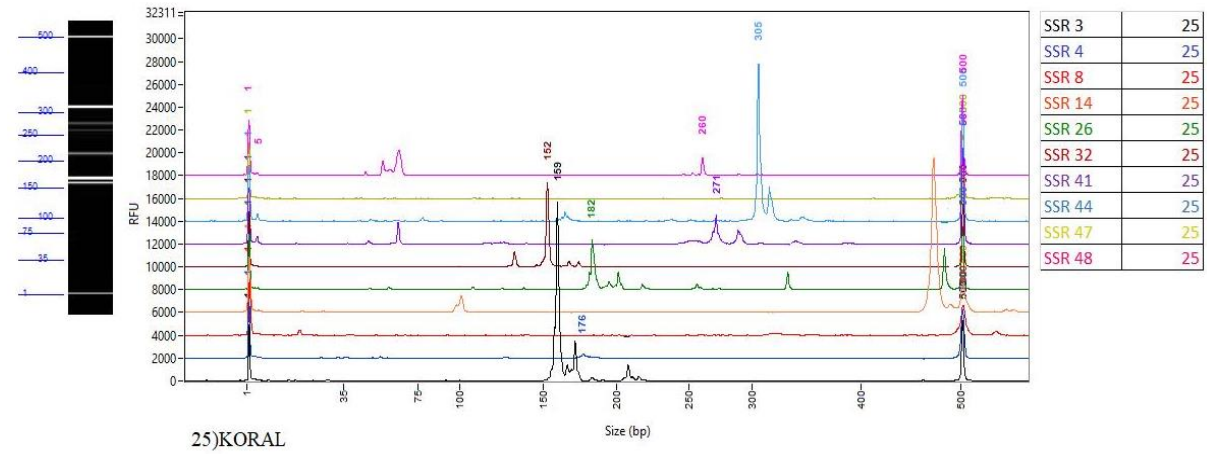
15)DEMİR

4- Demir rice variety molecular barcode with 10 SSR markers



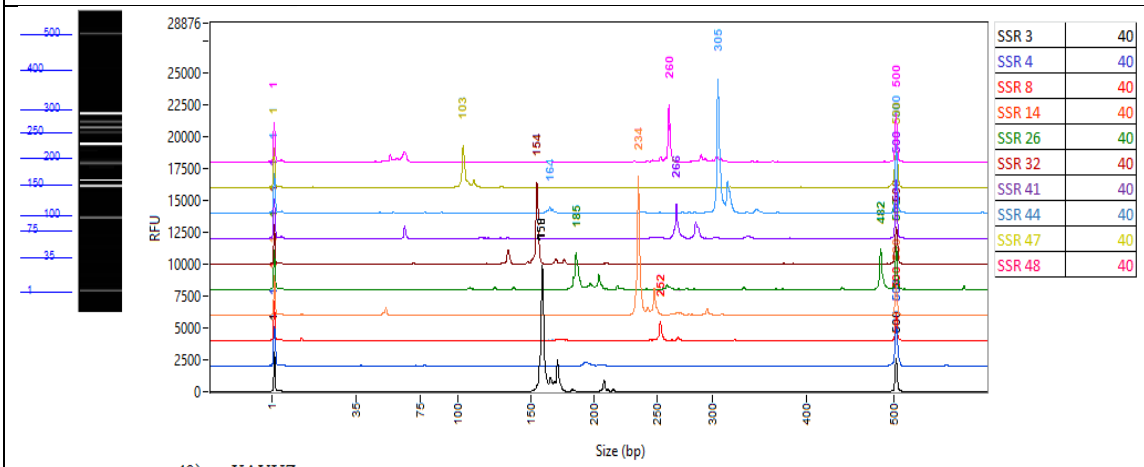
21)ŞUMNU

5- Şumnu rice variety molecular barcode with 10 SSR markers



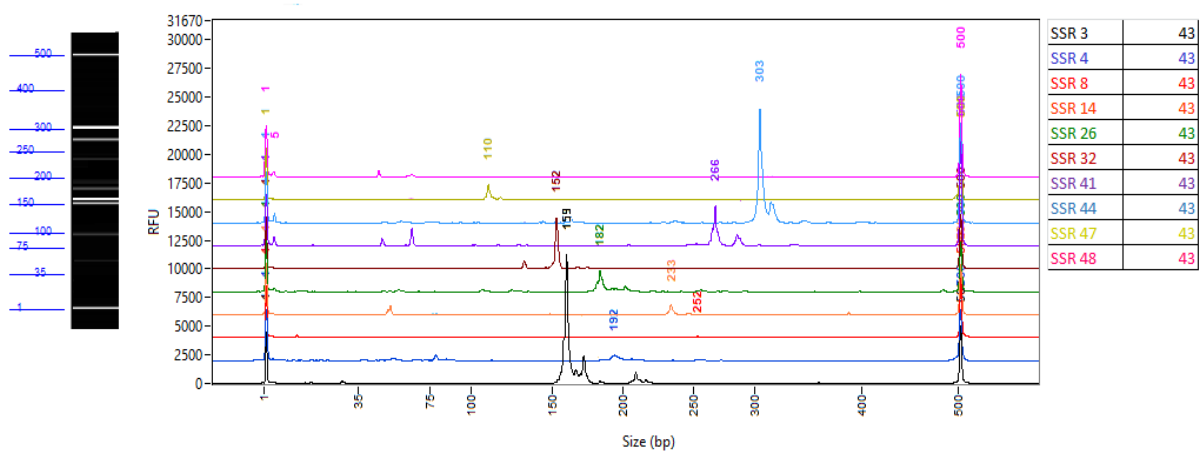
25)KORAL

6- Koral rice variety molecular barcode with 10 SSR markers



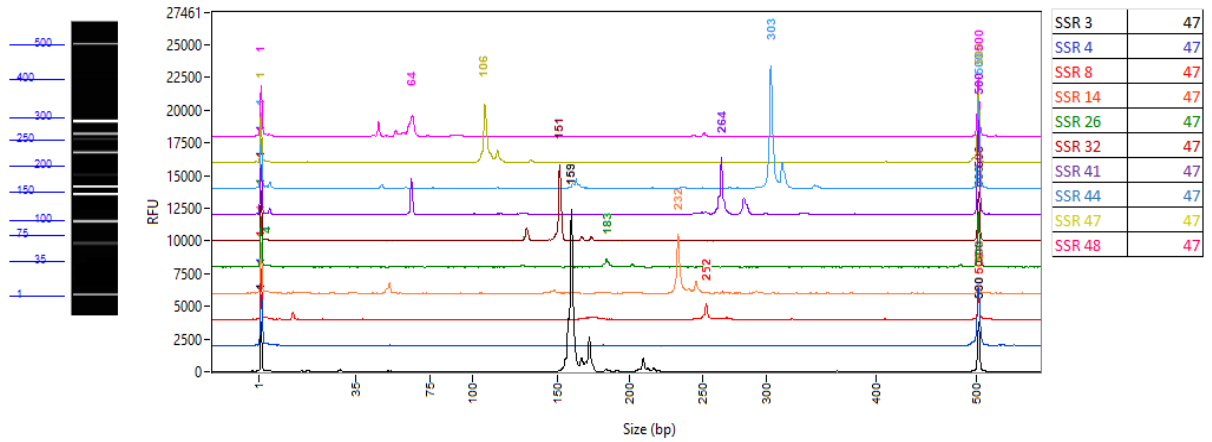
40) YAVUZ

7- Yavuz rice variety molecular barcode with 10 SSR markers



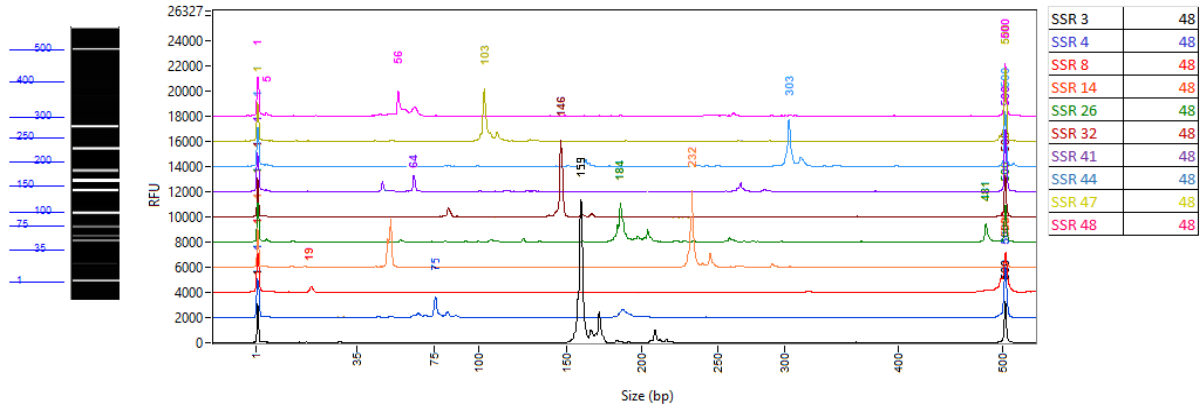
43) DURAĞAN

8- Durağan rice variety molecular barcode with 10 SSR markers



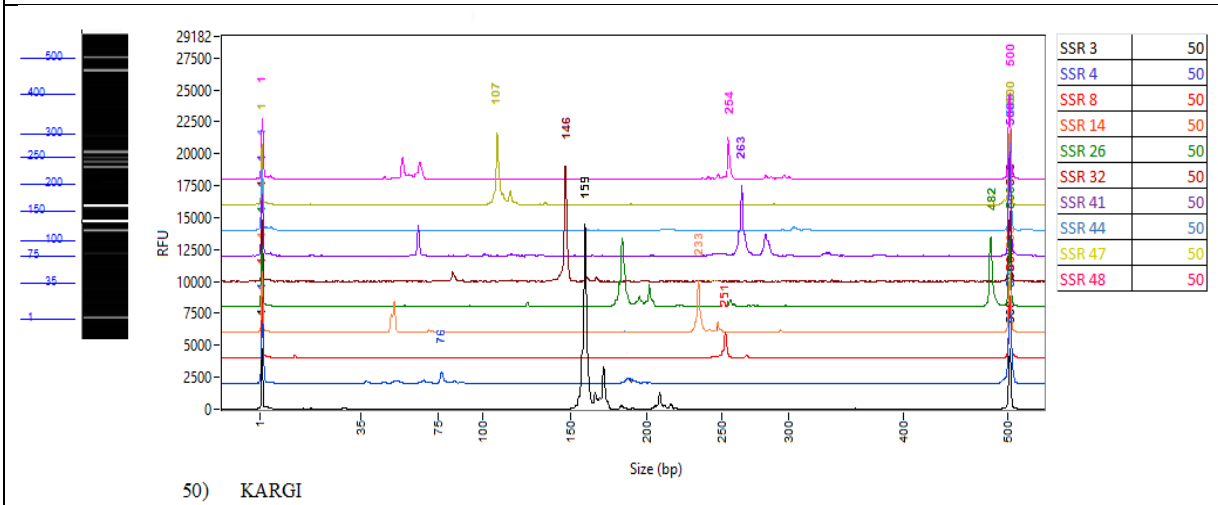
47) KRAL

9- Kral rice variety molecular barcode with 10 SSR markers



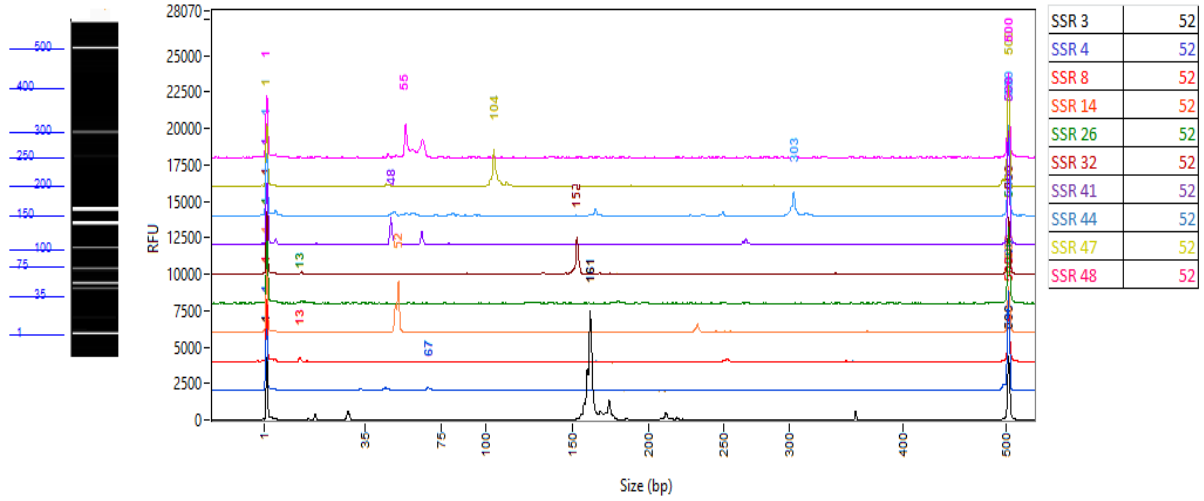
48) MERİÇ

10- Meriç rice variety molecular barcode with 10 SSR markers



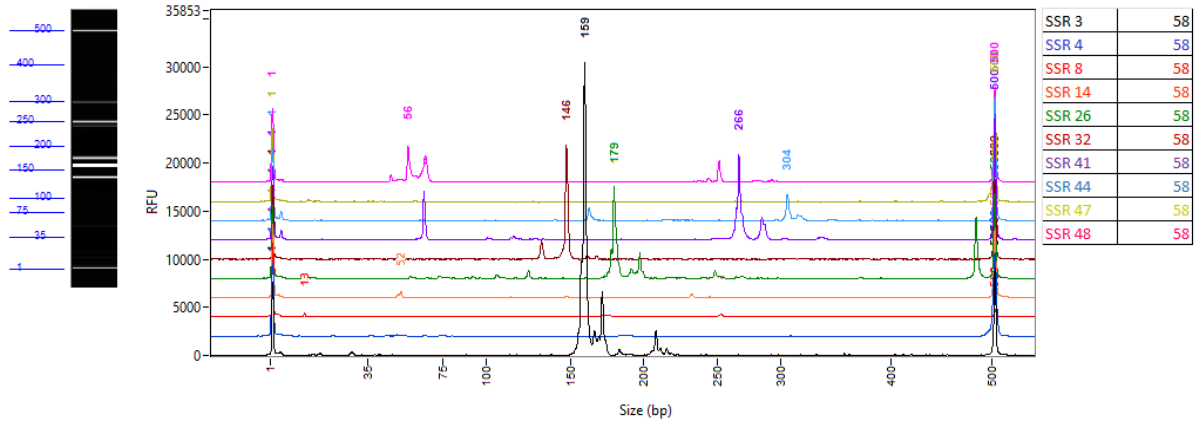
50) KARGI

11- Kargı rice variety molecular barcode with 10 SSR markers



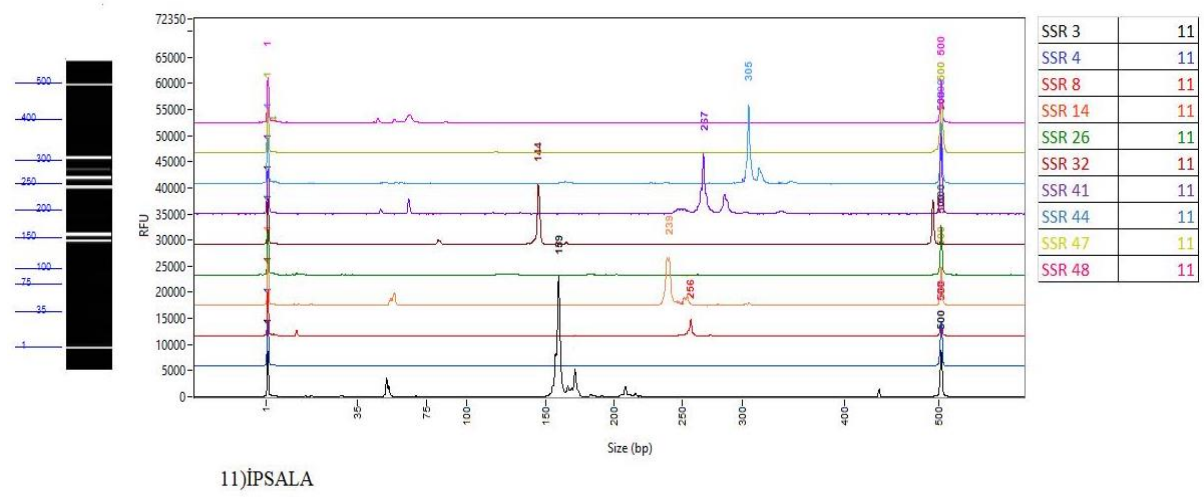
52) N1-4IT-IT-OT

12- N1-4IT-IT-OT rice variety molecular barcode with 10 SSR markers



58) BAFRAYILDIZI

13- Bafrayıldızı rice variety molecular barcode with 10 SSR markers



14- İpsala rice variety molecular barcode with 10 SSR markers

CONCLUSIONS

All registered and production permitted rice cultivars in Turkey were characterized in the study. There are molecular characterization results od some rice cultivars. While rice was selling and using non coating in Turkey, then there were not easy and fully to recognize for belonging which cultivars in the market. Based on study results, utilizing ten SSR markers it could be identified scientifically from one naked grain selling in the market belonging which cultivar studied in the research.

ACKNOWLEDGEMENT

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NICKEL ACCUMULATION AND ANTIOXIDANT ACTIVITY OF COMMON REED (PHRAGMITES AUSTRALIS) IN THE ALBANIAN PART OF LAKE OHRID

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ABSTRACT

The presence of some mines that produced nickel, chromium, iron and coal which now most are not functional is one of the main factors of metal contamination in the Albanian part of Lake Ohrid. In addition to this factor agriculture, chemical and metallurgical industry, serpentine soils endanger the lake. The purpose of our research was to evaluate the accumulation of nickel from the aquatic plant of the lake Common reed and to estimate the antioxidant activity of the leaves in the spring season. Concentration of heavy metals was determinated with Atomic Absorption Spectrofotometry (AAS) method whereas the total antioxidative activity with DPPH (2.2-diphenyl-1- picrylhydrazil) radical elimination method. The results showed that the largest accumulation of nickel was at the point of the former Fe-Ni mine 6.98 ± 0.5468 mg/kg and the difference was significant in comparison with entry of Pogradec ($p < 0.05$) and Lin village ($p < 0.01$). As far as antioxidant activity was concerned, it was highest at the point of the former Fe-Ni mine 431.88 ± 28.298 mg Trolox/g dry tissue weight but the difference was not significant compared to other points**.**

Keywords: *Ohrid, Ni, Antioxidant, Pollution, Mine, Reed*

INTRODUCTION

Nickel is the 24rt by weight, the fifth most abundant element after iron, oxygen, magnesium and silicon in the Earth crust. Although it is found in some oxidation states, Ni (II) is the form mostly found in the environment. This element has extensive commercial and industrial use. Usually used for the production of stainless steel. Nickel, which is quite undesirable in the environment, stems from natural resources and anthropogenic activities. Wind-blown dust, derived from the weathering of rocks and soils, volcanic emissions, forest fires and vegetation, represent natural resources. (Cempel and Nikel, 2016) Nickel is the essential nutritient for high plants without the presence of which cannot complete the life cycle. (Brown et al., 1987) But the presence of nickel in high concentrations in the medium has adverse effects on the plant. High concentrations of nickel cause chlorosis and necrosis in plants, preventing iron uptake and metabolism. (Molas and Baran, 2004) Reactive oxygen species, also known as oxygen-free radicals, can be described as a group of reactive molecules and free radicals that can be formed

during aerobic metabolic processes of cells (Bras et al., 2005 ; Czarn and Jarmuszkiewicz, 2006). Mitochondria is the organelle where occurs reactive oxygen species formation. Mitochondria has antioxidant mechanism to protect itself from harmful effects of reactive oxygen species. These mechanisms include reducement of reactive oxygen species formation and removal of existed reactive oxygen species (Czarn and Jarmuszkiewicz, 2006) There are proteins, enzymes (superoxide dismutase (Cu, Zn-SOD, Mn-SOD), catalase, glutathione peroxidase) and non-enzymatic antioxidants (vitamins (Vitamin C (ascorbic acid), Vitamin E (alpha-tocopherol), carotenoids), thiol antioxidants (glutathione, thioredoxin and lipoic acid), flavonoids, selenium, zinc) that play role in antioxidant defense mechanism of mitochondria (Jomova and Valko, 2011; Valko et al. 2006). Research carried out in the Albanian part of Lake Ohrid shows high values of nickel in sediment. (Vogel et al., 2011; Malaj et al., 2012) Sources of this metal may be mines located on the shore of the lake. Although they don't work, their wastes carry heavy metals to the lake after the rain. (Avramovski et al., 2003) Heavy metals can accumulate from aquatic plants with roots. (Zwolsman et al., 1990) One of these plants is Common reed (*Phragmites australis*) that grows at several points of the lake (Albrecht et al., 2008). This metals represent one of the environmental stress factors that stimulate the generation of reactive oxygen species (Sen A., 2012) The purpose of our research was to evaluate the accumulation of nickel from the stem of aquatic plant of the lake Common reed and to estimate the antioxidant activity of the leaves in the spring season.

MATERIAL AND METHODS

The research was conducted in the spring of 2015. The sampling was done at three points in Lake Ohrid on the side that belongs to Albania, precisely at the entrance of Pogradec (area occupied by *Phragmites australis*), near the former Fe-Ni mine and in the village of Lin. Lin village has served as a reference point due to the distance it has from the mine. The macrophytes samples have been collected by hand from the shore of the lake, then the leaves have been removed and the stems are placed in sterile glasses filled with the water taken from the lake. The prepared samples are brought to the laboratory in the ice containers at a temperature of about 5°C, then part of the stems and leaves are dried at room temperature while the rest is placed at a temperature of -20°C.

Determination of heavy metals

The heavy metals are determined by the atomic absorption spectrometry (AAS) method by the Perkin-Elmer 1200 B model apparatus. The samples are initially dried at room temperature and then subjected to liquid mineralization. The dry materials (0.5-1 g) are diluted with 10 ml of concentrated nitric acid (HNO_3) and 10 ml of concentrated hydrochloric acid (HCl) at a temperature of 400°C for about 1 hour. Then the mineralized samples were mixed with distilled water and filtered with Watman 0.45 μm filtration paper. The filter is placed in volumetric flask with a volume of 50 ml and is leveled up to the mark with distilled water. AAS calibration is done with a standard of 1000 ppm for each element.

Evaluation of Antioxidant Activity

The DPPH (2.2-diphenyl-l-picrylhydrazyl) radical scavenging assay and Trolox (2.5 mM in methanol) were used as reference substances following the protocol of Chizzola et al. The calibration curve was constructed using Trolox (0-50 mg/mL). The absorbance of the decolorizing process was measured at 515 nm. The results are expressed as the DPPH mg TE/g dm.

Statistical analysis

Diferences between the points are expressed using ANOVA and a Post Hoc Tukey test. Data were expressed as means ± standard error (SE) for each samples (n= 6) for heavy metals and (n=4) for antioxidant activity. P values of $p \leq .0.01$ were considered to be significant.

RESULTS AND DISCUSSION

The results of heavy metals showed that the largest accumulation of nickel in stem was at the point of the former Fe-Ni mine 6.98 ± 0.54 mg kg $^{-1}$ and the difference was significant in comparison with entry of Pogradec ($p < 0.05$) and Lin village ($p < 0.01$). As far as antioxidant activity was concerned, it was highest at the point of the former Fe-Ni mine 431.88 ± 28.29 mg Trolox/g dry tissue weight but the difference was not significant compared to other points. (Table 1).

Table 1. DPPH antioxidant activity in leaves and nickel values in stems of Common reed (*Phragmites australis*) at three sample points on the Albanian side of Lake Ohrid

	Entry of Pogradec	**Former Fe-Ni mine**	**Lin village**
mg TE / g dm	396.41 ± 54.52	431.88 ± 28.29	397.29 ± 65.71
Ni mg kg $^{-1}$	5.31 ± 0.4956	6.98± 0.5468 **	1.83 ± 0.3656

(The results are presented as Mean ± SD, significant to the reference point of Lin ** $p < 0.01$ and * $p < 0.05$) (n = 6)

Heavy metals in general and in this case chromium and nickel may have toxic effects on plants. According to Allen et al., nickel values over the value of 5 mg kg^{-1} are toxic to plants. In the current research, the toxic values of nickel have been ascertained at all points except the point of Lin. (Allen et al., 1989)

According to Bani et al., the plants collected in the serpentine soils from Librazhd-Pogradec region have shown great accumulation of nickel. This accumulation has changed from plant to plant where Alyssum markgrafite from Brassicaceae family has shown greater accumulation 5234 mg kg $^{-1}$ while lower accumulation has shown in Aegilops triuncialis from the L. Poaceae family 9.81 mg kg$^{-1.}$ According to them 2% of nickel comes from iron type laterites and a piece of nickel comes from chromium mines. Generally, the recorded values are high but in the plant belonging to the same family with P. australis is recorded a value close to the value obtained at the point of the former Fe-Ni mines. (Bani et al., 2013)

According to Zeneli et al., plants grown in industrial contaminated areas in Kosovo have shown bioaccumulation of some metals such as Pb, Cd, Zn, Mn and Fe. These metals have stimulated the production of reactive oxygen species. While plants grown in these areas have shown accumulation of heavy metals, the total antioxidative capacity was lower compared to the coparison group. According to them, Fe influences the reduction of total antioxidative capacity. (Zeneli et al.2013)

CONLUSIONS

The former Fe-Ni mine was the point where there was greater accumulation of nickel in stem. This value is a toxic value for the plant. The total antioxidant activity did not show much difference between the points. The lowest value is recorded at the entrance of Pogradec where except heavy metals in the creation of oxidative stress may have an impact agriculture.

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OXIDATIVE STRESS IN COMMON REED (PHRAGMITES AUSTRALIS) IN THE ALBANIAN PART OF LAKE OHRID

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ABSTRACT

Aquatic plants are constantly exposed to various environmental factors that cause reactive oxygen species (ROS) production. One of these factors that endanger the Ohrid Lake's plants is the accumulation of heavy metals by plants. Various metals like nickel, chromium increase ROS production and with it the malondialdehyde (MDA) values in the plants. The purpose of this research was to evaluate oxidative stress in common reed by measuring the values of MDA and to see the possibility of using of MDA as a bioindicator for the assessment of pollution in the Lake Ohrid. Plant samples were analyzed by Health Packer spectrophotometric method. The research conducted on three points at the entrance of Pogradec, at the former factory of ferro nickel and at the village Lin. Although not significant the greater values of MDA (4.8300 ± 0.5742 micromol/g wet tissue) in stem of common reed had in the factory point. Our results showed that a further study on MDA as a bioindicator on various parts of the plant and even in various aquatic plants should be continued.

Keywords: *Ohrid, MDA, plant, oxidative stress, common reed*

INTRODUCTION

Oxidative stress is one of the negative effects of environmental stress factors on high sessile plants. This stress caused by the overproduction of reactive oxygen species (ROS) may be the result of exposure to drought, salinity, heavy metals, nutritional disorders or radioactivity. In ROS are present the superoxide radical ($O_2^{\cdot -}$), the singlet oxygen ($^1O_2$), the hydroxyl radical ($OH^{\cdot}$), the hydroperoxyl radical ($HO_2^{\cdot}$), the hydrogen peroxide (H_2O_2) like that. These highly reactive and partially reduced oxygen particles can be produced in different parts of the plant such as chloroplasts, mitochondria, peroxisomes, plasma membranes, apoplasts, endoplasmic reticulum, and cell-wall. Their production occurs both during the normal metabolism of the plant and under the induction of environmental factors. Exposure to environmental stress factors increases the overproduction of ROS and this increase oxidative stress. Oxidative stress, which is defined as the imbalance of ROS production and antioxidant protection, causes damage in different parts of the cell such as proteins, lipids, carbohydrates and DNA. (Rao et al., 2006, Foyer and Noctor, 2005; Desikan et al. al., 2008; Gill and Tuteja, 2010, Sen, 2012) One of the environmental stress factors

that causes ROS overproduction in plants are heavy metals. (Sen, 2012) In moderate concentrations, heavy metals do not affect plant growth, but if the tolerance threshold passes, this may lead to the death of the plant. In their tissues aquatic macrophytes can accumulate heavy metals in considerable quantities. (Kovacks et al., 1984; Unadkat and Parikh, 2017) A typical aquatic aquatic plant that can accumulate heavy metals in different tissues in different parts of it like roots, tems, and leaves is common reed (*Phragmites australis*) (Bonnano et al., 2010). This plant is quite widespread along the shore of the Albanian part of Lake Ohrid. According to Albrecht et al, the macrophytes of this lake can grow in four zones of the so-called Clodophora zone, Chara zone, Phragmites australis zone, Potamogeton zone (Albrecht et al., 2008)

From this point of view, the purpose of this study was to evaluate the oxidative stress of the *Phragmites australis* stem in three points of the lake and to predict whether MDA could be a parameter of lake plants that could serve as a bioindicator for evaluation of pollution in the lake.

MATERIAL AND METHODS

Study area

The research was conducted in Ohrid Lake, which is one of the oldest lakes in the world located in Balkan Peninsula (Popovska and Bonacci 2007). Lake Ohrid is a transboundary lake shared between the Former Yugoslav Republic of Macedonia (FYROM) and the Republic of Albania (Fig. 1). The lake is located at 693.5m above sea level and has a maximum length of 30.4 km (N–S), a maximum width of 14.7 km (W–E), surface area of 358 km^2, and a tub-shaped bathymetry with a maximum water depth of 293 m, a mean water depth of ~151 m, and a total volume of 50 (Wagner et al. 2017). Despite the fact that the Lake Ohrid is under the influence of the anthropogenic factor this lake is still considered as an oligotrophic lake with high amounts of dissolved oxygen in the deep waters (Matzinger et al, 2007). Another special characteristic of this ecosystem is high biodiversity and extremely high endemicity rates, Ohrid Lake hosts various endemic species from the entire food chain (Albrecht and Wilke, 2008; Trajanovska et al., 2014).

Figure 1. Map of Lake Ohrid (https://www.google.com/maps/place/Lake+Ohrid)

Sampling

The research was conducted in the spring of 2015. The sampling was done at three points in Lake Ohrid on the side that belongs to Albania, precisely at the entrance of Pogradec (area occupied by *Phragmites australis*), near the former Fe-Ni mine and in the village of Lin. Lin village has served as a reference point due to its distance from the mine. The macrophytes samples have been collected by hand from the shore of the lake, then the leaves have been removed and only the stems are placed in sterile glasses filled with the water taken from the lake. The prepared samples are brought to the laboratory in the ice containers at a temperature of about 5°C, then part of the stems are dried at room temperature while the rest stalk is placed at a temperature of -20°C.

Determination of malondialdehyde

Malondialdehyde (MDA) was determined according to Heath and Packer 1968 with some modifications. The principle of this method is based on the reaction of MDA with thiobarbituric acid, whereby a colored complex is formed. Firstly, frozen samples of plant stems with countermeasure 0.5 g are homogenized with 10% tricloracetic acid (TCA). Then the homogenate was centrifuged at 7000 X g for 10 minutes. One ml homogenate is mixed with two ml of 0.5% thiobarbituric acid dissolved in 10% TCA. This mixture is placed at 95°C for 45 minutes and then cooled to room temperature. After centrifugation, the formed color is read at 532 nm in the spectrophotometer. Calculation of the MDA value is done with extinction coefficient 1.56×10^5 M^{-1} cm^{-1} expressed as micromol MDA per gram of wet tissue.

Statistical analysis

Diferences between the points are expressed using ANOVA and a Post Hoc Tukey test. Data were expressed as means ± standard error (SE) for each samples (n=3) for MDA. P values of $p \leq .0.01$ were considered to be significant.

RESULTS AND DISCUSSION

Although not significant the greater values of MDA (4.8300 ± 0.5742 micromol/g wet tissue) in stem of common reed had in the factory point. (Table 1)

Table 1. MDA values in sem of Common reed (*Phragmites australis*) at three sample points on the Albanian part of Lake Ohrid

	Entry of Pogradec	**Former Fe-Ni mine**	**Lin village**
µmol MDA/g wet tissue	3.9433 ± 0.3953	4.8300 ± 0.5742	3.5567± 0.7304

(The results are presented as Mean ± SD, significant to the reference point of Lin ** p <0.01 and *p<0.05)(n=3)

Lake Ohrid is endangered by pollution. The presence of old mining waste around the lake shore causes heavy metals such as chromium, nickel, molybdenum, and iron to be transported to the lake. They are mainly transferred to the lake after the rain. In addition to metals and agriculture, the inadequacy of water treatment at certain points of the lake threatens it with pollution. (Avramovski et al., 2003) Previous works has shown high values of chromium, nickel, iron and molybdenum in sediment. (Malaj et al. 2012; Vogel et al,) According to Malaj et al., 2012 mining points have had exceptionally high values of a number of metals in sediment compared to Lin and the points that were far from the mines. The presence of metal in high amounts of sediment causes them to accumulate from aquatic plants of roots. Their accumulation varies from plant to plant and their main accumulation is in the root. (Bonnano et al., 2012) One of these plants that grow in the lake Ohrid is common reed (Phragmites australis). (Albrecht et al., 2008) Heavy metals are considered as environmental pollutants that cause physiological reactions to plants. One of the risks that can cause plants is ROS production. ROS changes normal cellular physiology by degrading proteins, lipids, nucleic acids and enzymes. (Panda and Patra 2016) As a marker that evaluates the degree of lipid peroxidation is malondialdehyde MDA. There are numerous researches carried out on plants especially on leaves of plant during which MDA is estimated.

Malar et al., 2014 investigated the impact of lead on MDA in water hyacinths by treating it with different lead doses of 100 to 100 mg / L with a determination that there is an increase in MDA values up to 400 mg / L. But then at higher doses there was a decrease in MDA.

Jyknus et al., 2012 investigated the impacts of some heavy metals like Cu, Zn, Ni, Cr, Pb, Cd on oxidative stress and growth of spring barley. These metals induced MDA levels with increased metal concentration. In addition to the MDA increase, there has been a reduction of the dry biomass. A moderate increase in MDA has been the case with chromium and nickel treatment. Since Cr has been continuously increasing with dose increase, the highest dose of 1000 micromol has been shown to decrease.

A research conducted by Rahman et al., 2012 has shown that chromium treatment with different concentrations of 0.5 to 3 mg / L in mangrove seedlings has been continuously increasing MDA and the maximum value reached in 3 mg / L. The change was not significant only in the lowest dosage of treatment compared to the control.

Treatment with Co, Ni, Cd (as SO_4), Cr (as dichromate) and Pb (as nitrate) at the rate of 0.25 mM. has caused significant increase in MDA in the case of Cr, Co, Cd and Pb (Gopal and Khurana, 2013) .

CONCLUSIONS

Our results showed that a further study on MDA as a bioindicator on various parts of the plant and even in various aquatic plants should be continued in the Albanian part of Lake Ohrid.

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