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Brachytydeus armindae (Momen & Lundqvist, 2005); a new tydeid record (Acari : Prostigmata), with a revised key to *Brachytydeus* Thor species of Turkey

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ABSTRACT
Tydeoidea species (Acari: Prostigmata) are little and soft-bodied creatures and globally distributed. In the world, Tydeidae comprises 328 species in 30 genera, and *Brachytydeus* has the most number of species with 200. Tydeoidea presented by 23 species while *Brachytydeus* comprises eight species, gathered in orchards and shrub trees in Turkey. *Brachytydeus armindae* (Momen & Lundqvist 2005) (Acari: Tydeidae), found in association with *Thuja orientalis* L. (Cupressaceae) trees and stored wheat, is reported as a new species of tydeid fauna of Turkey. Distribution and host details of *B. armindae* are provided along with descriptive photos and illustrations. An updated key to *Brachytydeus* Thor species is given.

Keywords: *Mites; Tydeidae; Brachytydeus armindae; stored products; Thuja*

1. Introduction

Tydeoid mites (Acari: Prostigmata) are little and soft-bodied creatures with globally scattering. They live soil and herbaceous plants to relationships with invertebrates, slugs and backboned. They are mostly herbivore, mycophagist, pollen feeders, parasites on insects or decaying consumers. They are capable of surviving in regions with extreme climates like from the North to South Pole ecosystems (Krantz & Walter 2009). Tydeidae comprises 328 species in 30 genera, and *Brachytydeus* has a high amount of species with 200, subsequently *Tydeus* Koch have 50 species (Da-Silva et al. 2016).

Çobanoğlu & Kazmierski (1999), reported 4 *Brachytydeus* species collected form orchards and shrubs, *Brachytydeus amica* (Kazmierski 1998), *B. ferula* Baker 1944, *B. pulchra* (Oudemans 1929); *B. reticulata* (Oudemans 1928) were mentioned in Thrace region, and all around Turkey. Following that *B. livshitzi* (Kuznetzov 1974), *B. obnoxia* (Kuznetzov et Zapletina 1972, in Livshitz et al. 1972) and *B. paraobliqua* (Panou & Emmanuel 1996) were mentioned from hazelnut orchards in Northern part of country (Ozman-Sullivan et al. 2005; Akyazı et al. 2017). *Brachytydeus maga* (Kuznetzov & Livshitz 1973), *Tydeus kochi* (Oudemans1928), *Tydeus californicus* (Banks 1904) and *Tydeus plumosus* Karg 1975 were mentioned recently for Tydeidae family of Turkey which are found in association with stored wheat, vegetables and ornamental plants (Kumral & Çobanoğlu 2016; Ueckermann et al. 2019; Çobanoğlu et al. 2020). Till date, eight species were reported under *Brachytydeus* Thor (Ueckerman et al. 2019). This paper aims to report the occurrence of *Brachytydeus armindae* in Turkey and present a revised key for *Brachytydeus* genus.

2. Material and Methods

The mites were collected randomly from *Thuja orientalis* L. (Cupressaceae) trees in Istanbul and different storage facilities of stored wheat bran in Diyarbakır and Mardin during 2013-2014.

In storages, wheat seed and debris were gathered from the different level of the bulk with a split probe, depending on the size of the pile. Finally, they were mixed and taken into half kg amounts. The plant samples were taken by randomly to the shoots and green parts of the *Thuja* trees, in 2008.

The mite specimens were observed under a stereomicroscope and they were pulled out by a Berlese funnel. The collected mite specimen was preserved in 70% ethyl-alcohol. Samples were clarified with lactophenol solution, and microscopic preparations were made at Hoyer's medium. Mounted mites were detected by a phase-contrast Leica microscope and measured by a Leica software image analysing system.

The taxonomical characteristics measurements were made in micrometres (µm). The gnathosoma was measured from the base of the chelicerae to the tip. Palpus; from femur to tip of tarsus, the length of the body measurement was considered from the base of the chelicerae to posterior end of the body. The width of the body was measured at the level of setae (*c2*). Setae were measured from the setal base to their apex and the legs from trochanter to the end of the tarsal claw. Both setae and solenidia counts of the leg segments were considered and solenidia indicated in brackets.

The following publications were consulted for the identification of the species: Momen & Lundqvist (2005), Da- Silva et al. (2016) and Ueckermann et al. (2019). The voucher specimen were kept among the mite collection at Ankara University, Plant Protection Department.

3. Results and Discussion

Tydeiodea Kramer 1877
Tydeidae Kramer 1877
Brachytydeus Thor 1931

Brachytydeus armindae (Momen & Lundqvist 2005); Da -Silva et al. (2016): 10.
Tydeus armindae Momen & Lundqvist 2005: 229

Diagnosis: Opisthosoma striated transversal between setae *d1* to behind setae *f1*; some of the dorsal setae length, longer than half distance next behind.

Female (n= 2)

Dorsum (Figures 1-13, 15). Idiosomal length 305, width 218, with sparsely serrated dorsal setae, trichobothria (sci) filiform, slender and smooth. Setae *f2*, *h1*, *h2* and *ps1* longer than the other setae and blunt shaped. Dorsum simply striated, lack of reticulation. Prodorsum striated longitudinally, on opisthosoma transversal striation between setae c1 and d.

Dorsal setae lengths of: *vi* 17-23, *ve* 13-14, *sci* 45, *sce* 16, *c1* 14-15, *c2* 15-24, *d* 15-16, e 16, *f1 20-24*, *f2 20-27*, h1 *21-25*, *h2* 22- 26, *ps1* 26; *sci-sce* 39; *h1-h2* 24; *h2-c2* 46; *c1-c1* 42; *d-d* 28; *d-c1* 67 (Figs 1-3; 5-7).

Venter (Figures 14, 16). Distance of setae *3a* and *4a* longitudinally striated. Genital cleft flanked by 6 pairs of short setae *(g1*-g6) (9-5) and 4 pairs agential setae (*ag1-ag4*) (11-12). Anal opening surrounded by anal setae *ps2* (11-17).

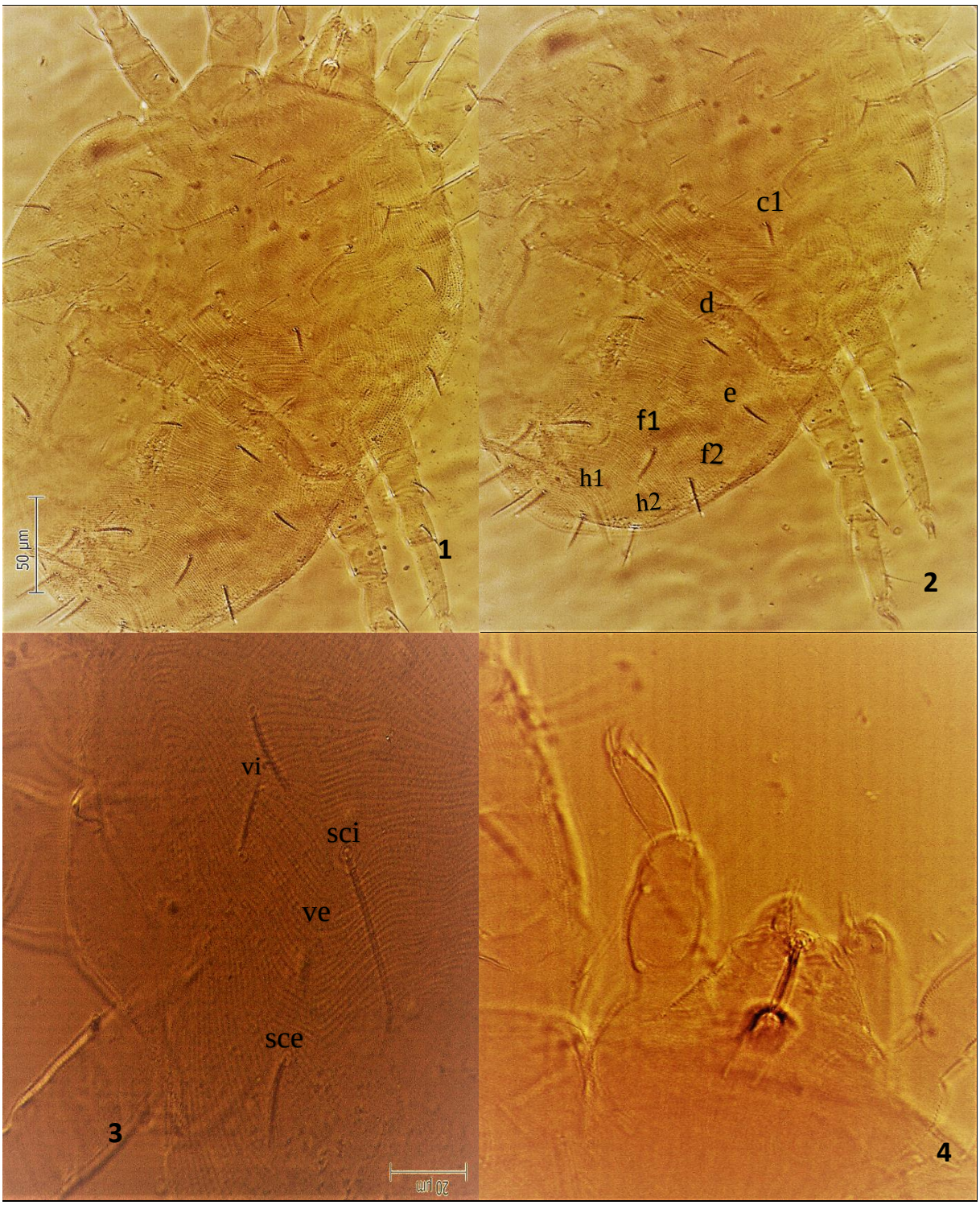
Gnathosoma (Figures 4, 8-9). Palp 71-81 long. The setal formula of palp (genua-tarsus) 2-2-6 (ω, not included in count), seta (*d*) forked distally. Palp tibial claw as long as palp tarsi.

Legs (Figs 10-14). Legs: I 170, II 132, III 140, IV158. Leg segments setal formulae: Tarsi 8(ω)-6(ω)-5-5, tibiae 3(+1)-2-2-2, genua 3-2-1-1, femora 3-3-2-1, trochanters 1-0-1-0. Solenidia on tarsi II (3) shorter than solenidia on tarsi I (8-11). All tarsi include claws and empodium with a well-developed claw. Seta (κ) on tibia I is forked.

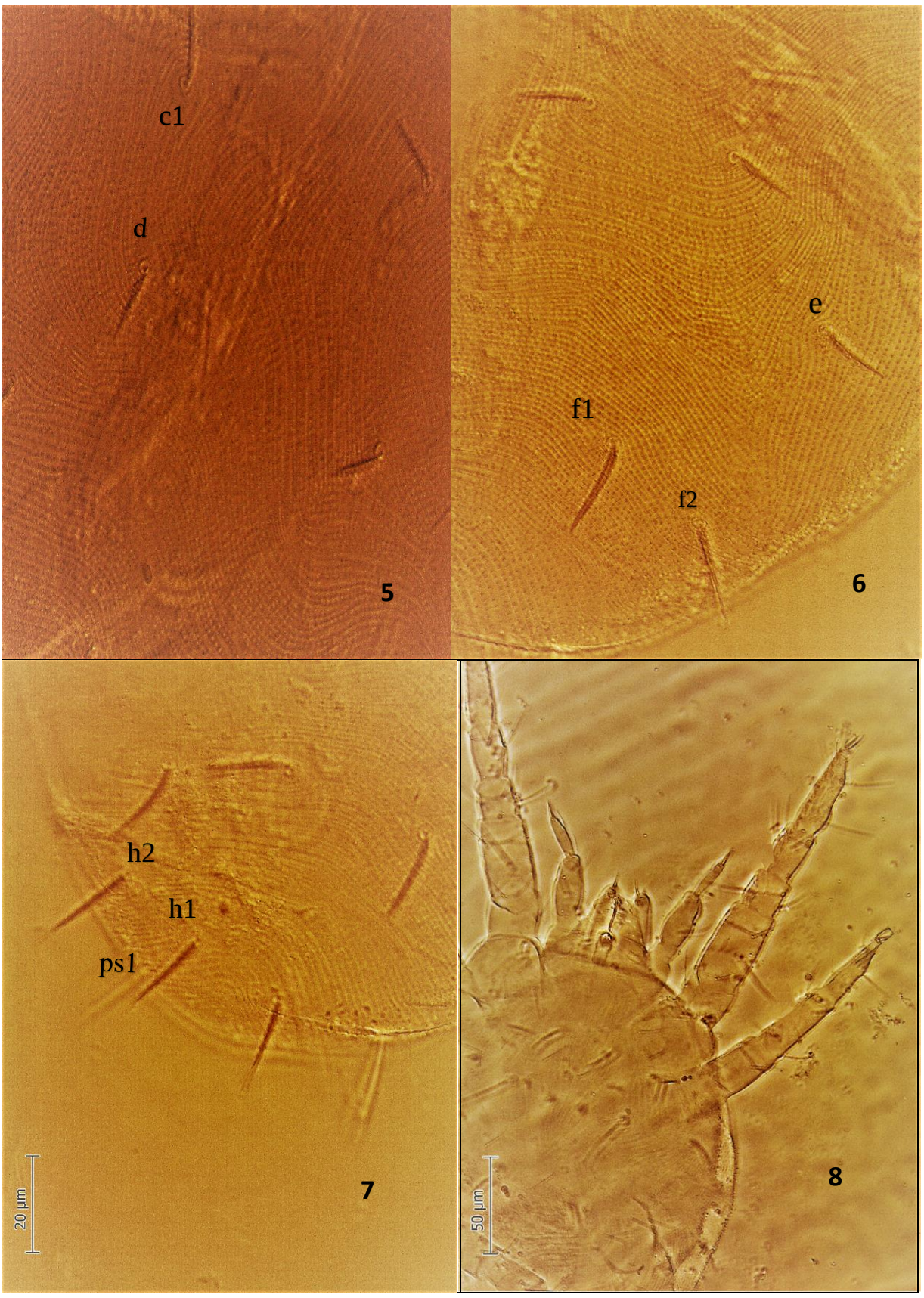
Material Examined: (1♀), (02.03.2008) *Thuja orientalis* L. (Cupressaceae), Kuleli-Istanbul; (40°58'46.2"N; 29°03'19.8"E); (1♀), (03-07-2014), wheat bran, Diyarbakır (37° 5´65.066"N: 40°1´40.041"E).

Distribution: It was reported from Sweden on lichens in mosses (Momen & Lundqvist 2005; Da-Silva et al. 2016) and Turkey with this study.

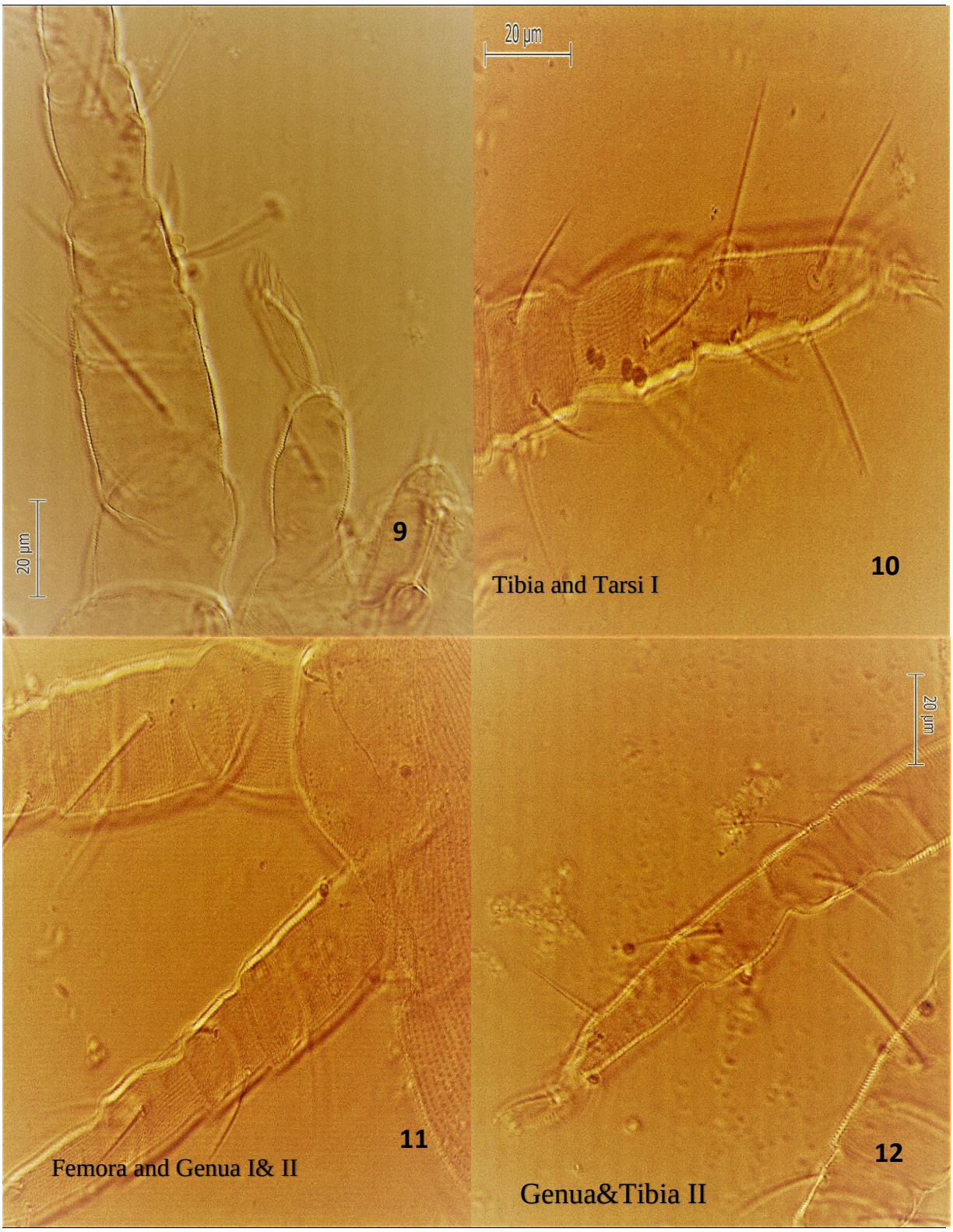
Remarks. *Brachytydeus armindae* specimens from Turkey is similar to the original description in all taxonomical characteristics. This species is separated easily from the other closest species by thick and blunt setae of *f2*, *h2* and *Ps2*. *Brachytydeus armindae* is a new record for the Tydeiidae fauna of Turkey.



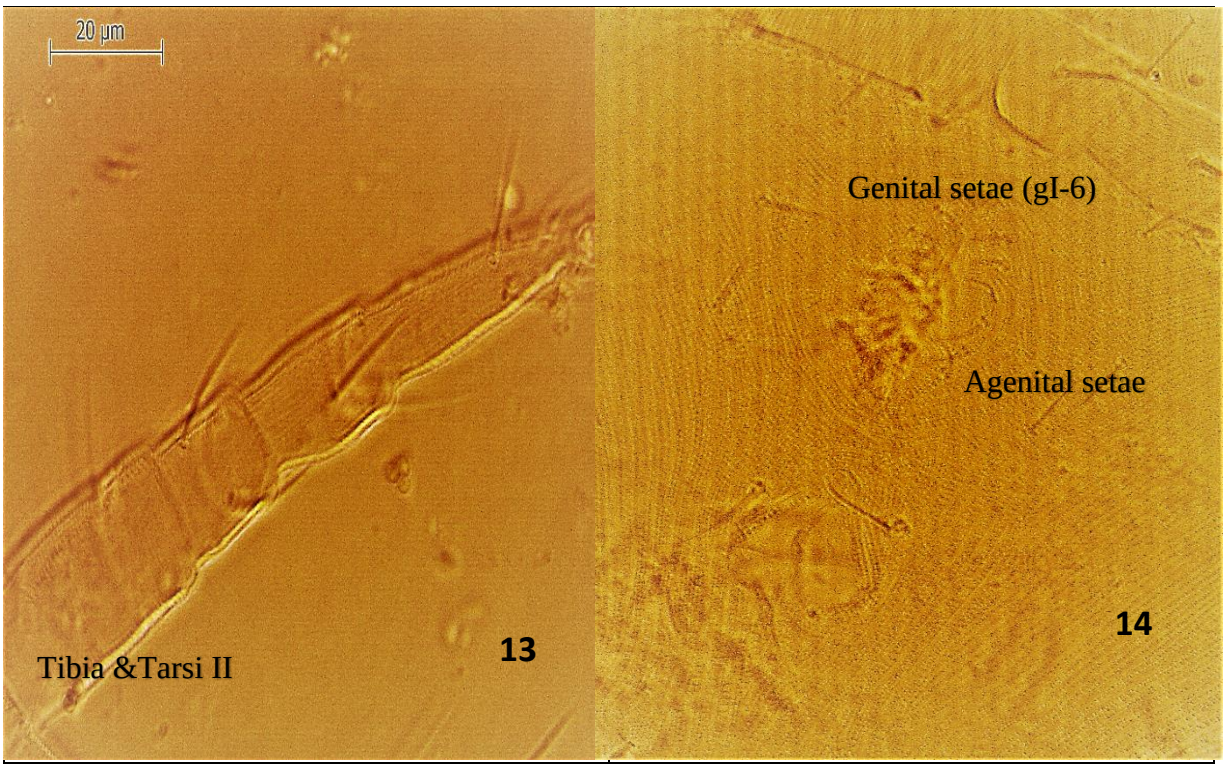
Figures 1-4. Brachytydeus armindae **(Momen & Lundqvist) (Female), 1. General view, 2. Idiosoma, 3. Prodorsum, 4. Chelicera.**



Figures 5-8. ***Brachytydeus armindae*** **(Momen & Lundqvist) (Female), 5. Dorsal stetae (*c1-d*), 6. Dorsal setae (*e, f1, f2*), 7. Opisthosomal setae (*h*ı*-h3, ps1*), 8. Gnathosoma.**



Figures 9-12. ***Brachytydeus armindae*** **(Momen & Lundqvist) (Female), 9. Palp, 10. Tibia and Tarsi I, 11. Femora and Genua I & II, 12. Genua & Tibia II.**



Figures 3-14. ***Brachytydeus armindae*** **(Momen & Lundqvist) (Female), 13. Tibia & Tibia II, 14. Ventral view.**

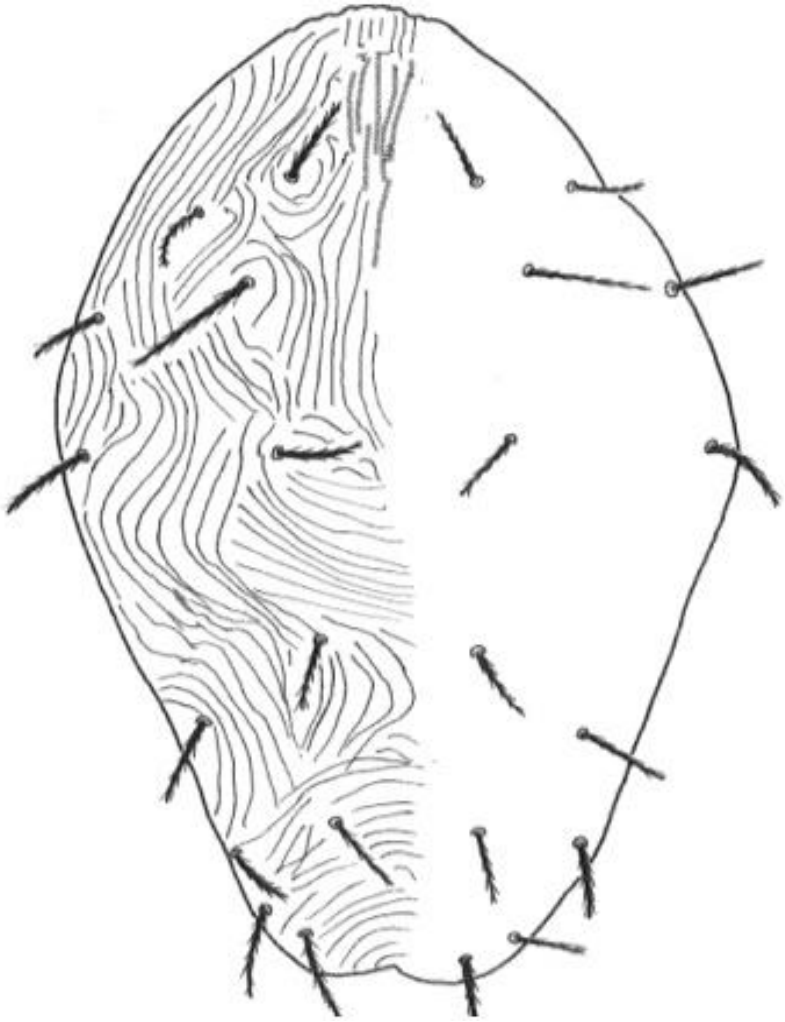


Figure 15- ***Brachytydeus armindae*** **(Momen & Lundqvist) (Female), dorsal view.**

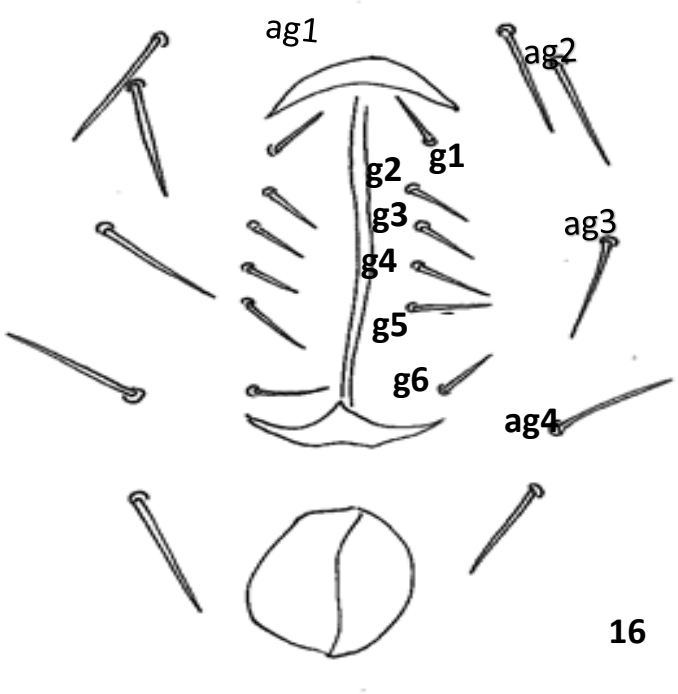


Figure 16- *Brachytydeus armindae* (Momen & Lundqvist) (Female), female genital area.

Key to the *Brachytydeus* Thor, species of Turkey, Females (Based on Ueckermann et al. 2019)

1 -Two setae on femur III ……..…*Brachytydeus* Thor……..2

-One seta on femur III…..……..……..……..……..…. *Tydeus* Koch

2 -Dorsum with completely reticulated…..…….…….....3

-Dorsum with smooth small areas, without complete reticulation…….4

3 -Dorsum with setae various-shaped caudally, at most half length of the distance between afterwards ……....……..…..…….…..…..*B. pulchra* (Oudemans)

-Dorsum with rod like setae, longer than half the distances or longer than distances between afterwards…..…….…….... *B. reticulata* (Oudemans)

4 -Hysterosoma striated with vertically between setae *d1* or extending to *f1*……..…… 5

-Hysterosoma striated with horizontally between setae *d1* ……..…..…..…..6

5 -Setae *h1-2* and *ps1* dull edge at end ……
……..*B. amica* (Kazmierski) (f2 apically sharped). *B. ferula* (Baker) (f_2 obtuse at the tip)
-Hysterosomal setae, short and hairy apart from *sci*, ……..…..……..*B. maga* (Kuznetzov)

6 -Dorsum of idiosoma with various little web like areas ……..…….....…..... *B. livshitzi* (Kuznetzov)

-Dorsum of idiosoma lack of web like areas ……..……..……..……..……..……..……..……..……..……..……..……7

7 -Hysterosomal setae mostly rounded edge with *f1*, *f2*, *h1*, *h2* and *ps1* clavate;……
……..……..……..…….. *B. paraobliqua* (Panou & Emmanouel)

-Hysterosomal setae mostly short in size, similar shape or some of the longer of blunt shaped……..8

8 -Hysterosomal setae short and similar in shape .....*B. obnoxia* (Kuznetzov & Zapletina)

-Some dorsal setae (*f2 h1, h2, ps1*) longer than others and blunt shaped distally. Prodorsal setae moderately notched ….……..……..……..……*B. armindae* (Momen & Lundqvist

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Investigating the Effect of Long-Term Sugarcane Cultivation on Some Soil Properties of Soils in Karoun Agro-industry Unit, Khuzestan Province, Iran

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ABSTRACT
The principal aim of the present research was investigation of the effect of long-term sugarcane cultivation on some chemical and physical properties of soil in Karoun Agro-industry Unit in Shoushtar city, Iran. The study was conducted in a factorial arrangement based on randomized complete block design with two field factors at 8 levels and three depths in three replications. Soil samples were collected and some of their physical (e.g., bulk density) and chemical (e.g., pH, EC, soil available phosphorus, organic carbon, soluble sodium and available potassium) Features9 were measured. Results revealed that land use change and long-term sugarcane cultivation reduced electrical conductivity, the amount of soluble sodium and available potassium contents of soil in different depths, while caused increasing the percentage of organic matter and available soil phosphorous. However, at similar experimental conditions, no significant changes were observed in soil pH. The soil bulk density contents in farms under continuous cultivation of sugarcane, was more than the control field and HARZA. Moreover, soil exchangeable potassium was identified as a sensitive indicator of long-term sugarcane cultivation.

Keywords: *Calcium carbonate, Cultivation, Electrical conductivity, Organic carbon, Soluble sodium, Sugarcane*

1. Introduction

Compact cropping and lack of suitable management approaches in agricultural fields all over the world have affected soil properties in vast areas and led to changes in soil quality. Cultivation of sugarcane (*Sacchrum officinarum L.*) was mechanized in the southwest of Iran in the late 1950s. Over the past 40 years, the sugarcane yield has been declined from 110 to 50 tons per hectare over the same period of time. The use of heavy machinery during planting, harvesting and transportation operations in soils with good texture leads to concern that soil compaction may reduce the productivity in the long term (Barzegar et al. 2005).

One of the most appropriate methods for evaluation of the quality of soil is studying the changes in soil properties through continuous use and analysis of the results. Selection of key indicators of soil quality is effective in evaluating these changes and determining the improvement, stability, or degradation of soil quality (Bünemann et al. 2018). Destruction of physical and chemical properties of soils is one of the most important problems in agro-industrial units. Despite the annual addition of chemical fertilizers, sugarcane crop yields have been declined. Naranjo et al. (2006) investigated the effect of 30 years of sugarcane cultivation on soils properties and they found that continuous cultivation reduced soil organic matter (SOM) and compacted the soils.

In the study in Ethiopia on the effects of 50 years of sugarcane cultivation on some of the physical and chemical properties of soil, Dengia and Lantinga (2016) found that SOM levels were 53 and 34% lower than other areas with depths of 0-30 and 30-60 cm, respectively. However, other factors such as pH and electrical conductivity (EC) of the soil were not significantly affected by long-term cropping, compared to the control land. Perhaps one of the possible causes of this decline is soil degradation. Therefore, the main soil quality indices are evaluated for the extent of changes that may be made with regard to the application of conventional long-term crops. The long-term cultivation period (6 to 7 months), high water consumption (30,000 cubic meters per hectare at 25 to 30 turns irrigation), and extensive use of heavy machinery in the planting stage and sugarcane harvesting,

may change soil properties. In order to determine how these changes are taking place, it is necessary to examine the land qualitatively and quantitatively in order to prevent further destruction of this vast God-given source. Due to the fact that few researches have so far been conducted on the long-term effects of cultivation on the physical and chemical properties of soil, this study aimed to investigate the effect of sugarcane mechanized cultivation in a 40-year period on some of soil characteristics in Karoon Agro-industry.

2. Material and Methods

2.1. Study area

This research was carried out to investigate the effect of long-term sugarcane cultivation on soil chemical and physical properties in Karoun Agro-industry Unit in Dimcheh with the geographical coordinates Latitude: 32° 02' 60.00" N Longitude: 48° 50' 59.99" E and 68 meters above mean sea level located at 12 kilometers to the west of Shoushtar, city in Khuzestan province, Iran (Figure 1). The total area of the land is 45,000 hectares (Moradi et al. 2014). This area has warm and dry climate conditions. The dominant soils of the area are classified in the large Calcic Haplousteps group (Staff 2014).

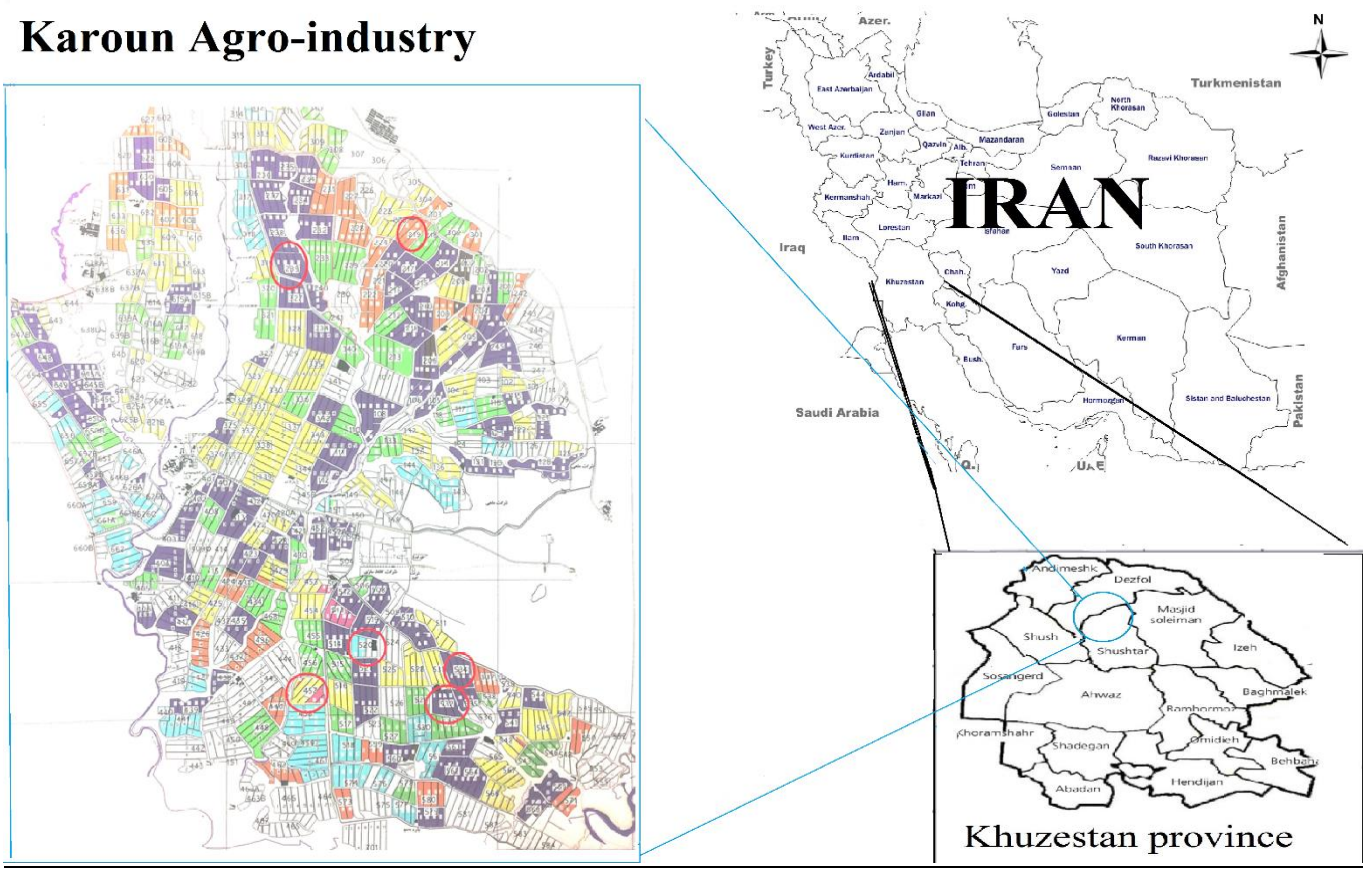

Figure 1- Location of the area under study in Khuzestan province

2.2. Analytical chemistry

The measured soil characteristics are included pH, electrical conductivity (EC) of saturation extract, soil available phosphorus measured by extraction with sodium bicarbonate, organic carbon obtained via wet oxidation method using potassium dichromate and concentrated sulfuric acid, soluble sodium of soil saturation extract and soil available potassium (extracted via ammonium acetate) using flame photometer, and the bulk density in soils. Data analysis was carried out as a factorial experiment in a randomized complete block design with two factors, farms in 8 levels including 6 farms numbered 219, 239, 457, 520, 532, 534, Harza report and control field, with soil depth treatment in three levels of depth including 0-30, 30-60, 90-60 cm in three replications.

2.3. Statistical analysis

Statistical analysis of data was performed by SAS 9.2 software and the comparison of means was made using Duncan's multi-domain test at 1% probability level. Figures were also drawn using Microsoft Excel software.

3. Results and Discussion

3.1. Electrical conductivity and pH of soil

The results showed that continuous cultivation of farms resulted in a statistically significant reduction in EC levels. The average EC in the surface horizon (0-30 cm) was decreased from 17.8 dS m^{-1} in uncultivated land to 0.85 dS m^{-1} in sugarcane-cultivated fields. By comparing the EC of the HARZA report and the sugarcane cultivated fields, no statistically significant difference was observed among them, indicating that the drainage and initial discharge of the farms at the early stages of Karoun Agro-industry Unit had reduced EC in soil via losing the salts (Figure 2). Studies of Landi et al. (2018) implied that land use change and long-term cultivation of sugarcane in northwest of Iran in both studies caused a significant decrease in soil EC. The results of the comparison between the Duncan's means showed that there is no significant difference between the pH of soils in the cultivated fields, control treatment, and Harza report. The average of soil pH in the 0-30 cm horizon of soil was found to be 7.7 in the control treatment and 8.05 in sugarcane cultivated farms. A similar trend was observed at depths 30-60 and 60-90 cm (Figure 3). Dengia and Lantinga (2016) stated that pH values of pristine land and cultivated land were not significantly different.

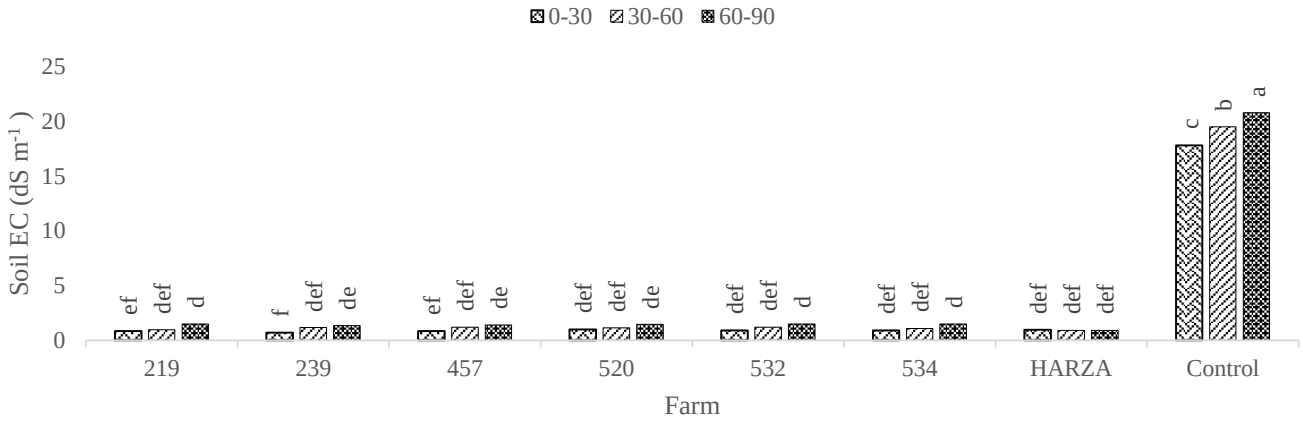

Figure 2- Comparison of means of electrical conductivity in farms under sugarcane and control and Harza report

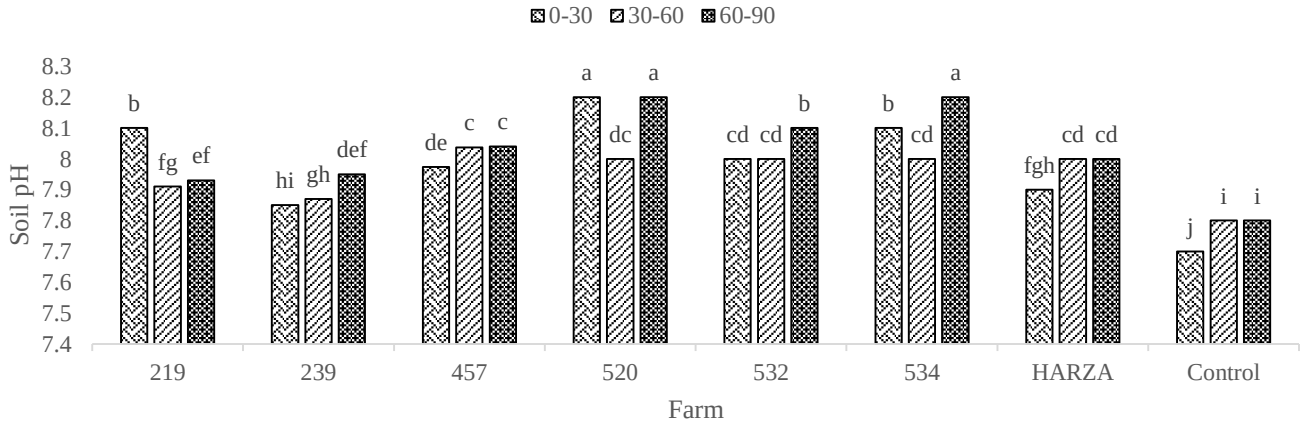

Figure 3- Comparison of means of pH in sugarcane cultivated soil and that of control and Harza report

3.2. Soil organic matter

The soil organic matter (SOM) values in soils under sugarcane at 0-30 cm of depth was significantly higher than that of soils in the control and HARZA farms: the SOM average at depths of 0-30 cm in the soils under sugarcane, the HARZA soil and the control soil were 1.45, 0.6 and 0.65%, respectively (Figure 4). It is obvious that the main reasons for SOM increase are cultivation practices which applied in the farms. The content of SOM was higher in lower depths of soil under sugarcane cultivation as compared with the adjacent pristine soils: the means of SOM in the 30-60 and 60-90 cm horizons in the cultivated soil were 0.62 and 0.41%, respectively. These contents were 0.33 and 0.42% in HARZA and control, respectively. This is probably due to sugarcane deep roots and its high production of biomass.

The presence of sugarcane roots and root discharges in different stages of growth can increase the SOM content of soils under sugarcane cultivation (Amerikhah et al. 2010). Silva et al. (2007) also found that long-term sugarcane cultivation in Brazil increased SOM. A decrease of SOM at deeper levels has been reported by many researchers, resulting from reduced root density (Blanco-Canqui and Lal 2007). Generally, due to the lack of SOM in pristine soils in arid and semi-arid areas, cultivation and irrigation can increase SOM content in such lands (Jafari et al. 2016).

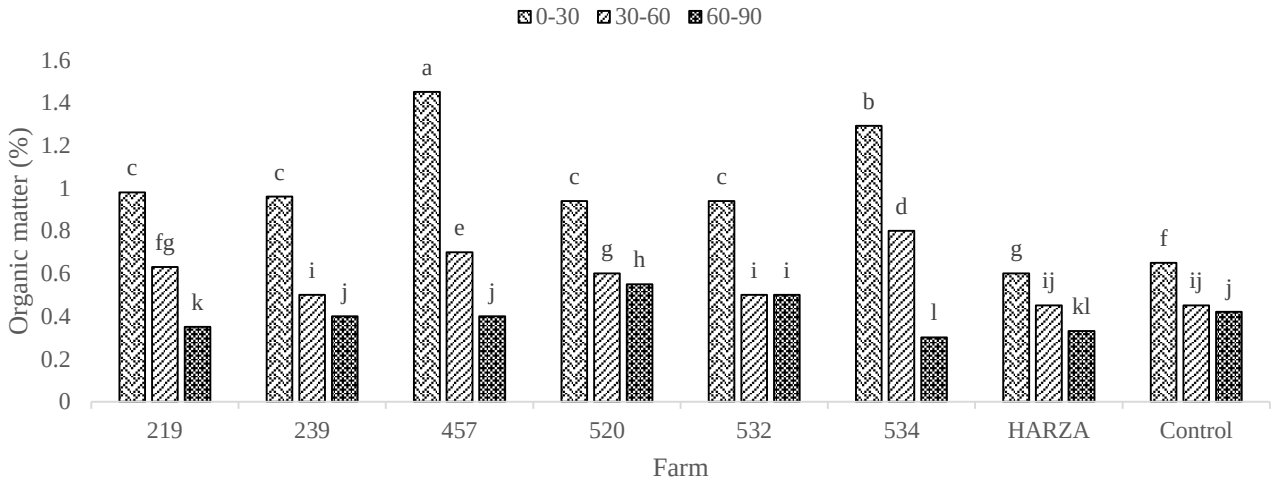

Figure 4- Comparison of means of SOM in sugarcane cultivated soil and that of control and Harza report

3.3. Soluble sodium

As can be seen in the results of the analysis of variance of soluble sodium in soils in different farms, there was a significant difference at the probability level of 1% error, but there was no significant difference resulting from the sampling depth (Table 1). Regarding the results of mean comparison, it was observed that the soluble sodium content of soil was not significantly different in the studied farms, but this difference was significant in comparison with the soil in the control and HARZA report (Figure 5). It seems that the pristine lands in studied lands are mostly very salty whose predominant salt, is mainly sodium chloride. Prior to cultivating sugarcane in the studied area, initial leaching has been conducted, leading to the loss of additional salts such as sodium, and continuous cultivation of sugarcane and heavy irrigation operations on these fields has also resulted in the leaching of soluble sodium through the drainage. In agreement with present study, the conversion of virgin land to sugar beet cultivation investigation showed that the soluble sodium content was reduced in the cultivated soil compared with the adjacent virgin lands (Rezapour et al. 2013).

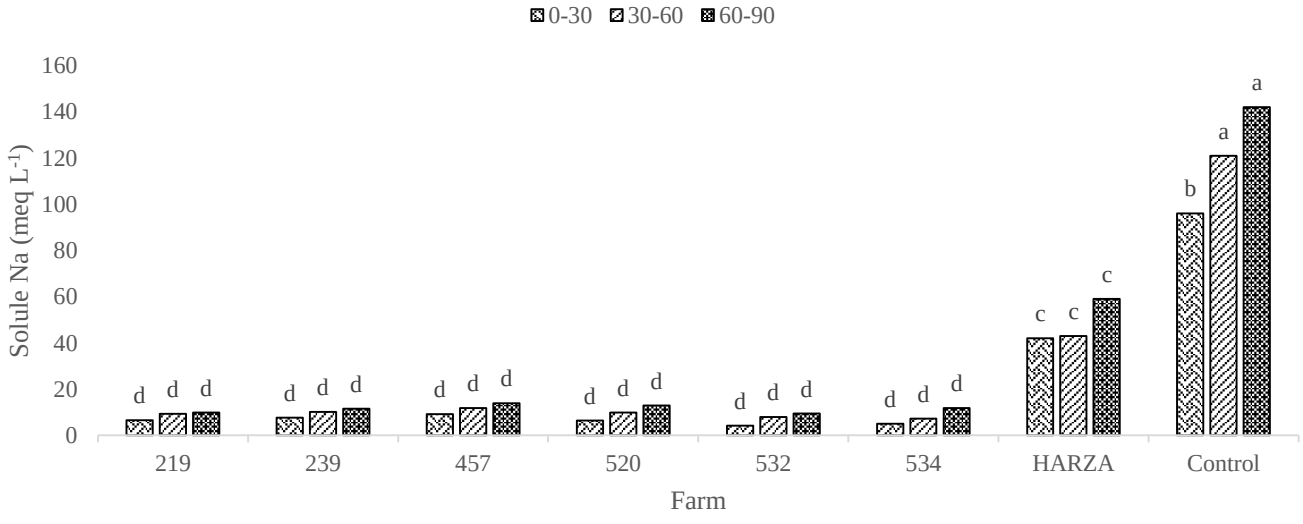

Figure 5- Comparison of means of soluble sodium in soils under sugarcane cultivation with those of control and HARZA report

Table 1- Analysis of variance of soil properties of the soils under long-term sugarcane cultivation

Sources of changes	*Degree of freedom*	*SOM*	*Available Phosphorous*	*CaCO3*	*Gypsum*	*SBD*	*Soluble sodium*	*Absorbable potassium*	*pH*	*EC*
Block	2	0.03**	2.22**	77.38 ^{ns}	0.01**	0.14**	994.58 ^{ns}	1191.45**	3.82**	0.69**
Farm	7	0.15**	17.50**	6.50**	3.07**	0.10**	22408.8**	25719.9**	0.12**	374.94**
Sampling depth	2	2.05**	203.75**	43.61**	0.53**	0.25**	179.35 ^{ns}	6414.78**	0.03**	3.89**
Farm * Depth	14	0.08**	8.18**	5.31**	0.21**	0.01**	190.26 ^{ns}	5202.01**	0.01**	0.64**
Total error	71	0.0007	0.02	0.59	0.0008	0.00008	2.21	9.62	0.001	0.09
CV (percentage)	-	4.21	2.50	1.75	4.99	0.57	6.78	2.20	0.41	9.11

3.4. Soil exchangeable potassium

The changes in land use from pristine lands to sugarcane-cultivated lands result in a significant decrease in available potassium in all three depths, especially at depth 0-30 cm (Figure 6). As a result, the average available potassium in the surface soil was decreased from 185 mg kg^{-1} in the control soil to 100 mg kg^{-1} in the cultivated fields. This reduction is more severe at depth 0-30 cm as compared with lower depths. As can be seen, the amount of potassium at 0-30 cm depth in HARZA report is greater than that of the control farm, which is significant as well, and seems to be due to the application of potassium fertilizer at the beginning of sugarcane cultivation. However, because of the continuous cropping of this plant and excessive discharge of this element from 0-30 cm depth of soil, it gradually decreased in amount compared to the soil of the control and HARZA report (Figure 6). The reason of this observation would be attributed to potassium uptake by sugarcane and the high demand for potassium in this plant. In general, it can be said that the reduction of available potassium in soils in sugarcane-cultivated lands is the excessive depletion of potassium from the soil, due to the absorption of this element by sugarcane as well as the leaching of this nutrient caused by heavy irrigation. The results of this study are consistent with the findings of Bostani and Savaghebi (2011), which reported a significant reduction in potassium content in soils under long-term cultivation of sugarcane. Van Antwerpen et al. (2007) also stated that uncultivated land had higher values of exchangeable K than cultivated land. According to these results, it can be concluded that long-term sugarcane cultivation without using potassium fertilizer causes excessive depletion of this element and leads to decreasing potassium content of the plant and thus reducing plant yield.

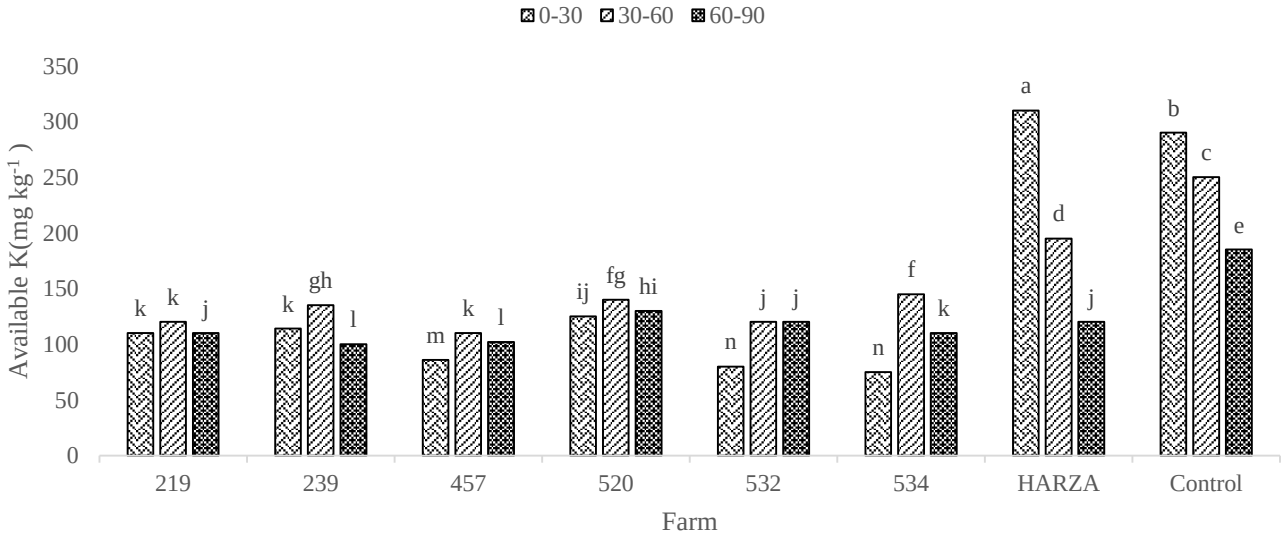

Figure 6- Comparison of the average absorbable potassium in the soil under sugarcane cultivation with the control and HARZA report

3.5. Soil available phosphorous

The soil available phosphorus content at 0-30 cm depth of sugarcane fields imply a statistically difference from that of control farm (Figure 7). The average amount of available phosphorus at 0-30 cm depth increased from 6 mg kg^{-1} in the HARZA report to 15.6 mg kg^{-1} in sugarcane cultivated fields. Meanwhile, there is no significant difference between the mean of available phosphorus in the farms under cultivation and the soil of the control farm and HARZA at the depth of 30-60 cm. The reason for the increased amount of phosphorus in soils under sugarcane cultivation is the addition of phosphorus fertilizers (triple super phosphate and ammonium phosphate) in sugarcane cultivation farms. However, the difference between the amounts of available phosphorus in different farms under sugarcane cultivation can be attributed to the difference management and applied amount of phosphate fertilizers in the farms (Figure 7). The increased amount of phosphorus in sugarcane cultivated fields in comparison

with the soil of the control and HARZA report can be attributed to the ability of phosphorus to form colloidal bonds with the soil grains and, therefore, no leaching of the element even in heavy irrigation operations conducted in such farms. This process leads to the gradual deposition of phosphorous in the soil and, consequently, a gradual increase of the element in the soil. Conversion of natural ecosystem to vegetable lands increased phosphorous proportion in the soils (Ahmad et al. 2017). Investigation the fate of phosphorous in Everglades agriculture soils implied that soil total phosphorous of sugarcane soil was higher than uncultivated soil as result of years of historical phosphorous fertilizer (Wright et al. 2012).

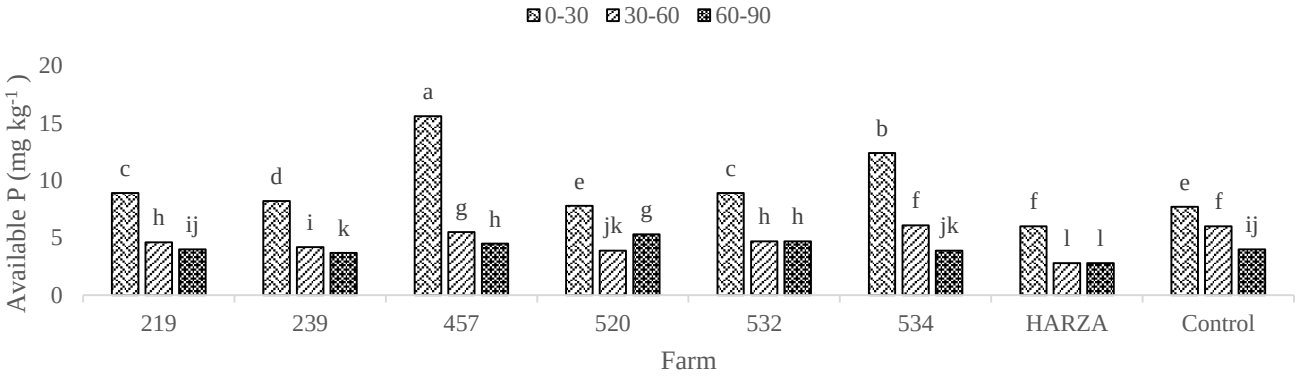

Figure 7- Comparison of means of available phosphorus in soils under sugarcane cultivation with those of control and HARZA report

3.6. Soil bulk density

The results of comparing Duncan's means showed that continuous sugarcane cultivation led to an increase in soil bulk density, especially at the depth of 30-60 cm. By comparing the soil bulk density of control farm (1.41 g cm^{-3}) and the soil of HARZA (1.32 g cm^{-3}) with the soil from continuously cultivated fields, it was observed that, in the early years, the texture of soil has turned porous and lighter, due to soil tillage. However, due to heavy tillage and transportation of tillage equipment in the field, the texture of soil has gradually become heavier and denser, which not only prevented the development of the root, but has also led to retaining water in the plant root area, resulting in reduced yield. The results are consistent with the findings of Moradi et al. (2014) as to increasing soil bulk density values, due to heavy tillage in Haft-Tapeh Agro-industry Unit.

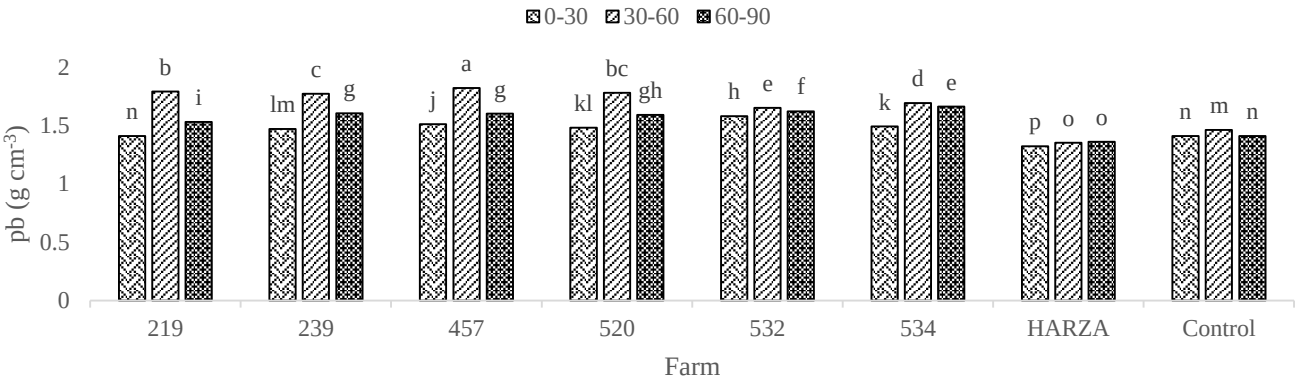

Figure 8- Comparison of means of soil bulk density in sugarcane farms with those of the control and HARZA report

4. Conclusions

The obtained finding revealed a direct relationship between changing land use and long-term sugarcane cultivation and physical and chemical properties of soil. Land use change, abundant irrigation and leaching, and long-term sugarcane cultivation had the ability of decreasing the salinity constraints and soluble sodium in pristine soils and increasing the organic matter content of the soil. These changes are greater in the surface depths of the soil than the deeper ones. While the amount of available phosphorus increased, due to long-term cultivation and fertilization, the available potassium greatly decreased in the farms under sugarcane cultivation. Since sugarcane consumes high amounts of potassium, the reduction of available potassium can be a limiting factor in its growth and yield. Also, the findings of this study showed that the heavy tillage operations by tillage equipment, caused a

gradual increase in soil bulk density in the depth of 30-60 cm in the farms, leading to an increase in the density of soil in this horizon. Consequently, the increased soil bulk density prevents the development of sugarcane root and, by creating damp conditions, prevents the availability of enough oxygen for the plant, resulting in the inability of the plant to absorb nitrogen and other nutrients from the soil and, therefore, reduced yield of the plant. In general, the results of this study showed that in order to prevent probable negative consequences and the depletion of nutrients in soil, especially potassium, and to increase soil bulk density, it is necessary to periodically evaluate the changes in the characteristics of sugarcane planted soils. The obtained results will provide a guidance to finding a suitable management techniques for maintaining soil quality.

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Aphid Species, Their Natural Enemies in Vegetables from Erzincan, Turkey: First Record of the Parasitoid Wasp *Aphelinus mali* (Haldeman) parasitizing *Lipaphis erysimi* (Kaltenbach)*

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ABSTRACT
Aphid species, their parasitoids and predators on vegetables (bean, cucumber, eggplant, melon, okra, pepper, pumpkin, tomato and watermelon) grown in Erzincan province, Turkey were determined. Surveys were carried out at weekly intervals from the seedling period until the end of the harvest period in Central and Üzümlü districts of Erzincan province in 2014-2016. 30-40 plants were randomly selected in the surveys and all parts of plants were investigated with a magnifier. Ten aphid, seventeen predator and seven parasitoids species were found. According to results, *Lipaphis erysimi* (Kaltenbach) (Hemiptera: Aphididae) was first recorded as a host of *Aphelinus mali* (Haldeman) (Aphelinidae: Aphelininae) in the world. In addition, parasitism rates of aphids were also established. The mean parasitism rate changed between 17.5% in 2015 and 4.39% in 2016. The relationship between parasitism rate, total parasitized aphid number and total aphid number were found. According to the analysis result, there was a very weak or a high positive correlation (r= 0.126-0.721) between total aphid number and total parasitized (mummified) aphid number, and not a correlation existed between parasitism rate and total aphid number as for years.

Keywords: *Aphid, Natural enemy, Parasitism rate, Vegetable, Turkey*

1. Introduction

One of the food groups that people need for healthy and balanced nutrition is vegetables and fruits. Vegetables; vitamins, minerals and other ingredients in terms of human nutrition are a very important place (Thompson & Kelly 1990).

Vegetable production could be decreased by few pest organisms. One of the most important of these pests is aphids. Aphids (Hemiptera: Aphididae) are recognized worldwide as economically important (Remaudière & Autrique 1985). Aphid species are polyphagous, feed on plant sap and multiply rapidly under favorable conditions. They feed on the leaves, shoots, branches, stems, fruits and roots of plants and cause serious damage and also lead to the formation of fumagine. This formation reduces the photosynthesis and respiration capacity of plants (Lodos 1986; Ölmez Bayhan & Ulusoy 2002; Tepecik et al. 2011). In addition to these damages, aphids also vectors to viruses and other similar organisms, which are often more important than other damages (Lodos 1986; Matheus 1993).

It is reported that there are 510 genus (that have reached 5.000 species) of aphids on the earth (Blackman & Eastop 2018). Important vegetable pest species of the Aphididae family (Hemiptera) are mainly belong to the genera *Aphis* (Linnaeus 1758), *Aulacorthum* (Mordvilko 1914), *Brevicoryne* (van der Goot 1915), *Macrosiphum* (Passerini 1860) and *Myzus* (Passerini 1860). Among these species, *Aulacorthum solani* (Kaltenbach 1843), *Aphis fabae* (Scopoli 1763), *Aphis gossypii* (Glover 1877), *Brevicoryne brassicae* (Linnaeus 1758), *Macrosiphum euphorbiae* (Thomas 1878) and *Myzus persicae* (Sulzer 1776) are the most important vegetable pest species in Turkey (Düzgüneş & Tuatay 1956; Çanakçıoğlu 1975; Göksu & Atak 1976; Ölmez Bayhan 2000; Toros et al. 2002; Sangün 2010).

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Although many studies have been carried out on vegetable aphids in Turkey, no comprehensive study has been done on vegetable aphids in Erzincan until now. This study was carried out to establish aphid species and their parasitoids, predators, parasitism rates on vegetables growing in Erzincan province. Thus, within the scope of integrated pest management and organic vegetable cultivation, basic data of biological control against aphids were found out. Potential natural enemies determined as a result of this work, will be tackle of later, and it will lead to subjects such as increasing of natural enemy activity and mass production.

2. Material and Methods

Aphid species and their parasitoids, predators were collected on plants of *Abelmoschus esculentus* L., *Capsicum annuum* L., *Citrullus lanatus* Thunb., *Cucurbita pepo* L., *Cucumis sativus* L., *Cucumis melo* L., *Lycopersicon esculentum* Miller, *Phaseolus vulgaris* L., *Solanum melongena* L. grown in greenhouse or open field in Erzincan province.

To determine aphid species on vegetable plants in Center and Üzümlü district of Erzincan province, surveys were carried out at weekly intervals from the seedling period until the end of the harvest period in 2014 and 2016. In surveys, according to the field size 30-40 plants selected by chance and all part of the plants were examined by means of magnifier. Samples collected to determine aphid species were brought to laboratory for diagnosis by puting into codded tubes including 70% ethyl alcohol.

In order to determine the parasitoids and predators, firstly vegetables containing aphids had been observed and then detected parasitized aphids and predators were collected and they were brought to the laboratuvary for further identification with their host aphids. While collecting immature stages of parasitized, suspect aphids thought to be parasitized and both predators and their hosts from vegetables, they carefully were swathed with paper towels and placed into polyethylene bags. Immediateley, samples were placed in a portable refrigerator to prevent damage from the hot, and then brought to the laboratory in order to rear adults.

Non-adult (immature stages) predators collected were reared to become adults in a culture box (the dimensions of the culture box was 30x35x15 cm and the top of them surrounded by tulle) in laboratory and they were daily controled. When it was needed, aphid infected fresh plant shoots were added into the culture box and then, obtained adult predators were diagnosed (Yumruktepe 1993; Aslan 2002). After cleaning all the pests except from the aphids, suspect aphids and aphid mummies were placed in parasitoid extraction boxes. Following that daily emerging parasitoids were transferred into eppendorf tube with 95% ethyl alcohol and diagnosed (Yumruktepe 1993).

All vegetable plants were randomly sampled to establish the rate of natural parasitism. During this study, while determining the rate of parasitism, total parasitized individual numbers obtained from the colonies including mummy was compared with total aphid numbers in the sample (Praslicka & Huszar 2005).

In this study, correlation and regression analyses were done on data by using SPSS 17.0 packet program to determine whether there was a relationship between total parasitized (mummified) aphids and total aphids and between parasitism rate and total aphids.

3. Results and Discussion

In conclusion, it was found that there were ten aphid species, *Acyrthosiphon pisum* (Harris), *Aphis craccivora* Koch, *Aphis fabae* Scopoli, *Aphis gossypii* Glover, *Aphis nasturtii* Kaltenbach, *Aphis spiraecola* Patch, *Aulacorthum solani* Kaltenbach, *Lipaphis erysimi* (Kaltenbach), *Myzus (Nectarosiphon) persicae* Sulzer and *Macrosiphum euphorbiae* (Thomas) (Aphididae) on grown vegetables in Center and Üzümlü district of Erzincan province in 2014-2016 (Table 1). It was found that aphids on *Capsicum annuum* L., *Cucurbita pepo* L., *Phaseolus vulgaris* L. was more intense in terms of distribution and intensity among other vegetable plants. The species with the highest number of host plants were identified as *A. gossypii* and *M. persicae*. *Acyrthosiphon pisum*, *Aphis nasturtii* and *L. erysimi* respectively determined only on the *P. vulgaris*, *C. pepo* and *Cucumis sativus* L. Holman (2009), found that total 42 aphids species on vegetables in Palearctic region and reported that these vegetable plants (bean, cucumber, eggplant, melon, okra, pepper, pumpkin, tomato and watermelon) were the host of *Acyrthosiphon pisum*, *Aphis craccivora*, *A. fabae*, *A. gossypii*, *A. nasturtii*, *A. spiraecola*, *Aulacorthum solani*, *L. erysimi*, *M. persicae* and *M. euphorbiae*. Bayram & Bayhan (2016), determined that *A. gossypii* species were found commonly and consisting of high population on watermelons in Diyarbakır province in Turkey. Alaserhat & Canbay (2017), found that *A. craccivora*, *A. gossypii*, *A. fabae* and *M. persicae* on pepper plant (*C. annuum*) in Erzincan province in Turkey.

Table 1- The aphid species detected in vegetables in Erzincan province in 2014-2016

Host plants	*Aphid species*
Phaseolus vulgaris L.	*Acyrthosiphon pisum* (Harris 1776)
	Aphis craccivora Koch, 1854
	Aphis fabae Scopoli, 1763
	Myzus (*Nectarosiphon*) *persicae* (Sulzer 1776)
Cucumis sativus L.	*Aphis gossypii* Glover, 1877
	Aphis spiraecola Patch, 1914
	Lipaphis erysimi (Kaltenbach 1843)
Solanum melongena L.	*Aphis gossypii* Glover, 1877
	Myzus (*Nectarosiphon*) *persicae* (Sulzer 1776)
Cucumis melo L.	*Aphis gossypii* Glover, 1877
	Aulacorthum solani (Kaltenbach 1843)
Abelmoschus esculentus L.	*Aphis craccivora* Koch, 1854
	Aphis gossypii Glover, 1877
Capsicum annuum L.	*Aphis craccivora* Koch, 1854
	Aphis fabae Scopoli, 1763
	Macrosiphum euphorbiae (Thomas 1878)
	Myzus (Nectarosiphon) persicae (Sulzer 1776)
Cucurbita pepo L.	*Aphis gossypii* Glover, 1877
	Aphis nasturtii Kaltenbach, 1843
	Aulacorthum solani (Kaltenbach 1843)
	Myzus (*Nectarosiphon*) *persicae* (Sulzer 1776)
Lycopersicon esculentum Miller	*Aphis spiraecola* Patch, 1914
	Macrosiphum euphorbiae (Thomas 1878)
	Myzus (Nectarosiphon) persicae (Sulzer 1776)
Citrullus lanatus (Thunb.)	*Aphis fabae* Scopoli, 1763
	Aphis gossypii Glover, 1877
	Myzus (*Nectarosiphon*) *persicae* (Sulzer 1776)

As a result of this study, seventeen predator species viz. *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae), *Deraeocoris (Camptobrochis) punctulatus* (Fallen), *Deraeocoris (Camptobrochis) serenus* (Douglas & Scott) (Hemiptera: Miridae), *Orius (Orius) niger* (Wolff) (Hemiptera: Anthocoridae), *Adalia bipunctata* (L.), *Adalia decempunctata* (L.), *Adalia fasciatopunctata revelierei* Mulsant, *Coccinella septempunctata* (L.), *Hippodamia (Adania) variegata* (Goeze), *Exochomus nigromaculatus* (L.), *Oenopia (Synharmonia) conglobata* (L.), *Stethorus punctillum* Weise, *Psyllobora vigintiduopunctata* (L.) (Coleoptera: Coccinellidae), *Episyrphus balteatus* De Geer, *Sphaerophoria scripta* (L.), *Scaeva dignota* (Rondani) (Diptera: Syrphidae) and *Leucopis* sp. (Diptera: Chamaemyiidae) were identified on aphid species found on the study (Table 2). While determining these species, it was observed that adult and pre-adult stages of predator species were fed directly on aphid species found on the study. In previous studies were done on these aphid species in Turkey, many other predatory species were determined including predator species were found in our study (Düzgüneş et al. 1982a,b; Zeren & Düzgüneş 1983; Ölmez Bayhan & Ulusoy 2002; Aslan & Uygun 2005; Ayyıldız & Atlıhan 2006; Aslan & Uygun 2007; Güleç 2011; Alaserhat 2015; Alaserhat & Canbay 2017). In addition, it was reported on the world that *Chrysoperla carnea*, *Chrysoperla pallens* (Rambur) (Neuroptera: Chrysopidae), *Geocoris pulvisculatus* Distant (Hemiptera: Lygaeidae), *A. bipunctata*, *A. fasciatopunctata revelierei*, *Brumoides sturalis* (F.), *Coccinella septempunctata*, *Cheilomenes sexmaculata* (F.), *Coccinella undecimpunctata* L., *Coccinella transversalis* F., *Harmonia axyridis* Pallas, *Hippodamia dimidiata* (F.), *Hippodamia (Adania) variegata*, *Menochilus sexmaculatus* (F.), *Microaspis discolor* (F.), *Micromus variegatus* (F.), *Oenopia kirbyi* Muls., *Oenopia quadripunctata* Kapur, *Oenopia sauzeti* Muls., *Pania luteopustulata* (Muls.), *Propylea quatuordecimpunctata* (L.), *Pseudoaspidimerus circumflexus* (Motschulsky), *Scymnus (Pullus) castaneus* (Sicard), *Scymnus guimeti* Muls., *Scymnus pyrocheilus* Muls., *Scymnus quadrillum* (Motschulsky), *Scymnus xerampelinus* Muls., *Spitocaria bisellata* (Muls.) (Coleoptera: Coccinellidae), *E. balteatus*, *Scaeva pyrastri* L., (Diptera: Syrphidae) fed on these aphid species found on the study (Agarwala & Ghosh 1988; Singh & Bali 1993; Irshad 2001; Brown & Mathews 2008; Guo et al. 2008; Sapathi 2009; Chaudhary & Singh 2012; Jaferi 2013; Vandereycken et al. 2015; Rocca & Messelink 2017).

Table 2- Predators of aphid species found on vegetables in Erzincan province in 2014-2016

Aphid species	*Predator species*	
	Familia	*Species*
Acyrthosiphon pisum (Harris 1776)	Anthocoridae	*Orius* (*Orius*) *niger* (Wolff)
	Miridae	*Deraeocoris* (*Camptobrochis*) *punctulatus* (Fallen)
	Coccinellidae	*Coccinella septempunctata* (Linnaeus)
		Hippodamia variegata (Goeze)
		Oenopia (*Synharmonia*) *conglabata* (Linnaeus)
Aulacorthum solani (Kaltenbach 1843)	Anthocoridae	*Orius* (*Orius*) *niger* (Wolff)
	Miridae	*Deraeocoris* (*Camptobrochis*) *punctulatus* (Fallen)
	Coccinellidae	*Adalia bipunctata* (Linnaeus)
		Adalia fasciatopunctata revelierei Muls.
		Coccinella septempunctata (Linnaeus)
		Hippodamia variegata (Goeze)
	Syrphidae	*Scaeva dignota* (Rondani)
	Chamaemyiidae	*Leucopis* sp.
Aphis craccivora Koch, 1854	Anthocoridae	*Orius* (*Orius*) *niger* (Wolff)
	Miridae	*Deraeocoris* (*Camptobrochis*) *punctulatus* (Fallen)
	Coccinellidae	*Coccinella septempunctata* (Linnaeus)
		Hippodamia variegata (Goeze)
		Oenopia (*Synharmonia*) *conglabata* (Linnaeus)
Aphis fabae Scopoli, 1763	Anthocoridae	*Orius* (*Orius*) *niger* (Wolff)
	Miridae	*Deraeocoris* (*Camptobrochis*) *punctulatus* (Fallen)
	Chrysopidae	*Chrysoperla carnea* (Stephens)
	Coccinellidae	*Coccinella septempunctata* (Linnaeus)
		Hippodamia variegata (Goeze)
Aphis gossypii Glover, 1877	Anthocoridae	*Orius* (*Orius*) *niger* (Wolff)
	Miridae	*Deraeocoris* (*Camptobrochis*) *punctulatus* (Fallen)
		Adalia bipunctata (Linnaeus)
		Adalia decempunctata (Linnaeus)
		Adalia fasciatopunctata revelierei Muls.
		Coccinella septempunctata (Linnaeus)
	Coccinellidae	*Exochomus nigromaculatus* (Goeze)
		Hippodamia variegata (Goeze)
		Oenopia (*Synharmonia*) *conglabata* (Linnaeus)
		Psyllobora vigintiduopunctata (Linnaeus)
		Stethorus punctillum Weise
		Episyrphus balteatus De Geer
	Syrphidae	*Scaeva dignota* (Rondani)
		Sphaerophoria scripta (Linnaeus)
	Chamaemyiidae	*Leucopis* sp.
Aphis nasturtii Kaltenbach, 1843	Miridae	*Deraeocoris* (*Camptobrochis*) *punctulatus* (Fallen)
	Coccinellidae	*Adalia bipunctata* (Linnaeus)
		Adalia decempunctata (Linnaeus)
		Coccinella septempunctata (Linnaeus)
		Hippodamia variegata (Goeze)
	Syrphidae	*Scaeva dignota* (Rondani)
	Chamaemyiidae	*Leucopis* sp.
Aphis spiraecola Patch, 1914	Miridae	*Deraeocoris* (*Camptobrochis*) *serenus* (Douglas & Scott)
Lipaphis erysimi (Kaltenbach 1843)	Coccinellidae	*Coccinella septempunctata* (Linnaeus)
		Hippodamia variegata (Goeze)
Macrosiphum euphorbiae (Thomas 1878)	Miridae	*Deraeocoris* (*Camptobrochis*) *serenus* (Douglas & Scott)
	Coccinellidae	*Coccinella septempunctata* (Linnaeus)
		Hippodamia variegata (Goeze)
Myzus (*Nectarosiphon*) *persicae* (Sulzer 1776)	Anthocoridae	*Orius* (*Orius*) *niger* (Wolff)
	Miridae	*Deraeocoris* (*Camptobrochis*) *serenus* (Douglas & Scott)
		Deraeocoris (*Camptobrochis*) *punctulatus* (Fallen)
	Chrysopidae	*Chrysoperla carnea* (Stephens)
	Coccinellidae	*Adalia bipunctata* (Linnaeus)
		Adalia decempunctata (Linnaeus)
		Coccinella septempunctata (Linnaeus)
		Hippodamia variegata (Goeze)
		Oenopia (*Synharmonia*) *conglabata* (Linnaeus)
		Stethorus punctillum Weise
	Syrphidae	*Episyrphus balteatus* De Geer
		Scaeva dignota (Rondani)
	Chamaemyiidae	*Leucopis* sp.

Aphelinus mali (Haldeman) (Aphelinidae: Aphelininae), *Aphidius ervi* Haliday, *Aphidius colemani* Viereck, *Aphidius matricariae* Haliday, *Lysiphlebus fabarum* (Marshall), *Binodoxys angelicae* (Haliday) and *Praon volucre* (Haliday) (Braconidae: Aphidiinae) were found on aphids as parasitoids (Table 3). According to earlier studies conducted in Turkey it was determined that *Binodoxys acalephae* (Marshall), *Praon dorsale* (Haliday) and *P. volucre* (Braconidae: Aphidiinae) were parazitoids of *A. pisum*; *Aphelinus* sp. (Aphelinidae: Aphelininae), *A. colemani*, *B. acalephae*, *B. angelicae*, *Ephedrus persicae* Froggatt, *Lysiphlebus cardui* (Marshall) and *L. fabarum* (Braconidae: Aphidiinae) were parazitoids of *A. craccivora*; *Aphelinus* sp. (Aphelinidae: Aphelininae), *Adialytus ambiguus* (Haliday), *A. colemani*, *A. matricariae*, *B. acalephae*, *B. angelicae*, *Diaeretiella rapae* (M'Intosh), *L. fabarum* and *P. volucre* (Braconidae: Aphidiinae) were parazitoids of *A. fabae*; *Aphelinus* sp. (Aphelinidae: Aphelininae), *A. colemani*, *A. matricariae*, *B. angelicae*, *Lipolexis gracilis* (Foerster), *L. fabarum* and *P. volucre* (Braconidae: Aphidiinae) were parazitoids of *A. gossypii*; *Aphelinus* sp. (Aphelinidae: Aphelininae), *A. colemani*, *P. volucre, B. angelicae*, *E. persicae*, *Lysiphlebus ambiguus* Halliday and *L. fabarum* (Braconidae: Aphidiinae) were parazitoids of *A. spiraecola*; *Aphelinus asychis* Walker, (Aphelinidae: Aphelininae), *Adialytus ambiguus*, *Aphidius abjectus* (Haliday), *Aphidius funebris* Mackauer, *A. colemani, A. ervi, A. matricariae, B. angelicae, Ephedrus cerasicola* Stary, *E. persicae, P. volucre, Lipolexis gracilis, D. rapae*, *Lysiphlebus cardui* (Marshall) and *L. fabarum* (Braconidae: Aphidiinae) were parazitoids of *M. persicae*; *Adialytus salicaphis* (Fitch), *P. volucre* and *B. angelicae* (Braconidae: Aphidiinae) were parazitoids of *M. euphorbiae* (Düzgüneş et al. 1982b; Zeren & Düzgüneş 1983; Ayyıldız & Atlıhan 2006; Güleç 2011; Alaserhat 2015; Alaserhat & Canbay 2017). Furthermore, it was reported that numerous parasitoid species (including these species) were determined on aphids in the world (Atwal et al. 1971; Singh 1980; Tomanovic et al. 1998; Stary et al. 2000; Wiackowski et al. 2001; Kavallieratos et al. 2004; Rakhshani et al. 2005, Tomanovic et al. 2005; Kavallieratos et al. 2006; Stary 2006; Rakhshani et al. 2007; Talebi et al. 2009; Kavallieratos et al. 2010; Mossadegh et al. 2011; Dassonville et al. 2012; Nazari et al. 2012; Barahoei et al. 2013; Rakhshani et al. 2013; Taheri & Rakhshani 2013; Anonymous 2018).

Table 3- Aphid and their parasitoid species on vegetables in Erzincan province in 2014-2016

Aphid species	*Parasitoid species*	
	Subfamily	*Species*
Aulacorthum solani (Kaltenbach)	Aphidiinae	*Aphidius colemani* Viereck
Aphis craccivora Koch	Aphidiinae	*Aphidius ervi* Haliday
Aphis fabae Scopoli	Aphidiinae	*Aphidius matricariae* Haliday *Lysiphlebus fabarum* (Marshall) *Praon volucre* (Haliday)
Aphis gossypii Glover	Aphidiinae	*Aphidius colemani* Viereck *Aphidius matricariae* Haliday *Binodoxys angelicae* (Haliday) *Praon volucre* (Haliday)
Aphis spiraecola Patch	Aphidiinae	*Praon volucre* (Haliday)
Lipaphis erysimi (Kaltenbach)	Aphidiinae Aphelininae	*Aphidius matricariae* Haliday *Aphelinus mali* (Haldeman)
Macrosiphum euphorbiae (Thomas)	Aphidiinae	*Praon volucre* (Haliday)
Myzus (*Nectarosiphon*) *persicae* (Sulzer)	Aphidiinae	*Aphidius colemani* Viereck *Aphidius ervi* Haliday *Aphidius matricariae* Haliday *Praon volucre* (Haliday) *Lysiphlebus fabarum* (Marshall)

Two parasitoid species viz. *Aphelinus mali* (Aphelinidae: Aphelininae) and *Aphidius matricariae* (Braconidae: Aphidiinae) were determined on *L. erysimi*. *Aphidius* sp., *A. colemani*, *Aphidius hortensis* Marshall, *A. matricariae*, *Aphidius rosea* Haliday, *Binodoxys brevicornis* (Haliday), *Binodoxys indicus* (Subba Rao & Sharma), *Diaeretiella rapae*, *Ephedrus* sp., *Ephedrus laevicollis* (Thomson), *Ephedrus plagiator* (Nees), *Lipolexis gracilis*, *Lysiphlebus fabarum*, *Lysiphlebus testaceipes* (Cresson) and *Praon volucre* (Braconidae: Aphidiidae) are pointed out as parasitoids of *L. erysimi* (Atwal et al. 1971; Singh 1980; Stary & Ghosh 1983; Stary et al. 2000; Stary 2006; Talebi et al. 2009; Mossadegh et al. 2011; Rakhshani et al. 2013). Consequence, *Aphelinus mali* (Aphelinidae: Aphelininae) was first recorded as a parasitoid's of *Lipaphis erysimi* (Hemiptera: Aphididae) in the world.

Aphids' parasitism rates in 2015 are given (Table 4). Starting from the first week of July and continued until the first week of November, natural parasitism with aphids on vegetables was observed. At the end of the vegetation, the maximum parasitism was recorded (third week of October–first week of November). The highest parasitism rate was recorded as 84.93% on 21 October 2015 with cultured sample, while the mean rate of parasitism was 17.15%.

After analyzing the 2015 data, it was found that, there was a very weak positive correlation (r= 0.126) between the the number of total parasitized (mummified) aphids and number of total aphids. Also, there was not a correlation between the parasitism rate and the number of total aphids. Regression analysis was performed on the data to detect the relationship between the number of total parasitized (mummified) aphids and the number of total aphids and a linear relationship between them was found (Figure 1).

Table 4- The rate of parasitism of aphids on vegetables in 2015 in Erzincan province

Host plant	*Date of sampling*	*Aphid species*	*Total aphid number*	*Total parasitized (mummified) aphid number*	*Parasitism rates (%)*
Abelmoschus esculentus	15.07.2015	*Aphis gossypii*	350	18	5.14
	28.07.2015	*Aphis gossypii*	620	38	6.13
Capsicum annuum	03.11.2015	*Aphis fabae* *Myzus (Nectarosiphon) persicae*	150	80	53.33
Lycopersicon esculentum	21.10.2015	*Aphis spiraecola* *Macrosiphum euphorbiae*	73	62	84.93
Phaseolus vulgaris	15.09.2015	*Acyrthosiphon pisum* *Aphis fabae*	480	38	7.92
	20.10.2015	*Acyrthosiphon pisum* *Aphis fabae*	105	16	15.24
Cucumis sativus	26.08.2015	*Aphis gossypii*	58	0	0
	08.10.2015	*Lipaphis erysimi* *Aphis gossypii*	342	31	9.06
Cucurbita pepo	28.07.2015	*Myzus (Nectarosiphon) persicae*	475	7	1.47
	06.08.2015	*Myzus (Nectarosiphon) persicae* *Aphis gossypii*	1250	46	3.68
	13.08.2015	*Aphis nasturtii* *Myzus (Nectarosiphon) persicae*	625	45	7.20
	09.09.2015	*Aphis gossypii*	440	25	5.68
Cucumis melo	08.07.2015	*Aphis gossypii*	1230	61	4.96
	28.07.2015	*Aphis gossypii*	185	16	8.65
	19.08.2015	*Aphis gossypii* *Aulacorthum solani*	306	23	7.52
Solanum melongena	03.11.2015	*Aphis gossypii*	200	107	53.50

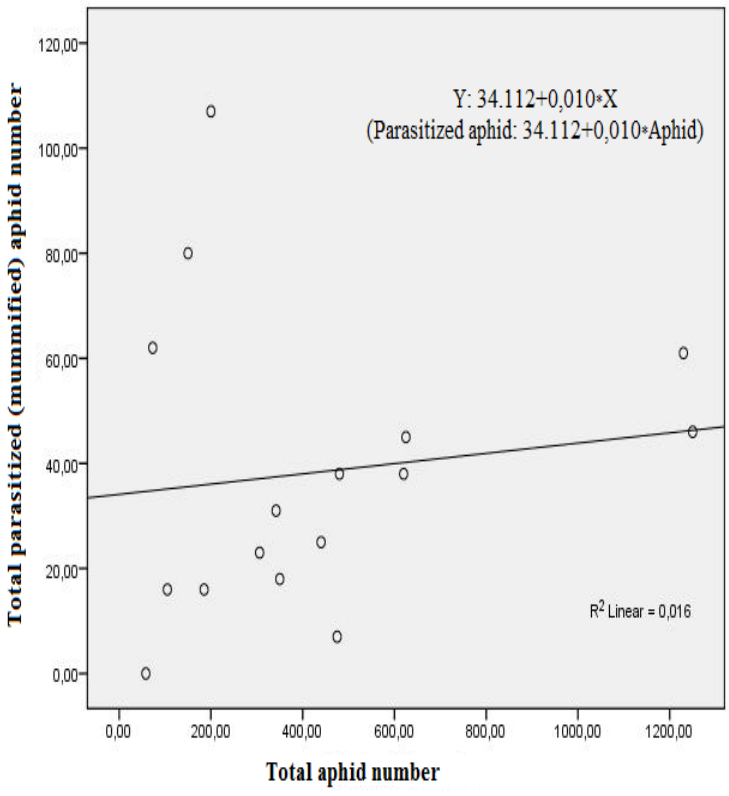

Figure 1- The relationship between the number of total parasitized (mummified) aphids and the number of total aphids in 2015

Parasitism rates in 2016 are given in Table 5. Starting from the fourth week of June and continued until the end of October, natural parasitism with aphids on vegetables was observed. Maximum parasitism was in 2016 first and fourth week October. The highest parasitism rate was recorded as 21.43% on 04 October 2016 with cultured sample, while the mean rate of parasitism was 4.39%.

Table 5- The rate of parasitism of aphids on vegetables in 2016 in Erzincan province

Host plant	*Date of sampling*	*Aphid species*	*Total aphid number*	*Total parasitized (mummified) aphid number*	*Parasitism rates (%)*
Capsicum annuum	28.10.2016	*Macrosiphum euphorbiae*	45	5	11.11
Lycopersicon esculentum	10.08.2016	*Macrosiphum euphorbiae*	59	1	1.69
	28.06.2016	*Aulacorthum solani*	64	0	0
Cucurbita pepo	11.08.2016	*Aphis gossypii*	2340	26	1.11
	15.08.2016	*Aphis gossypii*	450	17	3.78
	15.08.2016	*Aphis gossypii*	650	12	1.85
	23.08.2016	*Aphis gossypii*	480	15	3.13
	23.08.2016	*Aphis gossypii*	1280	38	2.97
Cucumis melo	13.07.2016	*Aphis gossypii*	270	8	2.96
	18.08.2016	*Aphis gossypii*	68	0	0
	25.08.2016	*Aphis gossypii*	750	41	5.47
	25.08.2016	*Aphis gossypii*	675	18	2.67
	31.08.2016	*Aphis gossypii*	30	1	3.33
	04.10.2016	*Aphis gossypii*	14	3	21.43

After analyzing the 2016 data, it was found that, there was a high positive correlation (r= 0.721**) between the number of total parasitized aphids and the number of total aphids. Also, there wasn't correlation between the parasitism rate and the number of total aphids. Regression analysis was performed on the data to detect the relationship between the number of total parasitized aphids and the number of total aphids and a linear relationship between them was found (Figure 2).

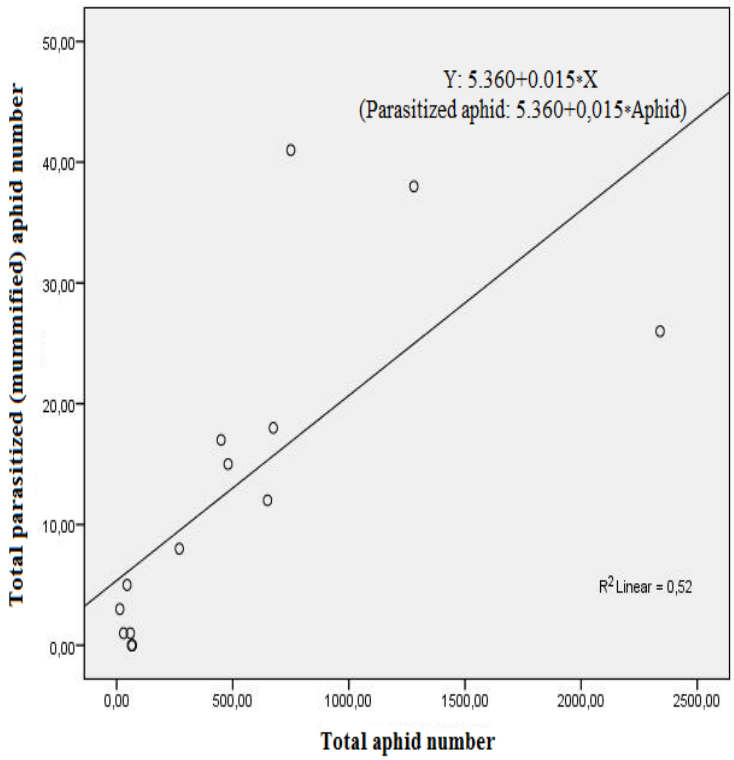

Figure 2- The relationship between the number of total parasitized (mummified) aphids and the number of total aphids in 2016

In our study, a very weak and a high positive correlation (r= 0.126-0.721) between total parasitized aphid number and total aphid number was found in both years. Additionally, not a correlation existed between parasitism rate and total aphid number in both consecutive years. Güçlü & Özbek (2002), indicated a correlation between the number of parasitoid species (*A. ervi* parasitoids of *Metopolophium dirhodum*) and the number of total aphids on *Rosa* spp. in Erzurum province in Turkey. On the other hand, Van Veen et al. (2002), determined a very strong correlation (r= 0.93) between the number of total parasitized aphids and the number of total aphids. Rakhshani et al. (2009), established that a high correlation between the number of parasitized aphids and the number of total aphids in the 5th and 6th cuts and in the 1st and 6th cuts of alfalfa respectively in 2004 (r= 0.954-0.794) and 2005 (r= 0.933-0.794). Furthermore, it has been indicated a low correlation in other periods of alfalfa. Alaserhat & Canbay (2017), conducted a study on aphid parasitoids on pepper (*Capsicum annuum* L.) in Erzincan province in Turkey in 2012-2013. They stated a very significant or a high positive correlation (r= 0.937-0.816) between the number of total parasitized

aphids and the number of total aphids, a very weak positive correlation (r= 0.163-0.064) between the rates of parasitism and the number of total aphids.

4. Conclusions

Consequently, ten aphid, seventeen predator and seven parasitoids species were determined on vegetables. Among the aphid, predator and parasitoid species were the most common species in nature respectively *A. gossypii*, *C. septempunctata* and *P. volucre*.

Aphelinus mali (Aphelinidae: Aphelininae) was recorded as a parasitoid of *L. erysimi* for the first time.

Natural parasitism rates of vegetable aphids were as 17.15% in 2015 and 4.39% in 2016. In 2015, producers did not carry out the necessary processes such as fertilization, irrigation and pesticide because their selling prices were low. Even, at the end of the season they did not harvest their vegetables and left them in the field. This led to increased parasitoid and parasitism rates.

It was found that the total aphid numbers were statistically related to the total parasitized (mummified) aphid numbers, while the total aphid numbers were not statistically related to the rate of parasitism. In 2015, the relationship between the total parasitized aphid numbers and the total aphid numbers showed variability widely. Especially, since the deviations of samples taken from at the end of the season in *C. annuum* (03.11.2015), *L. esculentum* (21.10.2015) and *S. melongena* (03.11.2015) were high, the correlation was also low. However, in 2016, the relationship between the total parasitized aphid numbers and the total aphid numbers didn't show varriabilty, so the correlation between them was found high.

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Relationship Between Resistance Against Neonicotinoids and Esterase Enzyme for *Myzus persicae* (Sulzer) (Hemiptera:Aphididae) Populations in South of Turkey

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ABSTRACT
In this study, the development of imidacloprid and acetamiprid resistance in *Myzus persicae* populations and the relationship between neonicotinoid resistance and esterase enzyme were investigated in seven *Myzus persicae* populations which is collected from greenhouse pepper production areas in 2018. In order to determine the resistance ratios of aphid populations against the insecticides, 1 control and 6 doses were used. Each insecticide dose was used in 3 replicates and 25 adult female individuals were used in each replication. For imidacloprid, the highest and the lowest resistance ratios were found to be 6.88 and 3.19-fold, in K-4 and D populations, respectively. For acetamiprid, the highest and the lowest resistance ratios were found to be 7.35 and 2.72-fold, in K-1 and E-2 populations, respectively. Also, highest and lowest esterase activities were found to be 2.60 and 1.75 mOD min^{-1} mg^{-1} in K-4 and E-2 populations, respectively. According to the results of this study, imidacloprid and acetamiprid resistance determined in some *Myzus persicae* populations may be related to esterase enzyme. However, detailed studies are required to establish a clear relationship between resistance and enzyme.

Keywords: *Acetamiprid, Esterase, Imidacloprid, Myzus persicae, Resistance*

1. Introduction

Myzus persicae (Sulzer) (Hemiptera: Aphididae), also known as green peach aphid, is a pest that causes significant crop loss in vegetables, tobacco, fruit and ornamental plants worldwide. This pest, harms the plant by absorbing its sap and releases a sweet-sticky substance during feeding. They cause fumagine as a result of saprophyte fungi adhere to this secretion. (Van Emden & Harrington 2007). *M. persicae* easily resists environmental pressures as a result of the high genetic diversity achieved through sexual reproduction. In addition, it is active throughout the year especially in areas with homogeneous conditions such as greenhouse production areas and can cause great damage by increasing the population density with parthenogenetic reproduction. (Blackman & Eastop 2007). *M. persicae* is one of the most important aphid pests in the world because of its host diversity, mechanism of damage, life cycle, as well as its ability to spread rapidly, transmit viral diseases and easily develop resistance to insecticides (Pavela 2018).

Insecticides are used commonly in Turkey to control against *M. persicae* due to their rapid efficacy. However the pest, develops resistance to insecticides in a short time due to its favorable biology. Thus, *M. persicae* has been reported to develop resistance to more than 75 chemicals (Sparks & Nauen 2015). *M. persicae* develops metabolic resistance to organophosphate and carbamate by increasing E4 or FE4 carboxylesterase levels (Devonshire & Moores 1982). In addition, it is possible to develop resistance to pyrethroid (kdr and super-kdr) and dimethyl carbamate (MACE) by target region mutations. (Eleftherianos et al. 2008). Neonicotinoid insecticides have been also used in the world for the last twenty years in the control of many pests including *M. persicae*. Neonicotinoid group insecticides are known as nicotinergic acetylcholine receptors and they inhibit the basic chemical transmission in the central nervous system of insects. (Afzal et al. 2015). Neonicotinoids belong to the group 4A in the IRAC MoA classification and are known as NACHR agonist. Especially in recent years, there are many records about *M. persicae's* neonicotinoid resistance in the world (Charaabi et al. 2016; 2018; Voudouris et al. 2017; de Little et al. 2017). Therefore, it is important to examine the resistance developed by *M. persicae* against neonicotinoids in detail.

In this study, resistance status of *M. persicae* field against imidacloprid and acetamiprid, two neonicotinoid insecticides commonly used in Turkey, have been examined. The levels of esterase enzyme that is known as an important detoxification enzyme has been also investigated.

2. Material and Methods

2.1. Myzus persicae populations

M. persicae populations were collected from greenhouse pepper production areas in Southern Turkey in April and May, 2018 (Table 1). Seven *M. persicae* populations were collected during the survey studies. The comparison population (susceptible population) was used to determine the resistance levels of the field populations. The susceptible population has collected from pepper production areas in 2006 and has been grown in climate controlled rooms without any pesticide application since then. *M. persicae* populations were cultured on hazelnut radish (*Raphanus sativus*) in climatic chambers with 26±1 °C, 60-65% humidity and 16:8 photoperiodic conditions.

Table 1- Collection dates, coordinates and hostplants of *Myzus persicae* populations

Populations	*Collection dates*	*Host plants*	*Coordinates*
K-1	01.05.2018	Pepper	36°32'39"N 30°27'41"E
K-2	01.05.2018	Pepper	36°28'33"N 30°35'25"E
K-3	01.05.2018	Pepper	36°31'09"N 30°35'23"E
K-4	01.05.2018	Pepper	36°30'83"N 30°34'72"E
E-1	01.05.2018	Pepper	36°67'63"N 29°91'43"E
E-2	01.05.2018	Pepper	36°63'82"N 29°88'22"E
D	01.05.2018	Pepper	36°25'59"N 30°02'74"E

2.2. Leaf-dip bioassays

In order to determine LC_{50} values of two insecticides that contains imidacloprid and acetamiprid against *M. persicae*, we used method no 19 that is recommended by IRAC (Insecticide Resistance Action Committee). In bioassay experiments, Confidor SC 350 (Bayer Crop Science) with imidacloprid active substance and Mospilan 20 SP (Sumi Agro) with acetamiprid active substance were used. First, 1% agar powder was boiled with distilled water and allowed to cool. Agar medium was poured into a 9 cm petri dish at a height of approximately 4 mm and waited to medium be hardened. To determine the LC_{50} and LC_{90} values in all aphid populations, a preliminary study yielded a dose of approximately 90-99%. In order to determine the resistance ratios of aphid populations, 1 control and 6 doses were used. Pure water was applied to the control group. Each dose of the insecticides was applied 3 replications and 25 *M. persicae* adult female invidivuals were used in each replication. Hazelnut radish leaves with a diameter of 3 cm were immersed to the previously prepared insecticide concentrations for 10 seconds using the leaf-dip method. The leaf discs were then placed into the petri dish with agar medium by means of forceps. For each population, 25 individuals from the aphid adults were placed on the leaf discs under binocular. The petri dishes were left to climate cabinets having 26±1 °C, 60-65% humidity and 16:8 photoperiodic conditions. Dead-live counting procedure was performed after 72 hours.

2.3. Statistical analysis

LC_{50} and LC_{90} values were determined in the POLO computer package program (LeOra Software 1994) by using the measurement data determined after 72 hours of *M. persicae* populations. For all populations used in the experiment, the LC_{50} and LC_{90} values were compared to the LC_{50} and LC_{90} values of the susceptible population and the resistance ratios of the populations were obtained for each insecticide. Resistance values were determined by the ratio of LC_{50}values determined in field populations to LC_{50}value of the susceptible population.

2.4. Esteraese activity

For determination of esterase enzyme activity in *M. persicae* populations, Devonshire et al. (1992) method was used. Each well of the microplates were loaded in with 20 mM phosphate buffer (pH:7) that containing 50 LL of 0.1% Triton X-100 (Boehringer Mannheim, especially purified) by using a multichannel micropipette. Adult aphids belonging to the populations to be tested were transferred to each well with a brush. The aphids were homogenized using a multiple homogenizer and the tissue was allowed to dissolve for 15 minutes.30 mg of Fast Blue RR Salt was weighed and then phosphate buffer (pH:6) added to 50 mL. After filtration through a No:1 Whatman filter, 500 L of 100 mM 1-naphthyl acetate solution was added. 200 µL of the prepared dye-substrate solution was taken into all wells with a multi-channel micropipette. Optical density (O. D.) values were obtained by reading in the kinetic microplate reader for a total of 5 minutes at 450 nm wavelength at 10 second

intervals. To calculate the total protein in the samples, Bradford's protein assay method was used, and bovine serum albumin (BSA) was used as a standard (Bradford 1976).

2.5. Detection carbocylesterase activity by electreophoresis

In the *M. persicae* populations, Ornstein and Davis (1964) method was used for the determination of carboxyl esterase by electrophoresis. For this purpose, one wingless adult aphids were homogenized in 25 µL homogenization solution (0.1 g sucrose, 1 mL 1.6% Triton X-100, 0.001% bromocresol purple) and 15 µL homogenate was loaded into each gel well. The gel, run at 250 volts for 1.5 hours, was taken up in 50 mL of Fast Blue RR salt solution [0.1 g of Fast Blue RR salt, 50 mL of 0.2 M phosphate buffer (pH:6)] containing 1 mL of 100 mM 1-naphthyl acetate. After staining for about 15 minutes, the gel was placed in 7% acetic acid for fixation. After 24 hours, it was photographed by using the imaging device.

3. Results and Discussion

3.1. Resistance results

The LC_{50} values and resistance ratios determined for imidacloprid in *M. persicae* populations are given in Table 2. The highest resistance rate to imidaclopride was determined in K-4 population with 6.88 fold and the lowest resistance rate was 2.26 fold in E-2 population. A low level of resistance to imidacloprid was detected in the populations D, E1 and E2. The LC_{50} values and resistance ratios determined for acetamiprid in *M. persicae* populations are given in Table 3. At the end of the study, 7.35, 6.80, 7.25, 6.51, 2.78, 2.72, 7.25 fold resistance development was determined for acetampirid in K1, K2, K3, K4, E1, E2 and D populations respectively. The highest resistance rate for acetamiprid was determined in the K1 population with 7.35 fold and the lowest resistance rate was found in the E2 population with 2.72 fold. Among the *M. persicae* populations, a lower level of resistance to acetamiprid was detected in E1 and E2 populations.

Table 2- LC_{50} values and resistance ratios determined against imidacloprid in *Myzus persicae* populations

Population	*n**	*Slope±SE*	*LC_{50} (mg a.i l^{-1}) (95% CL)*	*R***
K1	411	1.85±0.13	2.85 1.55-4.35	6.78
K2	419	1.79±0.13	2.32 1.11-3.68	5.52
K3	369	1.96±0.16	1.78 0.73-3.04	4.23
K4	411	1.96±0.13	2.89 1.70-4.24	6.88
D	409	1.80±0.13	1.34 0.52-2.32	3.19
E1	410	1.69±0.24	1.06 0.24-1.87	2.52
E2	405	1.56±0.23	0.95 0.22-1.70	2.26
Susceptible	402	0.95±0.34	0.42 0.03-0.96	-

*; Number of individuals used in the experiment, **; resistance ratio

Development of resistance by *M. persicae* against insecticides has been known since the early 1970s. This caused to problems in the control of aphids which becomes resistant to insecticides in the late 1970s and early 1980s (Moores 1995). Therefore, there are studies in which *M. persicae* has developed resistance to many insecticide groups including carbamates, organophosphates and neonicotinoids (Moores et al. 1996; Denholm & Jespersen 1998; Barber et al. 1999; Cassanell et al. 2005; Criniti et al. 2008). However, the number of studies that determine the development of resistance and resistance mechanisms against *M. persicae* insecticide Group in Turkey is quite limited.

In recent years, neonicotinoid insecticides have been used extensively in *M. persicae* control worldwide. In our study, resistance against imidacloprid and acetamiprid active ingredients have ranged 2.26-6.88 fold and 2.72-7.35 fold, respectively. Foster et al. (2008) reported that resistance development by *M. persicae* against imidacloprid, thiamethoxam, thiacloprid, clothianidin and dinotefuran was 11-, 18-, 13-, 100- and 6- folds, respectively. In another study, such values were found is 27.5- 30.14-and 41.31 folds for imidacloprid, thiamethoxam and thiacloprid, respectively (Puinean et al. 2010). Panini et al. (2014) identified 11.7-fold imidacloprid resistance in the 92H6 population of *M. persicae*. Therefore, neonicotinoid resistance by *M. persicae* populations collected from Turkey was found in accordance with the literature. There is no high level neonicotinoid resistance in *M. persicae* populations collected from pepper fields in Turkey. It is thought that the reason is the

rotation of neonicotinoid insecticides and other group insecticides using throughout the season. Therefore, it is thought that the resistance levels to insecticides with different mode of action should be investigated in future studies

Table 3- Determined LC_{50} values and resistance ratios to acetamiprid in *Myzus persicae* populations

Population	*n**	*Slope ±SE*	*LC_{50} (mg a.i l^{-1}) (95% CL)*	*R***
K1	418	0.79±0.13	3.13 1.65-4.86	7.35
K2	415	0.82±0.13	2.90 1.54-4.47	6.80
K3	416	0.74±0.12	3.09 1.54-4.91	7.25
K4	413	0.82±0.13	2.77 1.46-4.29	6.51
D	414	0.82±0.13	2.85 1.55-4.35	7.25
E1	413	0.87±0.22	1.18 0.21-2.41	2.78
E2	413	0.92±0.21	1.15 0.26-2.25	2.72
Susceptible	406	1.07±0.29	0.42 0.03-1.06	-

*; Number of individuals used in the experiment, **; Resistance ratio

3.2. Esterase activity results

The results of esterase enzyme activity determined by microplate assay method in *M. persicae* populations are given in Table 4. The highest esterase enzyme activity was detected in the K2 population with a value of 2.38 mOD min^{-1} mg^{-1} protein, while the lowest esterase enzyme activity was found in the E2 population with a value of 1.75 mOD min^{-1} mg^{-1}protein. The esterase enzyme levels of K1, K2, K3 and K4 populations were found to be statistically different when compared to the susceptible population ($P<0.05$). The esterase enzyme activities of the populations D, E1 and E2 were statistically within the same group as well as the susceptible population ($P<0.05$). In addition, esterase enzyme activities were higher in K1, K2, K3 and K4 populations for both imidacloprid and acetamiprid.

Table 4- Esterase enzyme activities in *Myzus persicae* populations

Population	*n**	*specific activity mOD min^{-1} mg^{-1} protein*	*R/S***
Susceptible	4	1.80 b***	
K1	4	2.35 a	1.30
K2	4	2.38 a	1.32
K3	4	2.01 a	1.11
K4	4	2.60 a	1.44
D	4	1.85 b	1.02
E1	4	1.95 b	1.08
E2	4	1.75 b	<1

*; Number of repetitions, **; Enzyme activity of tested population/enzyme activity of susceptible population, ***; The same letters indicate the same group statistically ($P<0.05$)

3.3. Elektroforesis

The gel results of carboxylesterase enzyme examined by Polyacrylamide Gel Electrophoresis (PAGE) are given in Figure 1. The esterase gel concentrations determined in the field populations of *M. persicae* were higher than the susceptible populations. Especially in the populations of K1, K2, K3 and K4, which have high resistance to imidacloprid and acetamiprid, revealedesterase gel bands with higher density than those of other populations. In the E2 population, esterase gel band with

enzyme density was determined. In addition, esterase enzyme activity in the E2 population was lower than that of other populations. Therefore, esterase enzyme activity and electrophoresis results have supported each other.

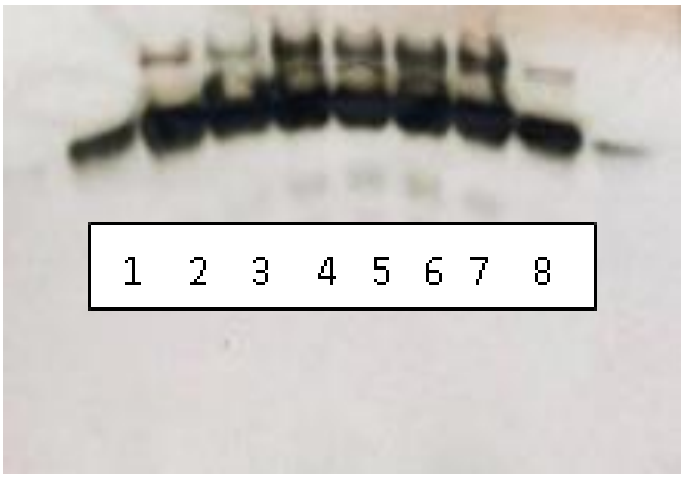

Figure 1- Carboxylesterase gel bands in *Myzus persicae* populations (1: susceptible; 2: K1; 3: K2; 4: K3; 5: K4; 6: D; 7: E1; 8: E2)

The esterase enzyme activities of *M. persicae* populations were found to be <1-1.44 fold compared to the susceptible population. Indeed, many studies, revealed a role for acetylcholinesterase, carboxylesterase or other esterase enzymes in insecticide resistance in aphids (Gao et al. 1992; Song et al. 1995).Wang et al. (2002) reported that the 8.1-fold imidacloprid resistance of *Aphis gossypii* (Hemiptera:Aphididae) may be linked with esterase enzyme. However, a 108.9-fold multiple resistance to fenveralate was also determined in this imidacloprid-resistant population. Therefore, both neonicotinoid and pyrethroid resistance increase the esterase enzyme activities. As a matter of fact, recent studies have shown that neonicotinoid resistance in *M. persicae* may be related to the increase of cytochrome P450 enzyme and R81T mutation in nAChR (Bass et al. 2011; 2014). It is noteworthy thatcarbamate, organophosphate and pyrethroid group insecticides have been also used to control pests in areas where *M. persicae* populations are collected in our study. Therefore, one could say that the increase of esterase enzyme could be related to also carbamate, organaphosphate and pyrethroid group insecticides as a result of cross-resistance development. Thus, *M. persicae* have been reported to develop metabolic resistance to organophosphate and carbamate by increasing carboxylesterase levels of E4 or FE4 (Devonshire 1989). Therefore, further studies would be helpful in considering this matter.

4. Conclusions

Taking these results into consideration, even if the development of neonicotinoid resistance in *M. persicae* populations is not very high, it is possible that the resistance will increase as selection pressure continues. This shows that *M. persicae*, who has rapid reproductive ability, may have problems in the control in the future. Therefore, at intervals of neonicotinoid development of resistance in *M. persicae* populations collected from production areas in Turkey it is thought that should be monitored regularly. In addition, further studies are needed to determine the mechanisms of resistance in *M. persicae* neonicotinoids in Turkey. In addition, rotation of insecticides which have different mode of action that will be use in production areas is one of the insecticide resistance management method.

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Meta-Learning-Based Prediction of Different Corn Cultivars from Color Feature Extraction

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ABSTRACT
Image analysis techniques are developing as applicable to the approaches of quantitative analysis, which is aimed to determine cultivar grains. Additionally, corn (*Zea mays*) grain processing companies evaluate the quality of kernels to determine the price of these cultivars. Because of this reason, in the study, a computer image analysis technique was applied on three corn cultivars. These were *Zea mays L. indentata*, *Zea mays L. saccharata* and a hybrid corn (Yellow sweet corn). These cultivars are commercially important as dry grains in Turkey. In the study, the grain color values were tested in the cultivars from Turkey's collection. One hundred samples were used for each corn cultivar, and 300 corn grains in total were used for evaluations. Each of nine color parameters (R_{min}, R_{mean}, R_{max}, G_{min}, G_{mean}, G_{max}, B_{min}, B_{mean}, B_{max}) which were obtained from original RGB color channels with maximum and minimum values was evaluated from the digital images of three different corn cultivar grains. The values were analyzed with the help of the Multilayer Perceptron (MLP), Decision Tree (DT), Gradient Boost Decision Tree (GBDT) and Random Forest (RF) algorithms by using the Knime Analytics Platform. The majority voting method was applied to MLP and DT for prediction fusion. All algorithms were run with a 10-fold cross-validation method. The success of prediction accuracy was found as 99% for RF and GBDT, 97.66% for MLP, 96.66% DT and 97.40% for Majority Voting (MAVL). The MAVL method increased the accuracy of DT while decreasing the accuracy of MLP partly for the fusion of MLP and DT.

Keywords: *Corn identification; Color measurement; Multilayer Perceptron; Decision Tree; Gradient Boost Decision Tree; Random Forest*

1. Introduction

In recent years, agricultural production has become a more critical sector, because human needs have increased in terms of food, energy and raw industrial materials. This increase has resulted in another increase in agriculture as technological practices. In modern agricultural production, in general, experts take an important role in getting information and making decisions, but this is not always possible. Because of this reason, expert systems are used for solving problems in agricultural production. One area of agricultural production which uses these expert systems is corn production. Corn is an essential product in the world's agriculture. This is because it may be used as human food and for animal breeding. The reason for this issue may be understood from FAOSTAT production quantities of corn for countries as the average of the period of 1994-2017 (Figure 1). In Figure 1, it can be seen that Northern America and Europe have the mass production level of corn of more than 5 863 440 tons. Additionally, the production share of corn by region as the average of the period of 1994-2017 may be seen in Figure 2. It can be observed that America produces 52.5% of corn in the world.

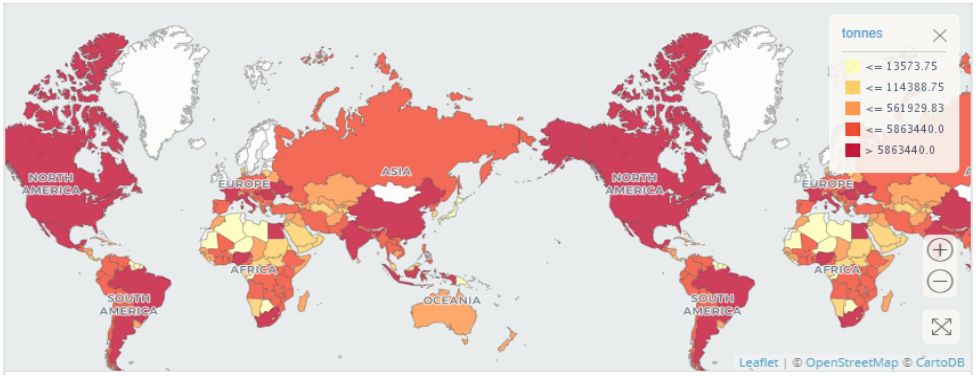

Figure 1- Production quantities of corn by country as the average of 1994 – 2017 (FAO 2019a)

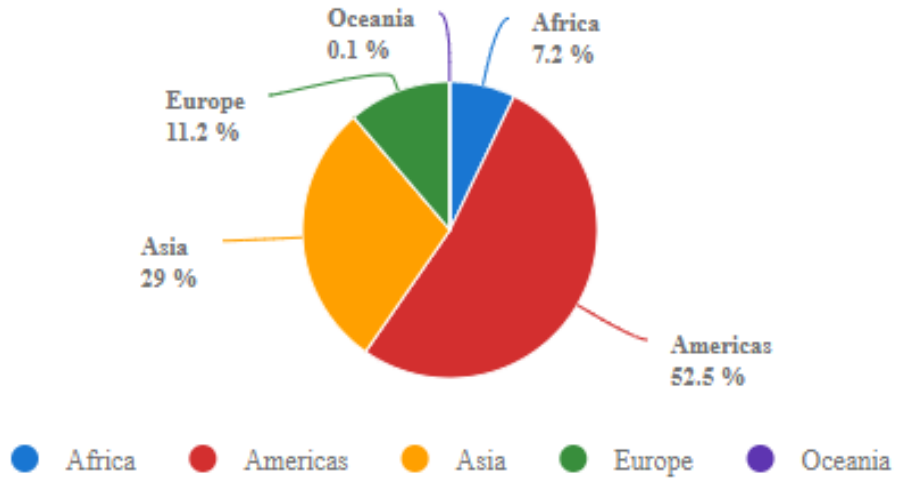

Figure 2- Production share of corn by region as the average of 1994 – 2017 (FAO 2019b)

The production of corn in agriculture reaches big amounts. This value varies from country to country in the world. The production area and production amount of corn in Turkey for 2001-2017 may be seen in Table 1 (TSI 2018). As seen in Table 1, Turkey produces 5 900 000 tons of corn from 639 084 hectares of production area. This statistic also shows that corn production is critical and has a large share amounts in Turkey's agricultural production like the case in the world. With this amount of production, the issue of corn, also corn cultivars, is becoming important for seed production of the world, as well as Turkey. Seed properties effect all agricultural production processes, as well as the quality and quantity of corn. The properties of corn cultivars are essential, and knowing about corn cultivars directly affects the agricultural production based on their production needs like water, soil and climate. It is the basic issue of seed studies in the scientific literature and the agriculture industry.

Table 1- Area and production of corn in Turkey (TSI 2018)

	Corn	
Year	*Sown area (Hectare)*	*Production (Tons)*
2001	550 000	2 200 000
2002	500 000	2 100 000
2003	560 000	2 800 000
2004	545 000	3 000 000
2005	600 000	4 200 000
2006	536 000	3 811 000
2007	517 500	3 535 000
2008	595 000	4 274 000
2009	592 000	4 250 000
2010	594 000	4 310 000
2011	589 000	4 200 000
2012	622 609	4 600 000
2013	659 998	5 900 000
2014	658 645	5 950 000
2015	688 169	6 400 000
2016	680 019	6 400 000
2017	639 084	5 900 000

In the scientific literature, Dechao (1996) worked on 11 different corn cultivars and their broken corn kernels positioned randomly in one frame, and then, they selected the morphological parameters detected with the BP moment learning algorithm. For this aim a three-layer feed forward neural network was formed, which could identify multiple or whole and broken corn kernels. Their classification accuracy was 93%. Ni et al. (1997) developed a machine-vision system to identify different types of corn kernels as crown and shapes. They used image processing techniques to enhance the object detection properties of images, and they reduced noise in the acquired images. Their system provided an average accuracy, approximately 87%, when they compared their results to human inspection. Tan´ska et al. (2005) and Stanisavljević et al. (2019) worked on measurement of the geometrical characteristics, size and surface colors of rapeseeds with the help of digital image and color analysis, and they expressed that an analysis of color of rape and sticky willy seeds in an RGB (red/green/blue) model showed differences in value ranges for distinguishing the seeds. Zhang et al. (2007) studied digital image processing for identification and detection of corn kernel surface cracks. A detection experiment was carried out by 50 kernels with cracks and 50 kernels without cracks. Their results indicated 90% to 94% detecting accuracy. Draganova et al. (2010) investigated an approach for identifying fusarium-infected maize grains by spectral analysis in the visible and near-infrared region, SIMCA models, parametric classifiers and neural classifiers, they also reported their system accuracy was good. Zhao et al. (2011) used a genetic algorithm and support

vector machine (SVM) to determine species. Their methods were optimized for cultivar recognition, and their algorithm was based on machine vision, which also improved determination accuracy with a performance percentage of 94.4%. Jiang et al. (2012) worked on corn seed purity, where their system was based on machine vision, and it was divided into three steps: the first step was image segmentation, the second step was feature extraction, and the third step was classification of corn seeds. Based on a classifier designed with SVM, their results showed 97.3% identification accuracy in the Nongda108 and 98% accuracy in the Ludan981 cultivars, better than 95% in previous studies. Yang et al. (2015) worked on classification of the purity of waxy corn seed varieties. Their study was based on the combined spectral, morphological and texture features extracted from visible and near-infrared (VIS/NIR) hyperspectral images. 150 kernels of each variety were captured and analysed with the images of both sides of corn kernels. Support vector machines (SVM) and a model of partial least squares–discriminant analysis (PLS-DA) were used to build the classification models for classification of seed varieties. The recognition accuracy was 98.2% and 96.3% for the germ side and the endosperm side, respectively, in the SVM model. It was more satisfactory than in the PLS-DA model according to their research. In their research, they also stated that their procedure has the potential for use as a new method for seed purity testing.

In the light of scientific literature and corn production needs of the world, also Turkey's agriculture, this study aimed to classify some corn cultivars based on their color values. Moreover, it was aimed to demonstrate the success of a potential industrial application with a pre-application by using image processing and analysis techniques in corn cultivar grains. It is also of great importance that the findings will be a source of inspiration for future studies and its contribution to the literature.

2. Material and Methods

In the study, an image analysis technique was applied on three corn cultivars. These were *Zea mays L. indentata*, *Zea mays L. saccharata* and hybrid corn (Yellow sweet corn). These cultivars are commercially important as dry grains in Turkey. One hundred samples were used for each corn cultivar. Corn grains were randomly selected from each variety, and a total of 300 corn grains were used for the evaluations. The corn cultivar grain samples can be seen in Figure 3.

Figure 3- Corn grain samples

2.1. Image processing and analysis

Corn grain images were captured by using a DSLR camera (Nikon D800) with an illuminated background. Images were acquired at a resolution setting of 300 dpi (1600 x 2400 pixels). In the study, color features were used for classifying grain varieties because it is known that color channels are widely used for this aim in the literature, while it is also known that the colors of a corn grain are not quite uniform. Because of this reason, each of the three-color channels with nine color parameters (R, G, B values which are obtained from the original RGB color channel with mean, maximum and minimum values) were evaluated from digital images of the grains of three different corn cultivars. These corn cultivar color channel values were evaluated by using the NI Vision Assistant software for classification of corn grains (Figure 4.). The reason for usage of this software was that the software has a magic wand tool for selection of corn grains, in which way the method does not need to isolate corn grains from their background by using a given threshold in contrast to the literature (Chen et al. 2010). The magic wand tool was set as 20 for edge detection of corn grains. Additionally, with the use of this software, the normalization needs of each color channel were eliminated in contrast to the literature (Chen et al. 2010). In this study, as a pre-application, before developing an industrial automation system, it was aimed to only determine the efficiency of the measurements with the help of the LabVIEW Vision Assistant module and other modules of the software for developing an optimum image measurement system.

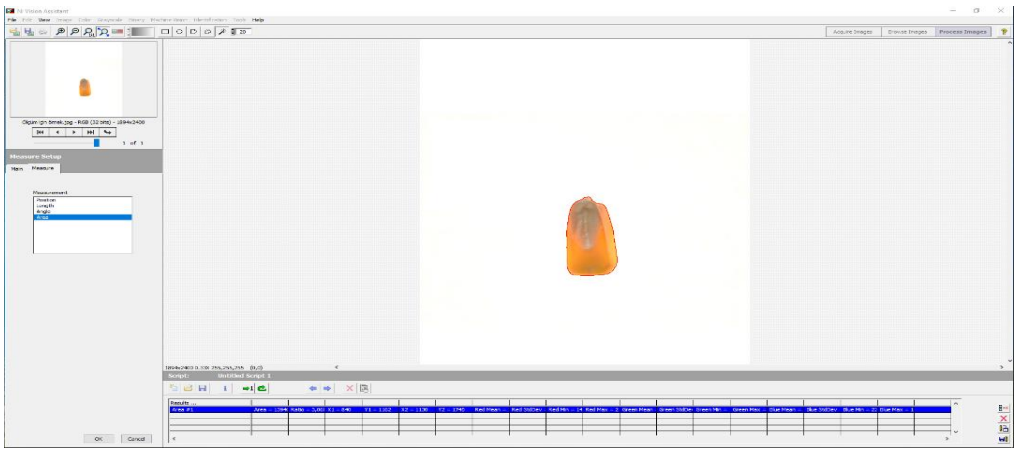

Figure 4- The evaluation process of color channel values of corn grain by using NI Vision Assistant software

All classification processes can be summarized as the steps of getting original corn images from corn cultivars, transferring these images to the NI Vision Assistant software, measurement setup process of the NI Vision Assistant software, area measurements, obtaining RGB data from digital images with mean, maximum and minimum values, saving the database as a txt file, txt to xls datasheet conversion for meta-learning operations, meta-learning of corn database, and getting corn cultivar classification results. These steps may also be seen in Figure 5.

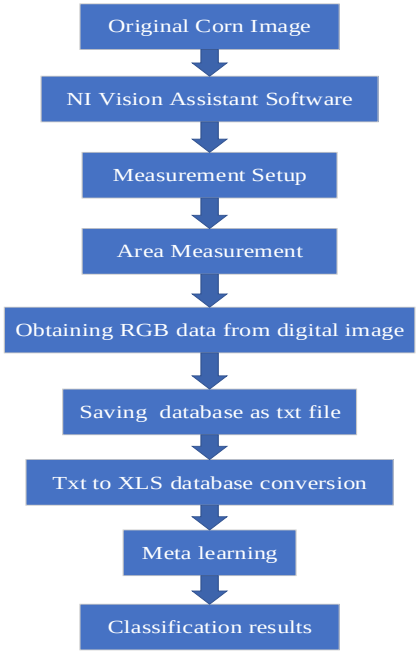

Figure 5- Classification steps of corn cultivars from digital images

2.2. Multilayer perceptron neural network

Since 1980, ANN has made progress thanks to developments in computer science. In addition to classification operations, it is also used in clustering and pattern definition operations. ANN refers to computer systems that perform the learning function of the human brain, just like the biological nervous system. In artificial intelligence, neural networks are frequently used in a wide range of implementations. A multilayer perceptron (MLP) is a feed-forward neural network structure that maps input training objects to target labels (Öztemel 2012; Silahtaroğlu 2016). In ANN, information is distributed over the connection weights between neurons. The hidden layer input is expressed as follows (Fakı 2015)

$$Hidden\ layer_{input} = w_1x_1 + w_2x_2 + w_3x_3 \quad (1)$$

In order to increase the ability of the ANN classifier to generalize learning and prevent overfitting, a small number of neurons and hidden layers were used to obtain the most appropriate network structure. Different ANNs were trained according to the algorithms used in this study. The ANN structure used in the study consisted of three hidden layers with 100 iterations and 10 neurons in each hidden layer (Table 2).

Table 2- Different ANN training results

Number of hidden layers	*Accuracy (%)*
1	96
2	97.33
3	97.66
4	96.66
5	97
6	92
7	77.33
8	84
9	79.66
10	70
15	61
25	33.66

2.3. Decision tree

Decision trees (DT) constitute an estimation model that represents the relationships between the properties and object values. They are frequently used in data mining methods. Decision trees, by sorting important attributes down the tree from the root to a leaf node, provided the classification of the instance. While each node is a decisive decision, each branch is finalized and results in a leaf. This process is then repeated for the subtree rooted at the new node. No branch continues with another branch (Mitchell 1997; Pandya & Pandya 2015; Köse 2018). In this study, for the Decision Tree, the gain ratio was selected as a measured quality, and Minimum Description Length was selected as a pruning technique.

The C4.5 tree is an improved version of the ID3 tree. It is an algorithm based on entropy and information gain same as the ID3 algorithm. Unlike the ID3 algorithm, pruning is performed in this algorithm (Köse 2018). Entropy is calculated by the following equation:

$$H(S) = \sum_{t=1}^{n} \rho i * \log_2(\rho i) \quad (2)$$

H: Entropy,
S: Source,
p: Probability (Silahtaroğlu 2016).
Gain information is obtained by calculating the differences between the weighted sums of the entropies of each sub-section (Silahtaroğlu 2016). The gain information is calculated by the following equation:

$$D = H(D) - \sum_{t=1}^{n} P(D_i)\, H(D_i) \quad (3)$$

D: Gain information,
H: Entropy,
p: Probability (Silahtaroğlu 2016).

2.4. Gradient boosted decision trees

Boosting is a prediction algorithm in machine learning based on the idea of combining a set of weak learners to create a single strong learner. Boosted trees is a classifier that is a combination of Boosting and Decision Trees in Meta-learning algorithms (Gupte et al. 2014; Kim et al. 2015). Likewise, GBDT is an ensemble method and a powerful supervised machine-learning technique that has been widely used in recent times mainly due to its high accuracy (Si et al. 2017). Formula 4 was used for multiple classification (Li et al. 2008):

$$\sum_{i=1}^{N} \sum_{k=0}^{K-1} -\log\left(P_{i,k}\right) 1_{yi=k} \quad (4)$$

2.5. Random forest

The random forest algorithm was developed by Leo Bieman, and it produces more than one tree to solve a question and creates different decision trees. RF is an ensemble learning method in meta-learning. These trees are an advanced version of the CART algorithm where many trees are created based on subsets of data. It is one of the popular meta-learning methods that provides simple and fast results in terms of understanding and application based on the collection of estimates from many decision trees.

It selects the observations and properties randomly to build various decision trees and takes the average of the results (Mitchell 2011). The Gini index is calculated through the following equation for producing a tree with the criteria of the RF (Classification and Regression tree) algorithm (Küçükönder et al. 2015).

$$\sum\sum j \neq i(f(C_i,T)/T)(f(C_i,T)/T) \quad (5)$$

2.6. Majority voting method in meta-learning

Meta-learning is an advanced technique in data mining that deals with the problem of computing a global classifier from the distributed database. In other words, this term is generally defined as learning from learned knowledge. Meta-Learning aims to learn from the predictions of classifiers on a common validation data set. At the end of classification, the meta-classifier works to train from the common validation data set (Figure 6) (Prodromidis et al. 2000). The selected Majority Voting (MAVL) method was deemed appropriate for showing correct high predictions. For this purpose, the 'Prediction Fusion' node in KNIME was connected between MLP and DT for majority voting application (Şeker & Erdoğan 2018). All nodes were connected as demonstrated in Figure 7. All algorithms were run with a 10-fold cross-validation method. At the end of the analysis, the accuracy, True Positives (TP), False Positives (FP), True Negatives (TN), False Negatives (FN), precision, recall, and F-means values were given in the relevant part of the study. Accuracy shows how accurately the system can predict by using the following formula (Takran et al. 2017):

$$Accuracy = \frac{TP}{(TP+TN+FP+FN)} \quad (6)$$

Recall or sensitivity or true positive rate (TPR) is the value that the system is predicting what rate is the passing assessment of the overall passing assessments. The recall is evaluated by using the following formula (Takran et al. 2017):

$$Recall = \frac{TP}{(TP+FP)} \quad (7)$$

Precision or Predicted Position Value (PPV) is the value which indicates how truly or correctly the system can predict (Takran et al. 2017):

$$Precision = \frac{TP}{(TP+FP)} \quad (8)$$

F-Measure is the overall efficiency assessment derived from the means of Precision and Recall (Takran et al. 2017):

$$F - Measure = \frac{2xPrecisionxRecall}{Precision+Recall} \quad (9)$$

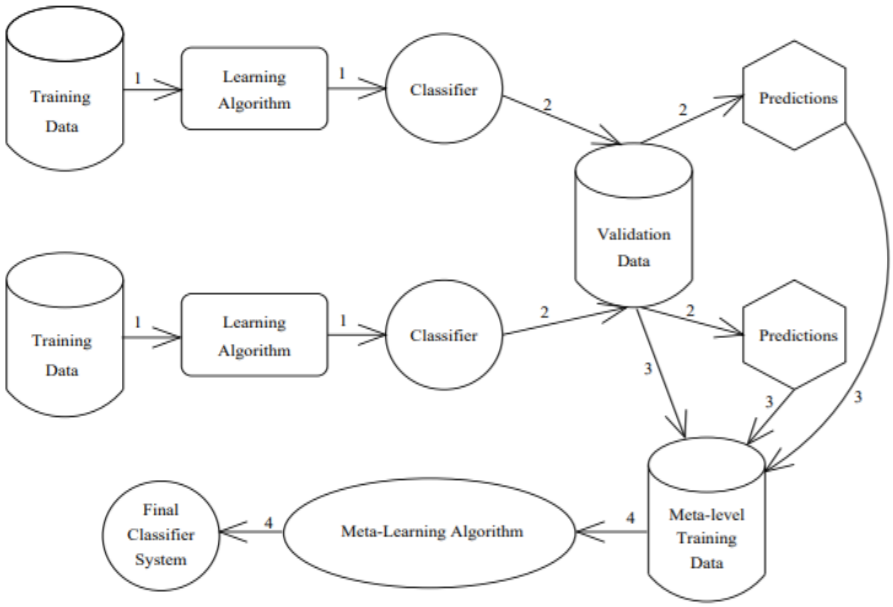

Figure 6- Meta-learning (Prodromidis et al. 2000)

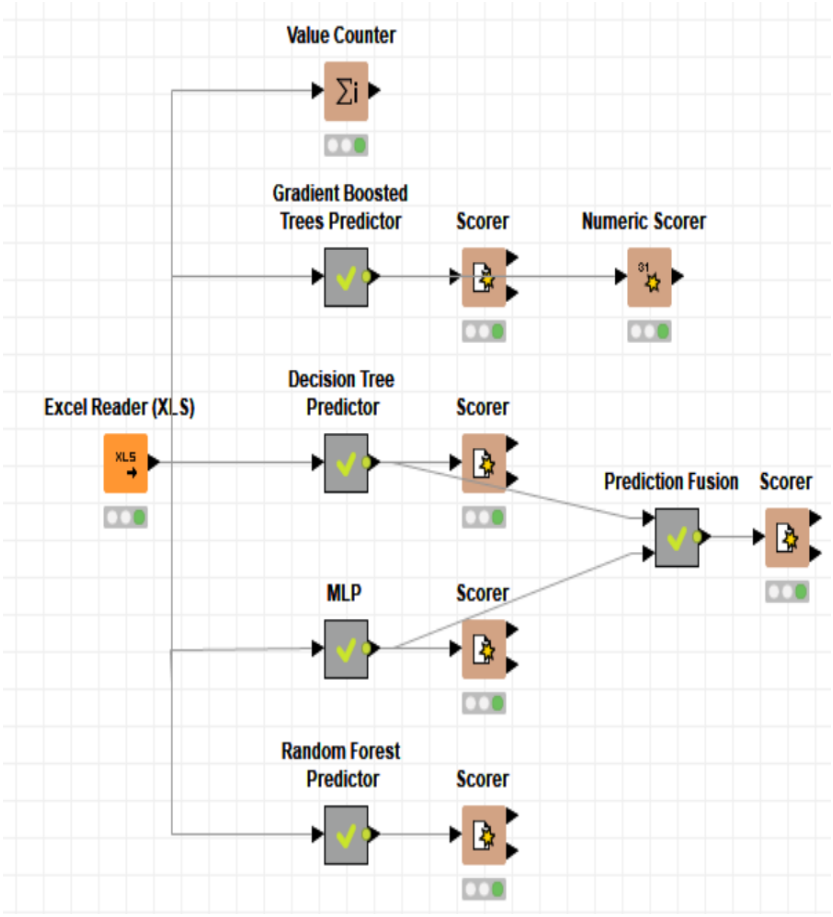

Figure 7- Workflow in KNIME

2.7. Training, validation and testing sets

Most machine learning techniques tend to overfit (Dietterich 1995; Shin et al. 2019). A way to prevent overfitting is to determine the optimum hyperparameter based on the data structure and use a large amount of training data. Moreover, adding validation steps or using well generalized architectures may also achieve success (Piotrowski & Napiorkowski 2013; Shin et al. 2019). In the study, an outer k-fold cross-validation loop was used for separating the data into training and testing folds in nested cross-validation and an inner loop for k-fold cross-validation with validation folds in the training folds. In order to evaluate the model, an independent testing dataset for the final model was kept, and these data were not provided before. The final accuracy was defined as the mean value of ten test accuracies obtained from ten training sessions, and this was 10-fold cross-validation. Whenever the training was running, the training datasets were randomly selected from the whole dataset (Shin et al. 2019).

The 10-Fold Cross Validation method refers to trying verification k times. In this method, for each 1/k of the data set, the previously unused part of the data set is used for testing, while the rest is used for training. Before applying the method, the k parameter must be determined. The k parameter specifies how many parts the data set will be divided into. k classification procedures are performed, and one of the parts divided at each step is reserved for the test process, while the remaining k-1 are used for the training of the classifier. The general classification result is obtained from the average of the classification results after k steps.

3. Results and Discussion

In the dataset, the R, G and B color channels were averaged to show the differences of all corn cultivars. The average color differences of corn cultivars can be seen in Figure 8. The confusion matrix and accuracy statistics of the supervised machine learning algorithms that were used are given in Tables 3 respectively. The success of prediction accuracy was found as 99% for RF and GBDT, 97.66% for MLP, 96.66% for DT and 97.40% for MAVL. The GBDT, RF, MLP, DT and MAVL algorithms were used, and in the comparison of the classification models, the Root Mean Square Error (RMSE), Mean Absolute Error (MAE), Root Relative Squared Error (RRSE), Relative Absolute Error (RAE) values were low, while the classification accuracy rate was high. As a result of the comparison, it was found that the classification model [MAE: 0.004, RMSE: 0.045% RAE:

0.915 and % RRSE: 9.458], which was formed based on the Random Forest algorithm, was a better classifier than the other algorithms with a prediction rate of 99%.

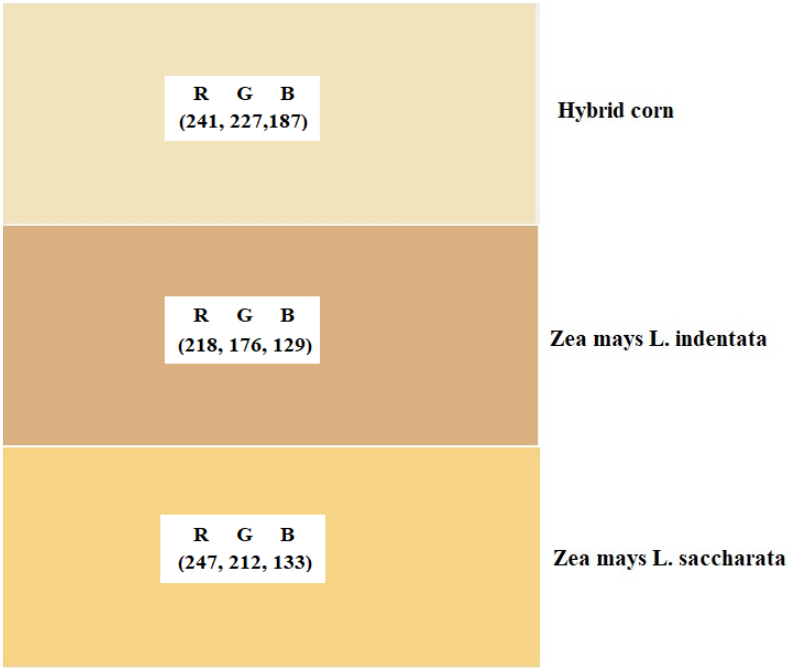

Figure 8- The average color values of corn cultivars for showing color differences (Anonymous 2019)

Table 3- Confusion matrix of the algorithms that were used

Algorithms	*Corn Various*	*Hybrid Corn*	*Indentata*	*Saccharata*	*Accuracy*
GBDT	Hybrid Corn	100	-	-	99%
	Indentata	1	99	-	
	Saccharata	2	-	98	
Random Forest	Hybrid Corn	100	-	-	99%
	Indentata	-	100	-	
	Saccharata	3	-	97	
MLP	Hybrid Corn	97	2	1	97.66%
	Indentata	3	97	-	
	Saccharata	1	-	99	
Decision Tree	Hybrid Corn	99	1	-	96.66%
	Indentata	4	95	1	
	Saccharata	3	1	96	
MAVL	Hybrid Corn	10284	385	106	97.40%
	Indentata	192	9342	-	
	Saccharata	97	-	9603	

The purpose of the meta-learning method is to contribute to high accuracy results when the low accuracy algorithm is evaluated together with another high accuracy algorithm. For this reason, Decision Tree, which has a low accuracy value, and Artificial Neural Network, which provides high accuracy values, were combined. As a result of MAVL, the decision tree prediction which had low accuracy was close to the artificial neural network prediction. The ANN and DT algorithm result was partly increased as from 96.66% to 97.40%. The disadvantage of the fusion process was that the ANN result was partly decreased as from 97.66% to 97.40%.

In the literature, Chen et al. (2010) worked on variety identification based on machine vision and pattern recognition for identifying five Chinese corn varieties according to their external features. They expressed that their classification accuracies of

corn varieties (BAINUO 6, NONGDA 86, NONGDA 108, GAOYOU 115, and NONGDA 4967) were 100, 94, 92, 88 and 100%, respectively, according to discriminant and neural networks analysis. Kurtulmus & Ünal (2015) investigated distinguishing rapeseed varieties using computer vision and machine learning based on SVM. They reported that the developed computer vision system provided an overall accuracy rate of 99.24% for the best predictive model in discriminating rapeseed variety. Additionally, Draganova et al. (2010) examined an approach for identifying fusarium-infected maize grains by spectral analysis in the visible and near-infrared region, SIMCA models, parametric classifiers and neural classifiers, and they stated that their recognition accuracy which was achieved for both classes of grains was 99.89% for the healthy and 93.7% for the infected specimens.

According to DT, *Zea mays L. saccharata* was a root node, and the distinctive attribute was G_{mean}. The decision tree visual results of Hybrid (Yellow sweet corn), *Zea mays L. Indentata* and *Zea mays L. saccharata* grains are given in Figure 9. As seen in Figure 9, the $Green_{mean}$ parameter provided a distinguishing feature in identification of the corn cultivars. It was seen that the cultivars could be identified at the values under and above the average value of 187.27, and then, the $Blue_{min}$ parameter was taken as the second order. It is possible to say that all cultivars under $Green_{mean}$ 90 and $Blue_{min}$ 5.50 could be classified as Hybrid corn.

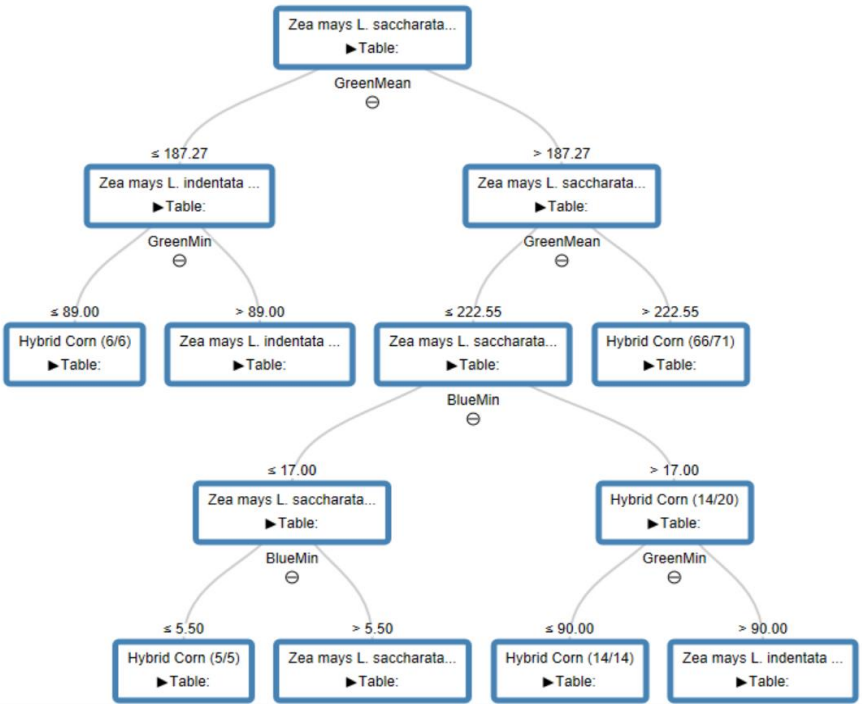

Figure 9- Decision tree visual results

4. Conclusions

As a conclusion, according to our research results and the literature, corn cultivars can be identified with the help of RGB color data, and these data may also be used for detecting the cracked and diseased ones among the healthy products. So, here, it can be clearly emphasized that, based on the differences and similarities, this algorithm can be used to distinguish different cultivars from other regions in future studies. However, it is not easy to argue that it could be possible to describe bulk products for increasing agricultural product quality. This is because, in bulk products, there are a lot of different parameters for color extraction like depth and 3D measurements. Furthermore, it may be stressed that the particular advantage of using meta learning here was making an optimization. Additionally, we think our results will be a source for future studies, and the contribution of this study to the literature is essential.

Abbreviations and Symbols	
TP	True positives
FP	False positives
TN	True negatives
FN	False negatives
GBDT	Gradient boosted decision trees
MLP	Multilayer perceptron neural network
MAVL	Majority voting
DT	Decision tree
RF	Random forest

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Assessment of Agricultural Sustainability in Sarikum Lake Basin, Sinop Province, Turkey

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ABSTRACT
The sustainability of agriculture in social, economic and environmental dimensions is important in terms of development policies. In this study, agricultural sustainability has been evaluated in social, economic and environmental dimensions. Twenty-one indicators were used and each dimension had 7 indicators. The research was carried out in Sarıkum Lake Basin of Sinop province in Turkey. The information obtained by face-to-face surveys with the farmers and the related statistics were used as data. The index method was used in the evaluation of these data. As a result, agricultural sustainability in the basin was found 13.95% in the environmental dimension, 9.64% in the economic dimension and 16.62% in the social dimension. The agricultural sustainability in Sarikum Lake Basin was determined as 40.21%. As a result of the study, in order to achieve economic sustainability it is necessary to increase the agricultural income, the ratio of irrigated land and the number of agricultural holdings with sufficient income. In order to ensure social sustainability, it is necessary to establish conditions to prevent migration and to ensure that farmers are satisfied with farming. In order to achieve environmental sustainability, it is necessary to increase the amount of protected area and to reduce wild animal damage.

Keywords: Sustainability; index; indicator; protected area; ecosystem

1. Introduction

Agricultural ecosystems provide food, feed, bio-energy and pharmaceutical raw materials for human beings and are the basis of human well-being (MEA 2005; Power 2010). It is estimated that the world population would be 9.2 billion in 2050, which is 7.6 billion in 2018 (WB 2018). Sustainability of agriculture is essential for the continuity of human existence.

Sustainable agriculture is only possible through making whole system sustainable; in social, economic and environmental aspects. The most common method used in the evaluation of sustainability is the index method. When the studies carried out to date were examined, it was observed that studies were carried out not only at the farm level (Gameda et al. 1997; Rigby et al. 2001; Van der Werf & Petit 2001; Frater & Franks 2013; Moore et al. 2014; Ryan et al. 2014; Waney et al. 2014), but also at regional, national and global levels (Trisorio 2004; Gomez Limon Jose & Riesgo 2008; Ceyhan 2010; Vecchione 2010).

The evaluation of agricultural sustainability around protected areas is particularly important in Turkey because of its rich biodiversity. The research area Sarikum Lake Basin, which includes Sarikum Lake Natural Park, has a rich biodiversity, being one of the world bird migration paths and having different ecosystems makes it one of the areas with high natural resource value and these values need to be protected. In addition, these characteristics in the Sarikum Lake basin cause the existing environmental balance to be more fragile.

2. Material and Methods

2.1 Research area

The research area is located in the Central Black Sea Region, in Sarikum area in the north of Sinop province (Figure 1). The basin has 6773.7 hectares and includes Sarikum, Basaran, Gumussuyu, Tekke, Yenicam, Selbeyi and Incirpinari villages.

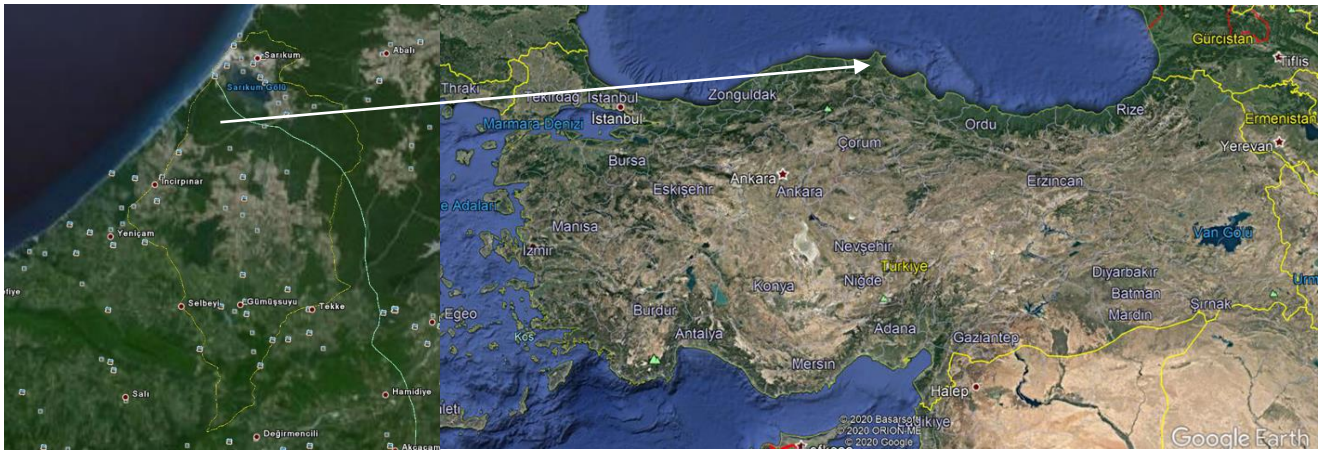

Figure 1- Sarikum Lake Basin

2.2. Data collection

The data were collected by face-to-face interviews through surveys with 40 farmers. The survey was conducted 2015 and the data for 2014-2015 production period was compiled. The farmers were selected randomly. The surveys were conducted to identify social, economic and environmental indicators.

2.3. Computation and data analysis

The proportional approach was used to determine sample size (Newbold 1995). The number of registered farmers in Erfelek and Merkez districts was 2060. The number of registered farmers in the study area (7 villages) was 413 (MAF 2015). Proportion of farmers in the study area, p= 0.2. The sample size was calculated 40 as the proportional sampling was used 90% confidence interval and 10% error margin.

$$n = \frac{Np\ (1 - p)}{(N - 1)\sigma^{2}_{\hat{p}_x} + p(1 - p)} \quad (1)$$

Where; N, Number of farmer, p= Proportion of farmers in the study area, variance, n, $\sigma^{2}_{p_x}$ Sample size.

Index method was used to assess agricultural sustainability. The Agricultural Sustainability Index (ASI) of the Sarikum Lake Basin was composed of social, economic and environmental dimensions (Gameda et al. 1997; Petrosyan 2010; Vecchione 2010; Moore et al. 2014; Ryan et al. 2014). The framework of the study was given Figure 2. and ASI steps were given Figure 3. Selecting indicators: In this study, group meetings were conducted in seven villages in the Sarikum Lake Basin before determining the indicators. The indicators were determined in the light of the data obtained from these meetings. The indicators used in this study were site-specific indicators and may not be applied in any another field (Waney et al. 2014). A total of 21 indicators were used in the agricultural sustainability index. Seven indicators were used under each dimension.

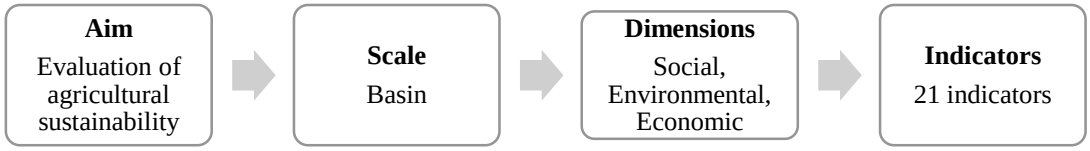

Figure 2- The research framework

Economic Dimension (Gameda et al. 1997; OECD 2002; Petrosyan 2010; Vecchione 2010; Gunduz et al. 2011; Demiryürek et al. 2013; Ryan et al. 2014; Lynch et al. 2019.): 1-Agricultural income competency level (%): The ratio of farms agricultural income was higher than poverty line. 2-Land productivity: It shows the ratio of farms higher agricultural income per da than average agricultural income per da in the basin.3-Fragmentation of land index: While the high fragmentation level has a positive impact on environmental sustainability, it has a negative impact on the economic dimension (Vecchione 2010). Therefore, Equation 2 was used to evaluate land fragmentation. 4-Crop diversification: Crop diversification decrease risks arising out of economic and environmental conditions (Zulfiqar & Thapa 2017). Equation 1 used to evaluate crop diversification. 5-Mechanization index: This index indicates a measure technological level of farms. It was the ratio of farms

with high level of mechanization to all farms (Vechione 2010).6-Land size (%): Soil Conservation and Land Use Law No. 5403 (Annex: 30/4 / 2014-6537 / article 5) refers to optimum size of farm land in Turkey (TR 2014). The optimum land size was 170 da. The ratio of the agricultural holdings have optimum land size. 7-Irrigated land (%): The ratio of irrigated land to the all land.

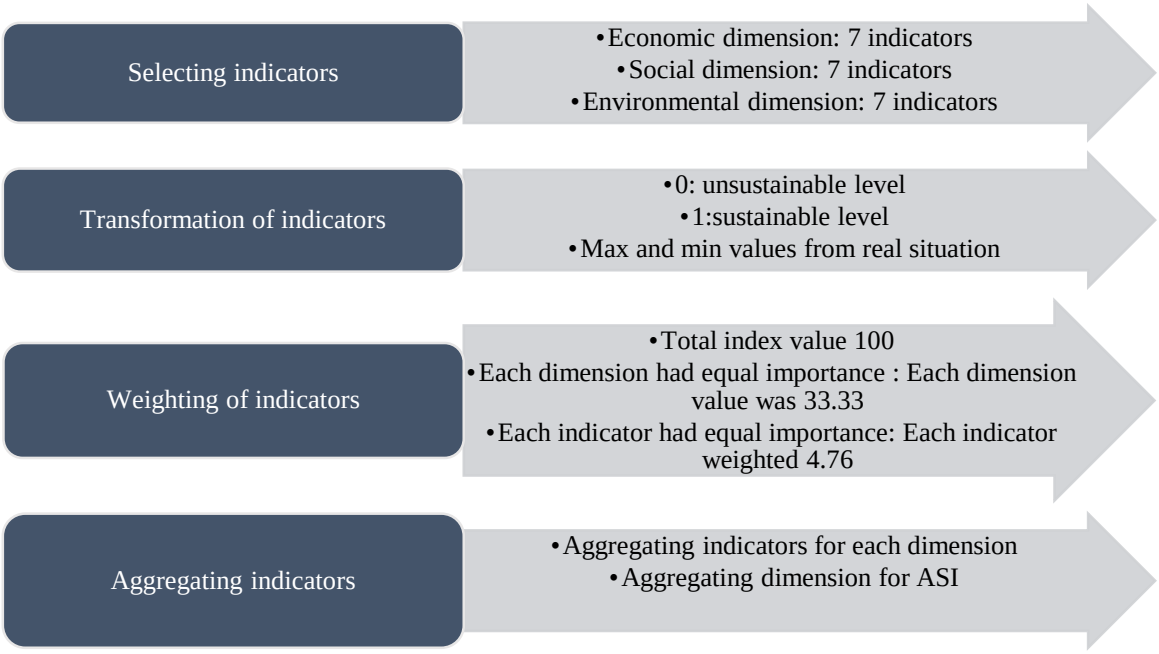

Figure 3- The steps of Agricultural Sustainability Index

Social Dimension (Gameda et al. 1997; OECD 2002; Petrosyan 2010; Vecchione 2010; Gunduz et al. 2011; Demiryürek et al. 2013; Moore et al. 2014; Ryan et al. 2014; Lynch et al. 2019): 1-Farm owner age index: The ratio between the numbers of farm owners with younger than sixty years and the all farm owners (%). 2-Gender distribution: Measures labour equality (Vecchione 2010). The ratio of female to all population. 3-Population resident: It is the number of residents in a particular geographic area (person/ha) (Vecchione 2010). 4-Organization level (%): The value was the ratio of the farmers belongs to any association, cooperative, union or professional chamber to the whole farmers. 5-Communication level (%): It was the ratio between the numbers of farmers who communicate agricultural organizations and the numbers of all farmers. 6-Satisfaction with dealing with agricultural activities (%): The ratio of farmers that were satisfied with dealing with agricultural activities to the total number of farmers. 7-Level of following agricultural innovations (%): The ratio of farmers following agricultural innovations to all farmers.

Environmental Dimension (Gameda et al. 1997; OECD 2002; Vecchione 2010; Gunduz et al. 2011; Demiryürek et al. 2013; Moore et al..2014; Ryan et al. 2014; Lynch et al. 2019): 1-Ratio of the farms with erosion problem (%): The ratio of the farms with erosion problems to the whole farms (Barrera Roldan & Saldivar Valdes 2002). 2-Use of pesticides (%): It was the ratio of the farmers using pesticides to all farmers (Zulfiqar & Thapa 2017). 3-Use of chemical fertilizers (%): It was the ratio of the farmers using chemical fertilizers to all farmers. 4-Level of soil analysis (%): The rate of the farms having soil analysis (Zulfiqar & Thapa 2017). 5-The protected area level (%): The ratio of the protected area in the total area (Barrera Roldan & Saldivar Valdes 2002). 6-Farmer's opinion about Sarikum Lake Natural Park as a protected area (%): The ratio of the farmers who are pleased to existence of protected area. 7-Ratio of wild animal damage (%): The ratio of the farms under wildlife damage to all farms.

However, it is necessary to consider the dynamics affecting the behavior of the indicators their spatial and temporal characteristics (Zhen & Routray 2003; Van Pham & Smith 2014; Mulligan et al. 2016). The second step of the ASI was transformation of the data (Figure 3). In the determination of agricultural sustainability, different approaches have been introduced (Rigby et al. 2001; Sulser et al. 2001; Ceyhan 2010; Petrosyan 2010; Vecchione 2010).

$$\text{Index} = 1 - \frac{[Maximum\ value] - [X]}{[Maximum\ value - Mininmum\ value]} \quad (2)$$

$$\text{Index} = 1 - \frac{[X] - [Maximum\ value]}{[Minimum\ value - Maximum\ value]} \quad (3)$$

X= The value of the variable used as the indicator (Barrera Roldan & Saldivar Valdes 2002; Ceyhan 2010; Demiryürek et al. 2013).

The third and fourth step of the ASI was given Figure 3. Each dimension of the ASI (social, economic and environmental dimensions) and each indicator of the ASI (21 indicators) had equal importance to eliminate influences of weights on the results (Figure 3). Each indicator multiplied by 4.76 to give final score out of 100. Total index value equal to 100, each dimension value equal to 33.3 and each indicator value equal to 4.76. These values also represent the max sustainability level (Figure 3).

3. Results and Discussion

3.1. Evaluation of structural characteristics of farms

Structural characteristics of investigated farms were given in Table 1. Average household size was about 4. Fifty-five percent of the population was female and 45% male. Average age of farm owner was 58 and they had about 5 years of education. They had an average of 64.6 da of land. Each farm was made of about five parcels of land, and each parcel was about 13.6 da in size (Table 1). The level of agricultural income adequacy was one of the most important indicators that show economic sustainability of agriculture. The value of this indicator was 1.05% (Figure 4). Waney et al. (2014) stated that the income was the most important indicator among the indicators determined by farmers. The main economic activity in the basin was agricultural activities. The high agricultural production cost was the most important reason of the low agricultural income level. Dellal et al. (2007) stated that the fuel costs rate in the total cost was varies 10%-20%. Land productivity index value determined as 2.00% (Figure 4). There were several reasons, which declined the land productivity. The production area of the high value-added products was very limited in the basin and family farming was common. Geographic features of the basin were the other factors that decreased the land productivity. Among the most important factors affecting the effective and sustainable use of agricultural land are its scale and fragmentation status. The number of parcels of agricultural land per holding was 5.9 and average parcel size of agricultural land was 12.9 da in 2016 in Turkey (TUIK 2018a). The less fragmentation of agricultural land had positive effect on the agricultural sustainability at economic aspect while it had negative effects at environmental aspect. The land fragmentation index value had the best score with 3.14% (Figure 4). Increasing the variety of crops in agricultural holdings had positive effects on agricultural sustainability in economic terms. The index of crop diversity was determined as 1.81% (Figure 4). The positive effect of crop diversity on the agricultural income was not demonstrated in the basin because the ratio of high market value crops was low. The mechanization index value determined as 1.29% (Figure 4). The low agricultural income level of the basin was the one of the most important reasons decreased the mechanization infrastructure investment. While the optimum farm size for dry conditions is stated as 170 da in Turkey (TR 2014), the average farm size of the Basin was 64,6 da. The number of agricultural holdings that met this condition in the basin was almost negligible and the indicator value was 0.24% (Figure 4). Türkten et al. (2014) stated that the average agricultural land size per agricultural holdings was 64.4 da in the Black Sea region. Thirty-one percent of the total land in Turkey was irrigated (TUIK 2018b). The rate of irrigated land in the basin was very low with 2.5% and the index value was the lowest with 0.12% in the economic dimension indicators of ASI (Figure 4). Due to the Mediterranean climate characteristics of the basin, the long drought period also decreased land efficiency and increased the need for irrigation.

Table 1- Characteristics of investigated farms

Characteristics of farms	*Mean*	*Std. Deviation*
Household size	3.93	1.82
Female	2.17	1.22
Male	1.76	0.89
Age of farm owner (year)	58.17	10.12
Education of farm owner (year)	5.39	1.80
Farm land (da)	64.61	41.90
Number of parcels	4.76	2.93

While assessment of agricultural sustainability, the social dimension has been often neglected because of difficult to measure. There was a need to develop methodologies for the evaluation of the social dynamics (Pinter & Herren 2006). Young farmers are extremely important roles to promote development in rural areas. It was determined that 78% of the farm owners was older than 50 years old in the basin. The fact that agricultural activities were based on labor force had increased the importance of young population in agriculture. In addition, wild boar pressure is very high on the agricultural lands in the basin. This situation increased the amount of labor needs in the agricultural holdings. The wild boar damage, the number of labor force and the low level of young population cause increase the amount of uncultivated land that can be forested in a short time. The ratio of female population determined as 55%. While women were actively involved in agricultural activities, they were not effective in decision-making processes in the agricultural holdings. The women who were actively involved in the production activities in the long term would bring about the fact that they were the decision makers in the business and had a

positive impact on agricultural sustainability in terms of gender equality. The economic structure of rural areas based on the agriculture and the socio-economic life and developments in these regions were a part of the agriculture. In some studies on rural migration show that factors related to agriculture were considered in the first place among the reasons of rural migration (Guresci 2009). The value of the resident population indicator was determined as 1.43% (Figure 4). When this indicator was evaluated together with other indicators (age, agricultural income, etc.), it could be foreseen that the resident population would continue to decrease in the basin. The main objective of the organization in agricultural was to increase the productivity of agricultural sector and to improve the producer's income and market position (İnan et al. 2000). Producer organization in the Turkish agriculture sector was not sufficient (MAF 2014). The organization level indicator value determined as 2.43% (Figure 4). The high level of relationship with agricultural organizations had a positive impact on agricultural sustainability. The index value of this indicator was 2.90% (Figure 4). The fact that farmers were in close contact with agricultural organizations was also an indicator of their willingness to make more conscious production in the basin, where a high proportion of elderly population and primary education common. The follow-up agricultural innovations showed the willingness to be open to new agricultural techniques and to implement it, and positively affected the agricultural sustainability. The index value of this indicator in the basin was 2.57% (Figure 4). In order to get positive results from development at local level, human-oriented, egalitarian and inclusive model must be established (Göymen 2004).

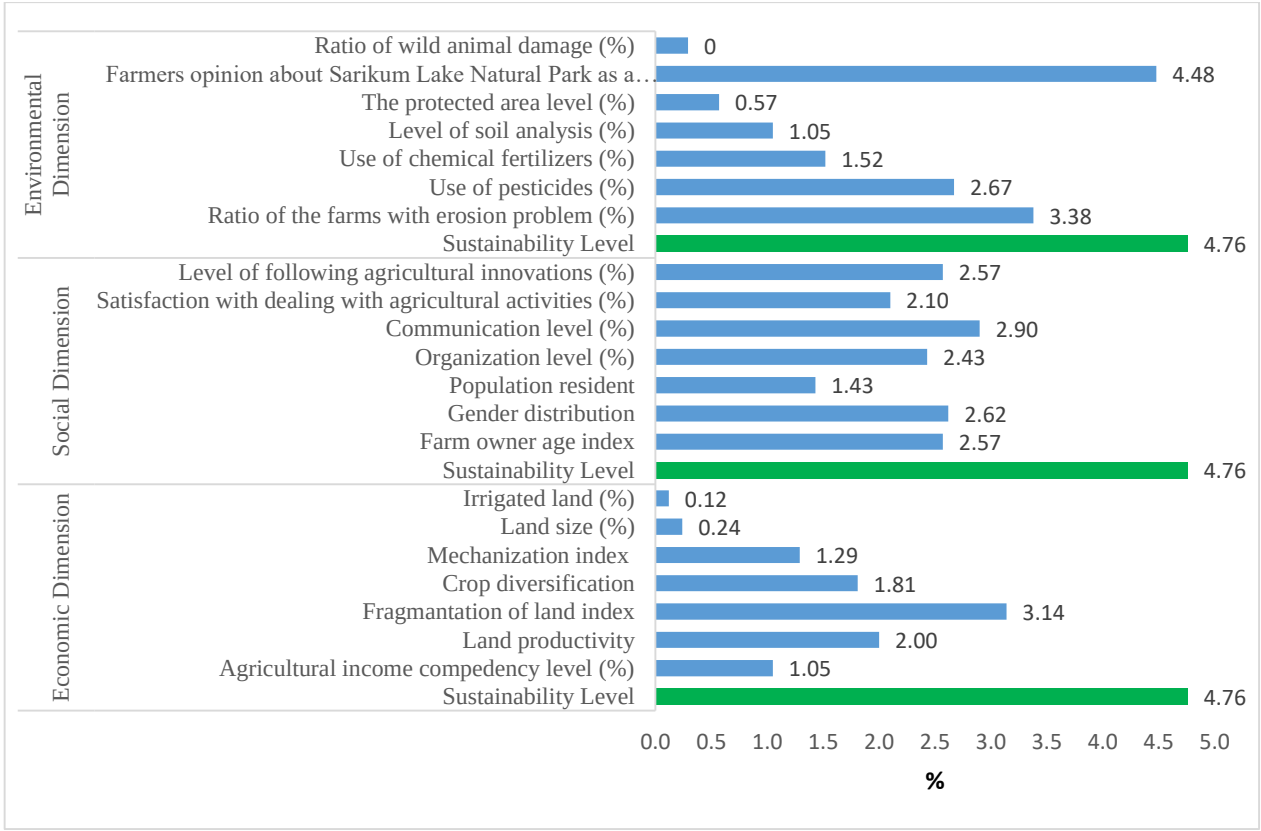

Figure 4- Agricultural sustainability indicators of Sarikum Lake Basin (%)

Twenty-nine percent of the agricultural holdings in the basin had erosion problems. The index value was 3.38% (Figure 4). The height of the basin varied between 0-440 meters. While 25.7% of the land was composed of flat and slightly slope areas, 46.2% of it is steep and steep slopes. The decrease in erosion risk in agricultural lands increased agricultural sustainability (Beşen et al. 2018). In Turkey, 59% of the cultivated land, while 64% of pastureland had erosion (MD 2014). The soil erosion reduced crop yields and increased the costs of production (FAO 2018). Waney et al. (2014) identified that the erosion control and the disease and pest control were the most important indicators at the environmental dimension for farmers. The high number of enterprises using pesticides had a negative impact on agricultural sustainability in the environmental dimension. The index value of the use of pesticides was 2.67% (Figure 4). The total amount of pesticide used was 54098 tons in 2017 in Turkey (TUIK 2018c). In recent years, biological control methods have more importance. Biological control methods should be supported in order to reduce the use of pesticides in the basin (Portakaldalı et al. 2015). Not only pesticide usage but also chemical fertilizer usage in unconsciously in agriculture harms the environment (Ataseven & Olhan 2010). The index value of chemical fertilizer use was 1.52% (Figure 4). The total amount of chemical fertilizer used was 13.09 billion tons in 2017 in Turkey (TUIK 2018d). Soil is essential for crop production. Neither food could be produced on a large scale, nor would livestock be fed without soil (FAO 2011). The index value of the soil analyses level was 1.05% (Figure 4). The rate of those using chemical fertilizers was higher than the rate of those having soil analysis. This indicated that the use of unconscious chemical fertilizers was widespread in the basin. The organic agriculture and the good agricultural practices have been

supported throughout the world in order to minimize the residues and to ensure sustainable environment (Türkten et al. 2014, MAF 2017). The fact that the Sarıkum Lake Basin has a rich biodiversity. The presence of protected areas in the basin affects the agricultural sustainability positively. The index value determined as 0.57% (Figure 4). The more effective protection can be provided by extending the protected area boundaries to the limits of the micro-catchment level. Adoption of protected areas by local people is very important in terms of preserving all natural values. It was determined that the people in the basin were satisfied with the presence of Sarıkum Lake Nature Park. Indicator value was 4.48% (Figure 4). Having a positive perspective about protected areas is a reflection of the importance given to environmental values. The high number of wild animals in forest areas increases wild animal damage in agricultural production areas and negatively affects agricultural sustainability in economic dimension. Wild animal damage indicator value was determined as 0.29% (Figure 4). It has the lowest value in the environmental dimension of agricultural sustainability. However, the presence of wild animals is an important source for the biological diversity of the Sarıkum Lake Basin and the presence of wild animals in terms of environmental sustainability creates a positive effect. On the other hand, the excessive increase in the population of a species can pose a threat to other species. It is necessary to control the wild boar population in order to protect the balance between the species and to reduce the wild boar damage on agricultural production areas. Solutions need to be developed to prevent conflicts between wildlife and rural populations. Thus, it can be ensured that nature and agriculture become factors that support each other, not competing with each other.

4. Conclusions

As a result of the research, economic sustainability had the lowest value while social sustainability had the highest value (Figure 5.) and the agricultural sustainability level of research area determined 40.23% (Figure 6). This value indicated that if the conditions of the area in social, economic and environmental aspects were not changed, the agricultural activities would be decrease. The sustainability of agriculture must be ensured in order to meet the increasing food demand and to protect all the benefits of agricultural ecosystem services.

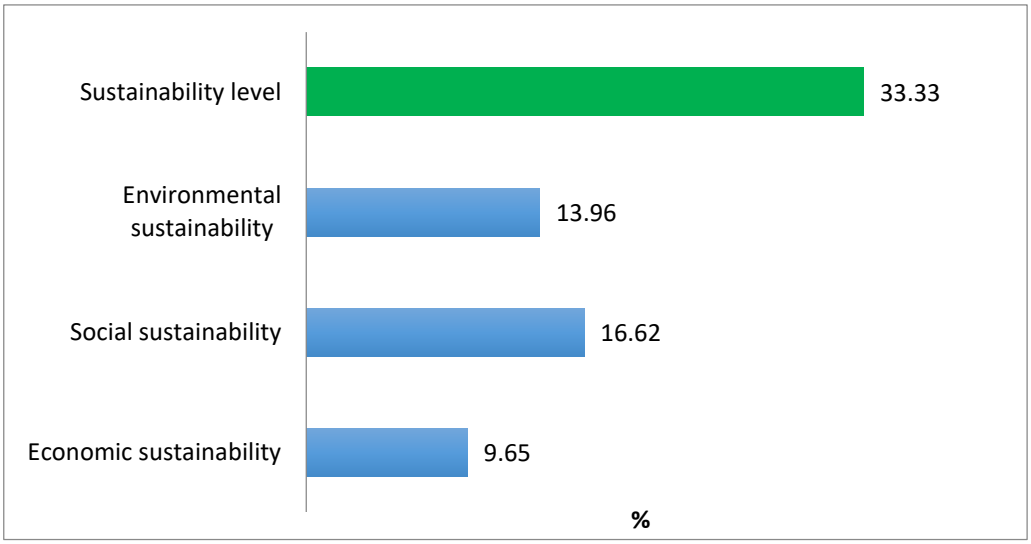

Figure 5- Economic, social and environmental sustainability level of Sarikum Lake Basin (%)

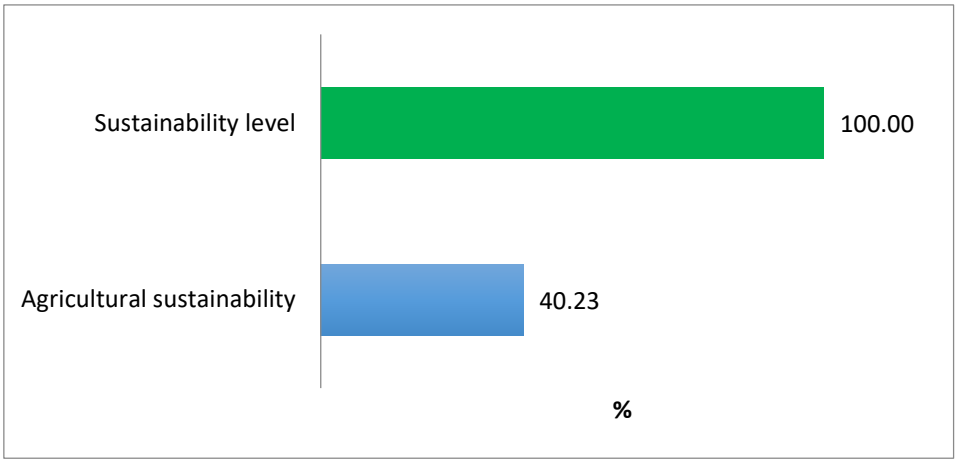

Figure 6- Agricultural sustainability level of Sarikum Lake Basin (%)

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Vigour Assessment of Dill (*Anethum graveolens* L.) Seed Lots in Relation to Predicting Seedling Emergence Potential

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ABSTRACT

The study aimed to test various vigour tests to correlate them with seedling emergence in two field sowing dates and two different sowing depths in controlled room emergence of ten dill seed lots. Mean germination time, accelerated aging; for 24 hours, 48 hours, and 72 hours, electrical conductivity readings at 16 hours and 24 hours, and EC readings after AA test were used as vigour tests, and results were correlated with seedling emergence percentages. Seedling emergence was tested in two sowing dates in the field and two different sowing depths in controlled room conditions. Controlled room seedling emergence values (80-95% in 2 cm, 76-92% in 4 cm) were higher than those of field emergence values (67-85% in the first sowing and 60-80% in the second sowing). Most of the vigour tests were significantly correlated with emergence potential. The highest correlation values were observed in EC16h and EC24h tests, significance between emergence and these two vigour tests were at P<0.001 three out of four sowings. The results indicated that EC16h can be a fast, practical and easy method of seed vigour discrimination in dill seed lots.

Keywords: *Accelerated aging, Electrical conductivity, Emergence correlations, Radicle emergence test, Seed vigour*

1. Introduction

High seedling establishment is essential for crop production to be profitable, and it is therefore widely accepted as a critically important trait for farmers. Seed lots having similar standard germination percentages can perform differently under the stressful conditions of commercial crop production. Seed vigour gives further information of any lot in the sowing conditions and is described as the sum total of those properties of the seed that determine the potential level of activity and performance of the seed during germination and seedling emergence (Marcos-Filho 2015).

Seed vigour and poor performance under field environments influence not only the number of seedlings that emerge in crops, but also the uniformity of emergence. For small-seeded vegetable crops, unpredictable timing of seedling emergence can disrupt planned schedules of production such as with dill, and insufficient and slow emergence can result in poor uniformity and yield (Finch-Savage 1995). Dill is a small seeded, leafy, short season vegetable crop species. It is grown throughout the year in the Mediterranean region either open-field or under plastic tunnels. Sowing in various field environments reduces the emergence of dill seeds, and stand establishment may not always be successful (Ozden et al. 2017). This is common, particularly when an aged seed lot is used. Therefore, the estimation of emergence potential of any lot (vigour assessment) helps farmers to plan sowing times and helps seed companies to arrange seed marketing. Seed lots differing in vigour (emergence potential) can be identified in various vigour tests, such as accelerated aging (AA) (TeKrony 2003; Guloksuz & Demir 2012), controlled deterioration (CD) (Basak et al. 2006), electrical conductivity (EC) (Matthews & Powell 2006; Demir et al. 2019; Ozden et al. 2020), mean germination time (MGT) and radicle emergence tests (RE) (Matthews & Powell 2011; Lv et al. 2016; Demir et al. 2019; Ozden et al. 2020). The tests have been shown to estimate the relative emergence of seed lots of many crops in the field and transplant modules. However, there is no study, to the best of our knowledge, on dill seeds. The objective of the present study was to investigate AA, EC, EC after AA and MGT as vigour tests to rank the relative emergence of seed lots of dill in field and controlled room conditions.

2. Material and Methods

Ten seed lots of dill (*Anethum graveolens* L. cv., Turkish *dereotu*) were obtained from commercial seed companies. The seed moisture content was determined according to ISTA (2017) rules. Seed lots were in sealed aluminium foil packets and were

stored at 5 °C until use. Before seed vigour tests, germination tests were carried out at 20 °C, for which 50 seeds of each lot were placed between paper towels (Filtrak, Germany) (20 cm x 20 cm), and wetted with 10 mL of distilled water. The germination tests were conducted with four replicates of 50 seeds in the dark. The paper towels were rolled and placed in plastic bags to prevent water loss. Radicle emergence (2 mm) percentages were calculated in daily counts during the germination test. Normal seedling percentages in the final count, at 14 days after the beginning of the test, were considered as standard germination (SG).

The MGT was calculated based on frequent radicle emergence counts using the formula;

$MGT = \sum n.t / \sum n$

Where n = number of seeds newly germinated (2 mm radicle emerged) at time t; t = days from planting, $\sum n$ = final germination

For the AA test, 40 mL of distilled water was added to each plastic aging box (11x11x4 cm) and 300 seeds were placed on a monolayer cheese cloth placed on the wire mesh tray (10x10x3 cm) inside the box (Hampton & TeKrony 1995). Seeds were aged at 45 °C for 24, 48 and 72 hours, after which they were dried on a laboratory bench for about three hours. The SG test was then conducted with four replicates of 50 seeds, using normal seedling development for assessment (ISTA 2017). The remaining 100 seeds in each period were used for EC measurements (EC after AA) after ageing.

In the EC measurements, two replicates of 50 unaged (EC) and aged (EC after AA) seeds were weighed and soaked in 40 mL distilled water for 24 hours at 20 °C in the dark. The EC of dill seeds were measured at 16 and 24 hours in both unaged (EC) seeds and seeds after accelerated aging (EC after AA). The EC of seed soak water was measured using a conductivity meter (Schott-Gerate GmbH, Hofheim) and expressed as $\mu Scm^{-1}g^{-1}$.

For controlled room emergence test (CRE), seeds (four replicates of 50 seeds/lot) were sown in 2 and 4 cm depths in peat moss (Plantaflor-Humus, Verkaufs-GmBH, Germany) in seedling trays (32x20x6 cm) and placed in the incubator at 20±2 °C. Light was provided at seedling level by cool fluorescent lamps (72 $\mu Mm^{-2}s^{-1}$) for 16 hours a day. The relative humidity in the cabinet was kept over 70% in order to eliminate evaporation from the surface. The number of emerged seedlings (unfolding cotyledons on the surface) was counted daily up to 25 days, and percentages of normally developed seedling were calculated.

For the field emergence test (FE), the seeds (four replicates of 100 seeds/lot) were sown in the experimental field of the Department of Horticulture, Faculty of Agriculture, University of Ankara, Turkey, on 9 September 2017 (first sowing) and 29 September 2017 (second sowing). The soil was a sandy loam (pH: 7.57, N: 0.09, P_2O_5: 5.5%, K: 0.16%, Na: 0.41%, Ca: 6.7%, organic matter: 1.1%). Daily minimum and maximum soil temperatures were recorded throughout both sowings and varied between 13 and 34 °C. The number of seedlings assessed to have emerged when the cotyledons had unfolded above the surface was counted daily for 35 days.

Statistical analysis was conducted using the Statistical Package for Social Sciences (SPSS) by using analyses of variance. Mean separation was made at the 5% level by the Duncan multiple range test. Correlation coefficients (r) of vigour tests with seedling emergence performance in controlled room and field conditions were also calculated.

3. Results and Discussion

The initial normal germination percentages of 10 seed lots were all above 90% (Table 1). MGT ranged between 3.5 and 4.7 days. Some of the seed lots that had the same normal germination showed differences in MGT. For example, lots 6 and 9 had a 91% germination value but MGT values were 3.7 and 4.7 days respectively.

The AA produced a wide range of normal germination percentages amongst the seed lots, indicating considerable differences in seed vigour (Table 1). The smallest range was observed in the 24 hours ageing period in which the lowest and the highest germination percentages after aging were 67 and 89%. These values were 37 and 70% at 48 hours of aging and 29 and 58% at 72 hours of aging. As the duration of the test increased, the germination speed between the lots also increased. There was a gradual decrease in the MGT of all lots as the AA period increased, and the lowest values were observed at 72 hours of aging.

Table 1- Standard germination (normal germination), mean germination time (MGT, h), Accelerated ageing after 24h (AA24 h), 48h (AA48h) and 72 (AA72h) hours of 10 commercially available dill seed lots. Means with different letters in the same column denote significant difference at 5% level

Seed lots	*SG (%)*	*MGT (day)*	*AA24h*	*AA48h*	*AA72h*
1	96 a	4.0 c	89 a	70 a	58 a
2	96 a	4.1 c	80 b	62 b	53 b
3	95 ab	3.9 bc	79 b	56 c	50 c
4	93 bc	3.5 a	72 c	50 de	35 f
5	94 ab	3.7 ab	73 c	45 f	29 i
6	91 cd	3.7 ab	70 cd	51 d	46 d
7	90 d	3.7 ab	70 cd	47 ef	30 hi
8	90 d	4.5 d	67 d	37 g	40 e
9	91 cd	4.7 d	67 d	50 de	33 fg
10	90 d	4.6 d	70 cd	51 d	32 gh
Range	90-96	3.5-4.7	67-89	37-70	29-58

The EC of soaking water after 16 hours (EC16h) and 24 hours (EC24h) were more contrasted between lots than were standard germination percentages, which ranged from 515 and 813 at 16 hours, and 521 and 883 at 24 hours (Table 2). EC measurements after accelerated aging increased as the aging time increased. The rate of EC increase was not the same in all seed lots. The EC readings of low-vigour seed lots such as lot 9 and 10 were higher than those of the high-vigour lots.

Table 2- Changes in EC after 16 (EC16h) and 24 hours (EC24h) and EC16, EC24h after Accelerated ageing after 24h and 48h in 10 dill seed lots. Means with different letters in the same column denote significant difference at 5% level

Seed lots	*EC 16h*	*EC 24h*	*EC16h /AA24h*	*EC24h /AA24h*	*EC16h /AA48h*	*EC24h/ AA48h*	*EC16h/ AA72h*	*EC24h/ AA24h*
1	515 a	521 a	565 a	576 a	547 a	561 a	621 a	636 a
2	552 b	600 b	603 b	618 b	662 b	661 b	650 b	670 b
3	626 c	655 c	684 d	731 d	757 e	763 e	767 d	799 de
4	700 e	698 d	690 de	702 c	697 c	716 d	749 d	813 ef
5	647 c	711 d	634 c	691 c	708 cd	691 c	747 d	790 d
6	654 cd	712 d	710 e	731 d	719 d	721 d	716 c	760 c
7	691 e	692 d	751 f	757 e	777 f	819 f	823 e	838 g
8	679 de	706 d	742 f	810 f	792 f	825 f	805 e	830 fg
9	756 f	751 e	786 g	833 f	878 g	896 g	811 e	864 h
10	814 g	884 f	885 h	946 g	990 h	1024 h	972 f	1005 i
Range	515-813	521-883	565-884	576-946	547-989	561-1024	621-972	636-1005

Seedling emergence in sowings at 2 cm and 4 cm depths in a controlled room ranged between 80 and 95% at the first sowing depth and between 76 and 92% at the second sowing depth (Table 3). Nine out of ten lots showed lower emergence percentages at deep sowing than at surface sowing depth. The differences between the two different sowing depths among the lots went up to 8% (Lots 4 and 6). Deep sowing was more discriminative of the seed vigour level of the lots, indicating the effect of mechanical stress on the vigour level. Field emergence percentages in the two sowings had very similar results, ranging between 67 and 85% in the first sowing, and between 60 and 80% in the second sowing. Most of the seed lots had the same or slightly lower emergence percentages in the two sowings. The differences varied only between 2 and 5% in nine lots. The final lot (lot 10) had a 7% difference between the two sowings.

Table 3- Changes in controlled room conditions in 2 cm and 4 cm depth sowings and field emergence percentages in two sowings of 10 dill seed lots. Means with different letters in the same column denote significant difference at 5% level

Seed lots	*CRE (%) (sowing depth,)*		*FE (%)*	
	2 cm	*4 cm*	*1. Sowing*	*2. Sowing*
1	95 a	92 a	85 a	80 a
2	91 b	92 a	78 b	75 b
3	88 c	82 b	75 c	72 cd
4	87 c	79 cd	72 de	75 b
5	82 de	78 de	74 cd	71 cde
6	84 d	76 e	71 e	73 bc
7	84 d	81 bc	73 cde	70 de
8	87 c	80 bcd	67 f	69 e
9	82 de	80 bcd	68 f	65 f
10	80 e	76 e	67 f	60 g
Range	80-95	76-92	67-85	60-80

Correlation values between seed vigour tests and two sowings in CRE and two in field conditions indicated several close associations (Table 4). EC16h, EC24h and EC24h/AA24h were highly correlated (P<0.001) in three out of four sowings. The rest of the vigour tests showed various levels of correlation but not at that level (Table 4). SG had a low level of significance, with two at 5% and two at the 1% level along with AA72h (three at 0.05, one at 0.01).

Table 4- Correlation coefficients calculated for MGT, AA (24, 48 and 72h), EC after 16h (EC16h), 24h (EC24h), EC16h after AA 24h and 48h and 72h and standard laboratory germination (SG) as estimation of seedling emergence percentages of field emergence and controlled room conditions in 10 dill seed lots

Seed Vigour Test	*CRE (Sowing depth)*		*FE*		*Total*		
	2 cm	*4 cm*	*1. Sowing*	*2. Sowing*	*	**	***
MGT	-0,212	-0,002	-0,425	-0.647*	1		
AA24h	0,831**	0,820**	0,951***	0.721*	1	2	1
AA48h	0,685*	0,764**	0,829**	0.554	1	2	
AA72h	0,860**	0,744*	0,702*	0.678*	3	1	
EC16h	-0.871***	-0.809**	-0.890***	-0.897***		1	3
EC24h	-0,915***	-0,836**	-0,880***	-0.922***		1	3
EC16h/AA24h	-0,785**	-0,713*	-0,862**	-0.923***	1	2	1
EC24h/AA24h	-0,799**	-0,731*	-0,887***	-0.958***	1	1	2
EC16h/AA48h	-0,815**	-0,664*	-0,852**	-0.985***	1	2	1
EC24h/AA48h	-0,774**	-0,637*	-0,843**	-0.969***	1	2	1
EC16h/AA72h	-0,795**	-0,708*	-0,799**	-0.933***	1	2	1
EC24h/AA72h	-0,838**	-0,776**	-0,842**	-0.927***		3	1
SG	0.728*	0.743*	0.849**	0.724*	3	1	

; P<0.05,; P<0.01,***; P<0.001*

The close and significant relations between EC16 and four sowing times are shown in Figure 1. The relationship between SG and sowings are also given in the same figure. EC16h was highly related to CRE percentages (R^2= 0.761, P<0.001, R^2=0.656, P<0.01) and emergence in field conditions (R^2=0.792-0.803, P<0.001), while SG was related to emergence percentages at much lower levels of significance (R^2=0.53- 0.55 in CRE; P<0.05, R^2=0.52-0.72, P<0.05-0.01 in field sowings).

The present study indicated that EC16 and EC24h were highly related to seedling emergence in controlled room and field conditions (P<0.001, 3 out of 4 sowing dates) (Table 4). Field establishment of some vegetables such as dill is usually performed with direct sowing of the seeds. As a result, rapid and uniform emergence of vigorous seedlings of the desired lot is a key event in ensuring high plant performance, affecting uniformity of development yield and quality of the harvested product. The main necessity to achieve that is the use of high-vigour seed lots. Standard laboratory germination tests indicate the emergence potential of any seed lot when the sowing environment is favourable for emergence. However, estimation of field emergence potential (seed vigour) of any lot is necessary, since deviations in ideal sowing conditions are common in the field. These deviations from ideal conditions are becoming more common due to climatic changes in various parts of the world. Thus, sub-optimum sowing environments are common because of low water availability, adverse soil characteristics and management practices. The use of commercially available seed lots with more than 90% of standard laboratory germination ensured a realistic approach to test emergence potential, since seed vigour is defined as the quality parameter that discriminates the lots with high germination percentages (Marcos-Filho 2015). Despite SG values being very high, accelerated aging after 24, 48 and 72 hours showed a great range (Table 1). The range was extended as the seed aging period lengthened.

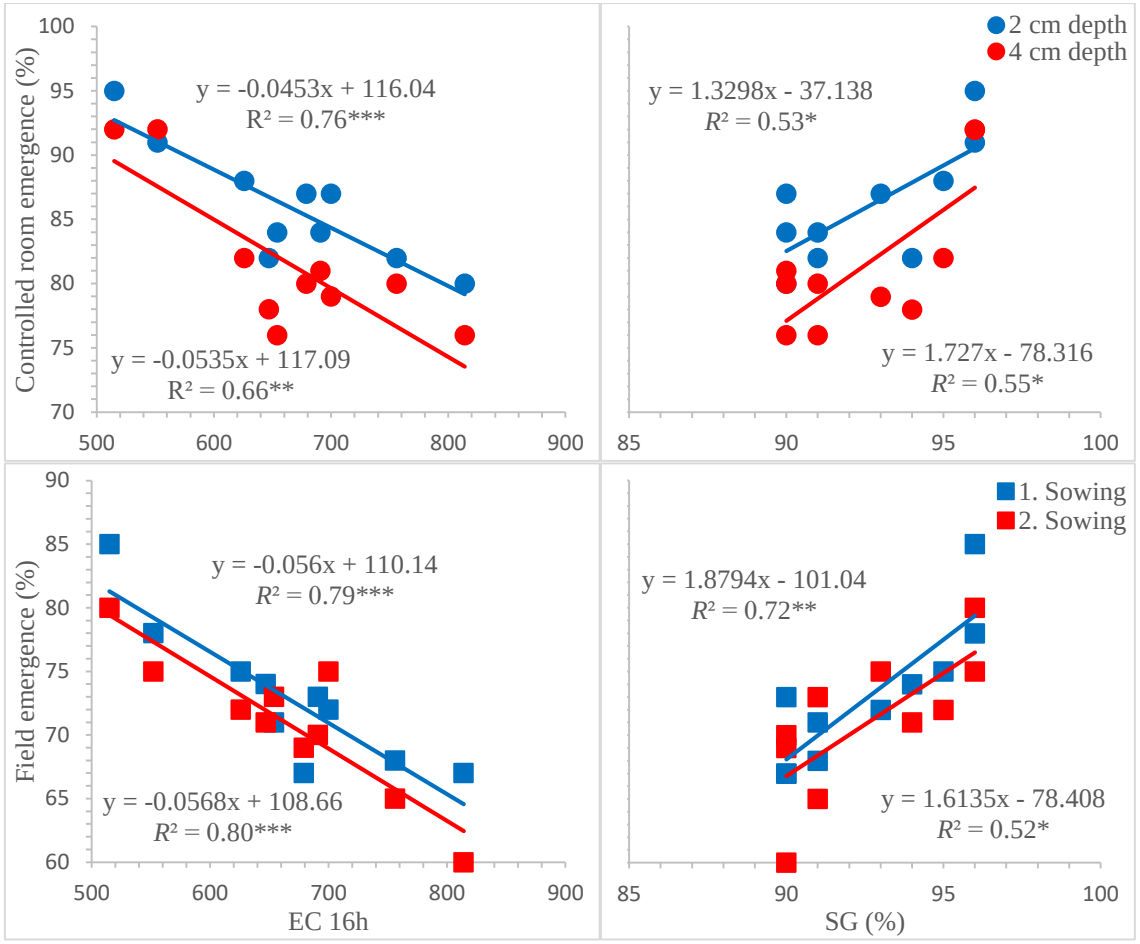

Figure 1- Relationship between electrical conductivity of 16 h (EC16h), and SG with field emergence in two sowings, 1. (■) and 2. sowing (■), and 2 cm (●) and 4 cm (●) depth sowings in controlled room emergence, of 10 dill seed lots. Significance: *: 0.05, **: 0.01, *:0.001**

The most obvious physiological manifestation of seed aging is the decline in germination rate, which is associated with seedling size and abnormalities. The accelerated aging test and electrical conductivity are two vigour tests that are currently validated by ISTA rules (ISTA 2017) for soybeans and garden peas and beans respectively (ISTA 2017). There are a number of studies in which AA is well related to the seedling emergence of horticultural and agronomical plant seeds (TeKrony 2003; Marcos-Filho 2015). Seeds in the accelerated aging test are exposed to high relative humidity and high temperature (100% RH, 40-45 °C). About 24-72 hours is basically suggested to be used with large seeded plants. In our study, AA with three different aging periods was correlated to seedling emergence (P<0.05-P<.001, r= 0.678-0.890) except in one case (AA48h, second sowing field emergence) (Table 4). This shows that the AA test can also be used with small seeded species. The reason that AA is less successful with small seeded plant seeds is that small seeds absorb water more rapidly, resulting in large variations and inequality among the seeds and lack of uniformity among the samples (Demir & Mavi 2007; TeKrony 2003). It has been suggested that this difference among seed lots promotes large differences between aging rates and great reductions in germination after aging. We have not measured the seed moisture contents after aging. However, we spread the seeds on just a single layer of mesh in an aging box to expose the seeds to high relative humidity. One precaution in aging small seeded species is to prevent seeds from dropping into the water underneath the mesh tray. Therefore, we put a cheese cloth under the seeds to prevent that.

Mean germination times have been used to assess seed quality in agricultural species (Demir et al. 2008; Khajeh-Hosseini et al. 2009; Mavi et al. 2010). In this work MGT was significantly related in only one case (the second sowing in the field) but had a relatively lower correlation with seedling emergence percentages (R^2= 0.647, P<0.05) compared to EC or AA tests (Table 4). MGT is generally calculated by frequent counts of germination, and MGT value extends as the delay between imbibition and radicle emergence. We suppose that more frequent counts during germination, particularly in the early stages of germination, would have given more discriminating results in MGT because seed lots in dill started to germinate in the very early hours of the test (12 hours).

Electrical conductivity has been described as a biochemical vigour test and associates with low germinability to high release of solutes in soaking (McDonald 1999; Matthews & Powell 2006; Demir et al. 2012; Ermis et al. 2016; Demir et al. 2019; Ozden et al. 2020). The principle of the EC test is that more deteriorated seeds release greater amount of solutes to the external environment. Under field conditions, leakage of exudates after sowing can stimulate the growth of pathogenic microorganisms and impair seedling emergence. Leakage during imbibition has been related to disruption of cell membrane systems and loss of semi-permeable structures (Matthews 1985). The negative correlation found between EC and seedling emergence (Figure 1) showed greater leakage in low-vigorous seeds. High solute leakage may result in an increase in the inoculum potential of various diseases such as *Pythium*, with dead tissue providing an initial site for infection (Matthews et al. 1988). The main advantage of using EC measurements as estimates of seed vigour level is the short time needed for assessment. This is particularly confirmed in this study for EC measurements just after 16 hours (overnight). The fast evaluation of seed vigour of the lots has great value for seed companies because in some periods of the year they need to test a large number of seed lots. Such tests save time, and are practical for farm based vigour assessments. Our results suggest that EC measurements can be used by seed producers to identify low-vigour lots.

4. Conclusions

In conclusion, we have shown that solute leakage from seeds using EC has the potential to predict seed vigour level in dill seeds. Higher levels of leakage were seen for dill lots that also showed low emergence not only in field (sub-optimum) conditions but also controlled room conditions indicating low vigour seeds. Differences among the lots can be detected within 16 hours (overnight) by EC readings. There may be a potential for a quick, cheap and simple vigour assessment method for testing seed vigour in farm-based tests.

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Effect of Production System and Slaughter age on Some Meat Quality and Digestive Tract Traits of Pheasants (*Phasianus colchicus*)

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ABSTRACT
Male and female mixed 200 pheasants were reared in intensive and outdoor conditions to assess the meat quality and some digestive tract traits. Color, pH, cooking loss, water holding capacity and drip loss were determined as meat quality traits. Production system did not have significant effect on most of meat quality traits. Conversely, most of these traits were affected by slaughter age. Water holding capacity significantly decreased but cooking loss increased by slaughter age. Total digestive system weight rate to body weight was decreased by slaughter age but did not differ between production systems. Small and large intestine lengths were significantly longer in indoor system and significantly decreased by slaughter age.

Keywords: *Pheasant, meat quality, digestive system, water holding capacity, pH*

1. Introduction

Chicken and turkey meat have reached the 93.5% of total poultry meat consumption in the World (Faostat 2017). Similar rearing conditions and same feed formulas have made the tastes of both turkey and chicken meat similar to each other. This prompted consumers to seek alternative poultry tastes. Game birds are good sources for the consumers and pheasants have highest rate in total consumed game meat (Bodnar et al. 2010). But it is also hard for people to reach these products. Therefore, it is important to produce game birds in intensive or semi intensive conditions. Poultry meat consumers are mostly interested in the production conditions of birds (Adamski et al. 2017) and animal welfare becomes more important in years. On the other hand, consumers' demands have to be met in these animal friendly production systems.

Pheasants are bred in intensive conditions for releasing to hunting areas. Farming of pheasants is also done for slaughter and meat production (Golze 2010). Meat quality of captured/ hunted (Hofbauer et al. 2010) or intensively reared (Kokoszynski et al. 2012) pheasants was investigated in previous studies. On the other hand, it was reported that by raising game birds under free-range systems, they could have a taste more similar to game-bird meat (Yamak et al. 2018). Meat quality of pheasants reared in extensive system was given by Franco & Lorenzo (2013). There is a need of detailed information on the comparison of meat quality of pheasants reared in outdoor and indoor systems. Also, the effect of production system on digestive traits of poultry species was shown before (Bartlett et al. 2015). Therefore, the effects of production system (indoor & outdoor) on some meat quality and digestive traits of pheasants were investigated in the current study.

2. Material and Methods

This study was performed at the Ondokuz Mayis University Agriculture Faculty Research Farm during May-August 2015. Experimental Animals Ethical Committee of the university approved the study. Eggs were collected from a flock of pheasants obtained from the Turkish Ministry of Forests and Water Affairs' Samsun Breeding Station. All eggs were transferred to the farm's hatchery on the same day which they were collected. Eggs were incubated for 25 days. After hatching, 200 day-old chicks were randomly selected and used in the experiment.

Chicks were randomly allocated to pens belonging to either an indoor or outdoor-access ('free range') production system that were interspersed within windowed houses, with 4 pens per system and 25 chicks per pen. The pens were in dimensions of 3.5 x 3.5 m. Wire mesh was used to keep birds from flying between pens. One round feeder and 1 round drinker was placed in each pen. Wood shavings used as litter in the pens. Infra-red heaters were used for heating of the house, while lighting was applied with economic white bulbs. During first three days, 24-h lighting was applied and incrementally decreased to 20 hours over Days 3-14 and then remained constant until 6 weeks, after which natural lighting (app.14 h day^{-1}) was applied until slaughter. Each replicate had its own outdoor area measuring 14x3.5 m separated by an exit door. The doors of pens were opened when pheasants reached 6 weeks of age and kept opened until the end of study. The floor of outdoor area was soil and some tree branches were placed on floor area for perching of birds. Pens were divided by wire mesh walls with height of 2 meters. Also, the top of outdoor area was covered with wire mesh for preventing birds flying.

Corn and soybean meal based feed and water were provided *ad libidum.* Until 12 weeks of age feed with given ingredients was supplied (190 g CP and 11.72 MJ ME, 10.0 g lysine, 4.0 g methionine, 11.0 g Ca, 7.0 g P, 120 mg Mn, 15 mg Cu, 100 mg Zn per kg). After 12 weeks to slaughter feed as given were provided (160 g CP, 11.30 MJ ME, 3.50 g methionine, 7.20 g lysine, 10.0 g Ca, 4.00 g P per kg).

All birds were wing-banded to identify individually. Live weights were recorded individually by an electronic bascule with the precision of 0.01 g (Hassas electronic scales, TEM TPG, İstanbul, Turkey) at hatch, 2, 4, 6, 8, 10, 12, 14, 16 and 18 weeks of age. Two males and two females per pen were slaughtered at 14, 16 and 18 weeks of age. Live weights were recorded prior to slaughter. Scalding (1 min. at 56 °C), picking, cold-water chilling, vent opening, evisceration and air-chilling were performed using semi-automated equipment. Hot-carcass weights were recorded; carcasses were then chilled for 12 hours at 4 °C, and cold-carcass weights recorded.

pH values of meat were measured on left breast and legs after carcasses chilled 12 hours at 4 °C. On each body part, measurements were repeated at three different points using a pH meter (Model PC 510, Cyber scan, Singapore). Similarly to pH, meat color (L* a* b*) was measured on left breast and legs, but measurements were repeated two different points of each part (Fanatico et al. 2007; Sarica et al. 2011) using a colorimeter (Konica Minolta CR-400 colorimeter). Mean values for both color and pH values were calculated and recorded.

Drip loss was evaluated by suspending 1 intact fillet in a sealed glass box for 48 h at 2-4 °C and expressed as percentage of weight loss during storage (Bianchi et al. 2007). Whole samples of both breast and leg muscles (20 g) were placed in uncovered aluminum pans and roasted in an electric oven pre-heated to 200 °C for 15 min until meat samples reached an internal temperature of 80 °C. Samples were cooled for 30 min to about 15 °C and dried on the surface with paper towels, and cooking loss was estimated as the percentage of the weight of the roasted samples with respect to the raw ones (Castellini et al. 2002). Meat water-holding capacity (WHC) was determined by calculating the weight-loss of a 1-g sample after centrifuging it for 4 min at 1500x*g* and drying it overnight at 70 °C (Castellini et al. 2002). Total digestive tract was weighed with an electronic bascule in precision of 0.01 g. Spleen, pancreas and craw was carefully separated from digestive tract and individually weighed with same bascule. an electronic bascule with precision of 0.01 g. Digestive tract was laid on a flat surface and total length was measured with a ruler with accuracy of 1 mm. Intestines were also measured with same method. The data about digestive tract organs was expressed as the percentage to body weight.

2.1. Statistical analysis

SPSS Software was used in the analysis. Analysis of variance with a factorial arrangement was used to test the effects of production system, age, sex and the interactions among these factors. Data was subjected to arc-sine transformation, and genotype and slaughter-age means were separated using Duncan's multiple range test. A level of $P<0.05$ was considered statistically significant.

3. Results and Discussion

Color and pH values measured at different slaughter ages were given in Table 1. Lightness (L*) values of breast meat were ranged between 50.18 and 57.95 while these values were between 46.69 and 54.32 for thigh meat. Production system only affected the thigh-meat yellowness and birds reared in outdoor had higher b* values (1.33 vs 0.53; $P<0.01$), whereas gender only affected thigh-meat redness and females had higher a* values (8.65 vs 7.74; $P<0.05$). All meat color traits were affected by slaughter age ($P<0.01$; $P<0.05$). Neither production system nor gender affected meat pH. Slaughter age had a significant effect on thigh meat pH ($P<0.01$).

L* value ranges from 0 to 100 which gives the lightness from black to white (Papadakis et al. 2000). Mean L* values of breast meat obtained in our study were found parallel with the findings of Hofbauer et al. (2010) and Kokoszynski et al. (2012) who found L values of breast meat between 51.4 and 54.2. There are also studies found the breast meat lightness lower. Fernye et al. (2017) and Dvořák et al. (2007) reported the breast meat lightness of pheasant breast meat to 49.27 and 41.19, respectively. Different factors could have effect on this variation. Breed, age, sex, diet, breeding practices, pH, total haem and

mygoblobin content are considered as factors affect meat colour. It is expected older birds to have higher myoglobin content (Wideman et al. 2016). This has to result with lower L* values at older slaughter ages. Contrarily, L* values of breast and thigh meat were decreased at 16 weeks of age and then increased to 14 week's level at 18 weeks. This could be related to slaughter and post mortem processing. Evolution time postmortem (Culioli et al. 1990), the residual blood amount is among the key factors which affect poultry meat color quality (Mohamed & Mohamed 2012). Also, it is reported that breast muscle has significantly lower myoglobin content than the thigh muscle. The number of red muscle fibres are higher in thigh muscle, while the breast meat is mostly composed of white fibres (Barbut 2001). Red fibres have high mygoglobin content than white fibres (Wideman et al. 2016). The numerical values of a* and b* range from -120 to +120 with a* ranging from green if negative to red if positive. Similarly, b* ranging from blue if negative to yellow if positive (Papadakis et al. 2000). According to this, it is normal to thigh meat had higher a* values than breast meat. Breast and thigh meat redness determined in this study found similar to the findings of Kokoszynski et al. (2012) who found a* value 3.8 for breast meat and Kotowicz et al. (2012) who found it 10.5 for thigh meat. Also, thigh meat had lower L* values than breast meat in our study. This could be related to pH levels of both breast and thigh meat. Fletcher (1999), reported that pH of meat has a strong effect on meat color with higher pH values resulting in a darker meat. pH levels of both breast and thigh meat were found higher than reported values for pheasant meat. Kotowicz et al. (2012) reported pH of breast and thigh meat 5.57 and 5.95 respectively, while, Kokoszynski et al. (2012) found pH of breast and thigh meat as 5.74 and 6.57. It is thought that this is due to glycogen concentration in the muscles which could be related to struggling during slaughter (Debut et al. 2003). Yellowness (b*) of breast and thigh meat was found lower than the reported values of previous studies. Kotowicz et al. (2012) determined the breast meat b* value between 12.7 and 15.3; and thigh meat yellowness between 6.34 and 9.98. Fernye et al. (2017) and Kokoszynski et al. (2012) found the yellowness of pheasant breast meat between 4.8 and 7.59. This could also be related to pH. Allen et al. (1998) showed that lightness (L*) and yellowness (b*) were found to correlate negatively to pH, whereas redness (a*) had a positive correlation.

Table 1- Color and pH values of pheasant meat at different ages

Production System	*Slaughter Age(weeks)*	*Gender*	*Breast color*			*Thigh color*			*Breast pH*	*Thigh pH*
			L	*a*	*b*	*L*	*a*	*b*		
Free-range	14	M	56.18	2.90	2.88	53.90	7.85	1.33	6.75	7.22
		F	55.12	2.84	2.88	49.07	7.31	1.09	6.82	7.23
	16	M	51.01	3.04	1.23	50.75	8.56	0.44	6.80	6.97
		F	50.97	3.29	3.01	46.69	9.51	0.21	6.75	6.93
	18	M	55.87	2.35	2.80	52.60	9.37	1.42	6.65	6.91
		F	54.22	4.73	4.82	51.95	9.51	3.47	6.56	6.97
Indoor	14	M	54.71	2.81	2.37	54.32	8.40	1.32	6.84	7.21
		F	56.09	2.23	2.67	54.22	7.48	0.77	6.67	7.21
	16	M	50.18	3.25	0.66	49.70	7.47	1.57	6.79	7.02
		F	51.71	3.23	1.36	48.41	7.35	0.72	6.68	7.03
	18	M	57.95	4.43	4.75	51.76	10.27	1.85	6.71	6.91
		F	53.82	3.26	3.12	50.53	8.56	1.52	6.77	7.16
		SEM	0.615	0.154	0.270	0.518	0.123	0.172	0.021	0.020
					Effects					
Production system		FR	53.89	3.19	2.93	51.32	8.13	1.33	6.72	7.04
		IN	54.07	3.20	2.49	51.49	8.25	0.53	6.75	7.09
Slaughter age		14	55.52a	2.69b	2.69b	52.88a	7.76b	1.13b	6.77	7.22a
		16	50.96b	3.20ab	1.56c	49.63b	7.40b	0.41c	6.76	6.99b
		18	55.46a	3.69a	3.87a	51.71ab	9.43a	2.07a	6.68	6.99b
Gender		M	54.32	3.13	2.97	52.17	8.65	0.80	6.76	7.04
		F	53.65	3.26	2.44	50.64	7.74	1.06	6.71	7.09
Production system			NS	NS	NS	NS	NS	*	NS	NS
Slaughter age			**	*	**	*	**	**	NS	**
Gender			NS	NS	NS	NS	**	NS	NS	NS

G; Gender, *; P<0.05, **; P<0.01, SEM; Standard Error of Means, NS; Insignificant, FR; Free-range, IN; Indoor, M; Male, F; Female, a, b, c; Means within columns with no common superscript letter differ significantly

Water holding capacity (WHC), Drip Loss (DL) and Cooking Loss (CL) of pheasant meat at different ages were given in Table 2. Breast meat WHC was ranged between 72.61% and 74.41% and was not affected by production system, slaughter and gender. Thigh meat WHC values were found higher than breast WHC and only significantly affected by slaughter age (P<0.01) and decreased by maturation. Production system and gender did not significantly affect DL or CL of breast and thigh meat. These traits were significantly affected by slaughter age. Drip loss increased at 16 weeks of age and then decreased at 18 weeks of age. But cooking loss increased at older ages. The water binding properties of meat was evaluated by measuring drip loss, WHC and cook loss (Allen et al. 1998). The WHC of meat is an important trait in terms of eating quality and has an influence on product yield (Cheng & Sun, 2008). Breast meat WHC was found around 73% and was not affected by production system, slaughter and gender in our study. Our finding was in parallel with the result of Kokoszynski et al. (2014) who found the breast meat WHC of pheasants 72% at 17 weeks of age. Contrary to our finding they found that WHC increased

by delayed slaughter age. Thigh meat had higher WHC than breast meat and found around 75%. Kokoszynski et al. (2012) reported that protein hydration, which is higher in leg muscles, causes increased water binding Also, the WHC was decreased at older slaughter ages. Kokoszynski et al. (2014) reported same decreasing tendency for leg muscle WHC of pheasants at different slaughter ages.

Table 2- Water holding capacity, drip loss and cooking loss of pheasant meat at different ages

Production System	*Slaughter Age*	*Gender*	*WHC (%)*		*Drip Loss (%)*		*Cooking Loss (%)*	
			Breast	*Thigh*	*Breast*	*Thigh*	*Breast*	*Thigh*
Free-range	14	M	73.19	77.12	3.88	3.30	10.89	13.10
		F	74.38	76.22	4.18	3.50	10.28	11.87
	16	M	73.67	75.47	4.62	3.90	10.43	13.85
		F	73.54	75.14	4.82	4.25	12.37	14.40
	18	M	74.03	73.98	3.55	2.49	13.89	17.16
		F	72.61	74.51	3.36	1.95	17.62	17.00
Indoor	14	M	73.58	78.19	2.72	3.74	10.34	12.64
		F	74.41	75.67	4.19	3.17	12.58	13.77
	16	M	73.77	76.53	4.67	4.53	11.64	12.47
		F	73.44	76.91	4.67	3.85	10.81	12.18
	18	M	73.44	75.28	3.19	1.88	14.14	16.73
		F	74.08	72.28	2.88	1.60	12.18	14.21
SEM			0.147	0.281	0.121	0.119	0.356	0.259
Effects								
Production system		FR	73.57	75.41	4.07	3.23	12.58	14.56
		IN	73.79	75.89	3.72	3.13	11.95	13.67
Slaughter age		14	73.89	76.80a	3.74a	3.43b	11.02b	12.84b
		16	73.60	76.01a	4.69a	4.13a	11.31b	13.22b
		18	73.54	74.17b	3.25b	1.98c	14.46a	16.28a
Gender		M	73.61	76.10	3.77	3.31	11.89	14.32
		F	73.74	75.21	4.02	3.05	12.64	13.91
Production system			NS	NS	NS	NS	NS	NS
Slaughter age			NS	**	**	**	**	**
Gender			NS	NS	NS	NS	NS	NS

WHC; Water holding capacity, FR; Free-range, IN; Indoor, NS; Insignificant, M; Male, F; Female, **; P<0.01, SEM; Standard Error of Means, a, b, c; Means within columns with no common superscript letter differ significantly (P<0.05)

Water loss is s problem during processing of meat. It is frequently expressed as drip loss, expressible water, cook loss, and cooling loss depending upon the stage during processing in which it was measured Cheng & Sun (2008). Poor WHC caused more drip loss (Warriss 2000). In line with this, drip loss of breast meat was found higher than thigh meat, because breast meat had lower WHC. Cooking loss was significantly increased by slaughter age both for breast and thigh meat. Cooking of meat denatures different meat proteins and this denaturation causes changes which results in cooking loss (Honikel 1998). High WHC results in less water loss during cooking and good juiciness when chewing (Tlhong 2008).

Immune system of birds develops after hatch by develop of digestive tract. Development of digestive system in poultry species could be at different rates due to many factors (Lilja 1983). The species of the bird, gender, age and physiological status of the birds are the most important factors on this rate (Wasilewski et al. 2015). Feed has also an effect on development of digestive tract (Gille et al. 1999). Production system did not significantly affect total digestive tract weight ratio to body weight and length in our study (Table 3). This could be related to feed formula because birds fed with same feed formula in both systems. Similarly, the rate of digestive tract weight to body weight did not differ between males and females. But males had longer digestive tract than females (Table 3, P<0.01). Digestive tract length significantly increased at older ages, but the rate of weight to body weight was significantly decreased (P<0.01). These were expected results related to body weight. Digestive organs grow more rapidly than body weight at younger ages (Sell et al. 1991). Therefore the rate of digestive tract decreases at older ages. Rates of other digestive organs (spleen, pancreas and craw) to body weight were similarly affected by only slaughter age. They were affected by neither production system nor gender. Findings of Yovchev et al. (2012) were in parallel with our results. Small and large intestine lengths of birds reared in free-range system were significantly longer than indoor. Bartlett et al. (2015) reported that birds reared in free-range system or on pasture had access to consume forage, insects etc., and this could cause intestines to develop better. The differences of intestine lengths between both sexes were not found significant. Both small and large intestine lengths significantly decreased at older ages. These results were parallel with the findings of Yovchev et al. (2012) who found small intestine lengths 11.6 cm, 11.2 cm and 10.5 cm at 15, 17 and 19 weeks of ages, respectively.

Table 3- Effect of production system and slaughter age on some digestive tract traits of pheasants at different ages

Production System	*SA*	*G*	*BW (g)*	*TDT Weight /BW (%)*	*TGT Length (cm)*	*Spleen /BW (%)*	*Pancreas /BW (%)*	*SIL (cm)*	*LIL (cm)*	*CL (cm)*	*Craw weight /BW (%)*
Free-Range	14	M	1008.3	2.67	124.88	0.06	0.19	96.92	9.32	13.75	0.27
		F	806.9	2.43	118.63	0.06	0.21	94.80	9.27	12.55	0.25
	16	M	1160.8	2.35	141.70	0.06	0.18	103.35	9.87	16.50	0.31
		F	873.4	2.27	127.70	0.08	0.17	102.60	9.35	15.52	0.33
	18	M	1258.1	2.39	147.33	0.05	0.15	94.20	8.75	13.72	0.23
		F	953.5	2.21	123.25	0.05	0.17	92	9.02	12.87	0.34
Indoor	14	M	967.1	2.40	123.30	0.05	0.19	116.07	10.12	13.52	0.24
		F	785.8	2.49	120.85	0.05	0.18	122.60	9.82	13.60	0.28
	16	M	1110.5	2.30	135.93	0.07	0.15	98.27	10.60	16.45	0.24
		F	867.1	2.46	127.68	0.06	0.16	99.80	10.10	16.42	0.33
	18	M	1205.6	1.93	135.93	0.06	0.13	99.70	9.90	15.40	0.33
		F	962.9	2.01	120.88	0.05	0.15	88.70	8.70	12.77	0.29
SEM			8.803	0.031	1.124	0.02	0.005	1.315	0.143	0.272	0.009
Effects											
Production System			NS	NS	NS	NS	NS	*	*	NS	NS
	FR		1010.0	2.39	130.57	0.06	0.19	97.31	9.27	14.15	0.28
	Indoor		983.2	2.27	127.43	0.06	0.17	102.52	9.87	14.70	0.28
Slaughter Age			**	**	**	*	**	*	*	**	*
	14		892c	2.50a	121.91b	0.06ab	0.19a	105.10a	9.64ab	13.36b	0.25b
	16		1003b	2.35a	133.25a	0.07a	0.16b	101.01a	9.98a	16.22a	0.30a
	18		1095a	2.14b	131.84a	0.05ab	0.15b	93.65b	9.09b	13.69b	0.29ab
Gender			**	NS	**	NS	NS	NS	NS	NS	NS
	M		1118.3	2.34	134.84	0.06	0.16	101.42	9.76	14.89	0.27
	F		874.9	2.31	123.16	0.06	0.17	98.42	9.38	13.96	0.30

*; P<0.05, **; P<0.01, BW; Body weight, TDT; Total digestive tract, SIL; Small intestine length, LIL; Large intestine length, CL; Cecum length, SEM; Standard Error of Means, NS; Insignificant, M; Male, F;Female, a, b, c; Means within columns with no common superscript letter differ significantly

Pheasants are game birds and preferred for niche markets. Rearing them in indoor systems became popular to meet the demand. On the other hand, the reason of consumers' preference is their gamey taste. Also, they are still not fully domesticated birds. Therefore, free-range systems could be more suitable for pheasant rearing in overall terms. This could be related to increased welfare conditions in free-range area for these non-domesticated birds. In conclusion, non-domesticated birds like pheasants have to be reared in animal friendly or organic production systems. This production model could have advantages both for consumers and producers.

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Investigation of Alkaloids in Opium Poppy *(Papaver somniferum* L.) Varieties and Hybrids

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ABSTRACT
This study aimed to investigate the proportions of morphine, thebaine, noscapine, codeine, oripavine and papaverine alkaloids in poppy varieties and hybrids. Nine parents and 36 hybrid lines were used in the trial. The trial was carried out in 2016 and 2017 in four replications according to the Randomized Block Trial Design. According to the average results of the two years, in parent and hybrid combinations, morphine was found to vary between 0.42% and 1.66%, thebaine between 0.01% and 0.53%, noscapine between 0.01% and 0.31%, codeine between 0.03% and 0.17%, oripavine between 0.00% and 0.12% and papaverine between 0.00% and 0.10%. In the study, Ofis NM x Ofis 1, Ofis 1 x TMO T, Ofis 2 x TMO T, Ofis NM x Ofis 1, Ofis NM x TMO T hybrid combinations were identified as superior hybrids compared to the others.

Keywords: *Alkaloids; Diallel hybridization; Morphine; Poppy; Papaver somniferum L.*

1. Introduction

Papaver somniferum L. species (2n= 22) the opium poppy, is a traditionally cultivated annual cultivar. It is grown in Turkey, India, Australia, Spain, France and Hungary as legal main producer countries under the supervision of the United Nations to obtain opium and opium alkaloids for medicinal purposes in World (TMO 2014). India produces opium gum, whereas other countries produce dried opium capsules. The average legal opium poppy cultivation area in 2014-2020 is 79 thousand ha on the basis of main producers in the World, the average morphine equivalent raw material production was 483 tons (INCB 2020). Average most poppy cultivation has been realized with share of 52% in Turkey and with a share of 15% in Australia.

Poppy cultivation for a long time in Anatolia geography, including Sumerians and many civilizations since dates back to 4000 BC and it used for various purposes (Tan 2008). There are 7 species of poppy family in Turkey. Studies report that there is a total of 58 papaver taxa including 36 species and 22 subspecies and varieties, 15 of which are endemic (Guner et al. 2012).

Opium poppy makes a significant contribution to the country economy in terms of making up both morphine and derivatives export. In Afyon Alkaloids Factory with its 25.000 tons/year dried un-incised poppy capsule processing capacity, which was established to commercialize opium poppy and meet the legal alkaloid requirement of the market, approximately 80-100 tons of morphine is produced annually based on the proportion of morphine in capsules. Morphine and its derivatives are used in domestic pharmaceutical industry and the rest 95 % is exported for medical purposes (TMO 2017).

Opium poppy (*Papaver somniferum* L.) plant has two important products in terms of use. One of the products is capsules and the alkaloids in them, and the other is seeds and oil. Researchers have identified 80 different alkaloids of medical importance in opium poppy capsules, from which a large number of important alkaloids are obtained (Celik 2011; Mishra et al. 2013; Marciano et al. 2018). A large number of alkaloids are obtained from opium poppy (*Papaver somniferum* L.), and morphine, codeine, thebaine, noscapine, oripavine, and papaverine are among the most important ones. These alkaloids are used as active ingredients of many drugs. Yazici et al. (2017) the alkaloid contents of some poppy varieties and genotypes reported that the rate of morphine varied from 0.53% to 0.58%, the codeine 0.02% to 0.08%, the oripavine 0.0005% to 0.005%, the thebaine 0.006% to 0.02%, the noscapine 0.02% to 0.19% and papaverine 0.01% to 0.09% in the varieties. In the genotypes was reported the rate of morphine varied between 0.15% and 0.60%, the codeine 0.001% to 0.21%, the oripavine 0.00% to 0.01%, the thebaine 0.001% to 0.08%, the noscapine 0.005% to 0.20%, and the papaverine 0.004% to 0.21%. Morphine is the most important alkaloid that is found in the maximum amount in the poppy capsule. Arslan et al. (2000)

morphine proportions were found between 0.25% and 0.89% in 353 samples collected from various regions. Kara (2017) stated that the morphine content of fifteen poppy cultivars varied between 0.47% and 1.00% in the autumn sowing and between 0.45% and 0.97% in the spring sowing, respectively. Morphine is a strong analgesic (pain killer), utilized to relieve severe pains and to prepare the patient for surgery. Codeine is a very good antitussive (cough controller) as it eliminates the cough response. Thebaine is the most poisonous opium alkaloid. It is not used as a drug; it has a stimulant effect on the central nervous system. Noscapine is the second most common alkaloid after morphine in opium, and it is cough sedative. Papaverine has a pronounced antispasmodic effect on striated and especially non-striated muscles. Oripavine, on the other hand, is used in the treatment of cocaine addiction (Tanker & Tanker 1990; Baser & Arslan 2014; Inal 2015; UNODC 2018). This study aimed at examining the proportions of morphine, thebaine, noscapine, codeine, oripavine, and papaverine alkaloids in opium poppy varieties and hybrids.

2. Material and Methods

In the study, nine different opium poppy varieties (Ofis 96, TMO 1, Huseyinbey, Celikoglu, Ofis NM, Ofis 1, Bolvadin 95, Ofis 2, and TMO T) were used for half diallel crosses (Table 1). The genetic material of the study consisted of nine parents and 36 hybrids obtained through half diallel hybridization method [n. (n-1) / 2]. Nine opium poppy cultivars used as parents were sown in the trial field of the Directorate of Middle Black Sea Transition Zone Agricultural Research Institute under Tokat-Kazova conditions on 27 February 2015 to obtain the hybrid seeds to be used as the study material. The varieties used in the trial were planted as three replications in two rows of 3 m length and 45 cm row spacing. 10 cm plant spacing was obtained with later thinning, and 1 m space was left between varieties for the ease of performing hybridization and inbreeding operations. To ensure that the blooming dates of the varieties to be used as fathers and mothers coincide, all varieties were sown again in two rows one month later. F1 hybrid seeds obtained through hybridization and inbred parents were divided into two groups, and they were sown in the trial field of the Directorate of Agricultural Research Institute in 2015-2016 and 2016-2017 vegetation periods in four replications according to the randomized block trial design. The experiment was carried out in two rows of 3 m length and 45 cm row spacing, and 10 cm plant spacing with later thinning. In the study, adequate amount of samples were extracted from capsule shells of each combination, they were ground to powder, and then their alkaloid (morphine, thebaine, noscapine, codeine, oripavine and papaverine) ratios were determined using HPLC analysis method (Kucuk 1996).

Table 1- Parents used in the trial and 9x9 half diallel hybridization combinations

Parents			
Item No		*Item No*	
1	Ofis 96	6	Ofis 1
2	TMO 1	7	Bolvadin 95
3	Huseyinbey	8	Ofis 2
4	Celikoglu	9	TMO T
5	Ofis NM		
Hybridization combinations			
1x2	Ofis 96 x TMO 1	3x7	Huseyinbey x Bolvadin 95
1x3	Ofis 96 x Huseyinbey	3x8	Huseyinbey x Ofis 2
1x4	Ofis 96 x Celikoglu	3x9	Huseyinbey x TMO T
1x5	Ofis 96 x Ofis NM	4x5	Celikoglu x Ofis NM
1x6	Ofis 96 x Ofis 1	4x6	Celikoglu x Ofis 1
1x7	Ofis 96 x Bolvadin 95	4x7	Celikoglu x Bolvadin 95
1x8	Ofis 96 x Ofis 2	4x8	Celikoglu x Ofis 2
1x9	Ofis 96 x TMO T	4x9	Celikoglu x TMO T
2x3	TMO 1 x Huseyinbey	5x6	Ofis NM x Ofis 1
2x4	TMO 1 x Celikoglu	5x7	Ofis NM x Bolvadin 95
2x5	TMO 1 x Ofis NM	5x8	Ofis NM x Ofis 2
2x6	TMO 1 x Ofis 1	5x9	Ofis NM x TMO T
2x7	TMO 1 x Bolvadin 95	6x7	Ofis 1 x Bolvadin 95
2x8	TMO 1 x Ofis 2	6x8	Ofis 1 x Ofis 2
2x9	TMO 1 x TMO T	6x9	Ofis 1 x TMO T
3x4	Huseyinbey x Celikoglu	7x8	Bolvadin 95 x Ofis 2
3x5	Huseyinbey x Ofis NM	7x9	Bolvadin 95 x TMO T
3x6	Huseyinbey x Ofis1	8x9	Ofis 2 x TMO T

3. Results and Discussion

Genetic diversity is important in plant breeding. Presence of genetic variability is the basic requirement for developing high alkaloid content and better yield varieties in opium poppy. The results of the variance analysis showed that were significant ($P \leq 0.01$) differences among the parents and their F1 progenies in traits studied except for noscapine in the first year (Table 2).

Table 2- Mean squares of variance analysis regarding alkaloid ratios of F1 hybrid combinations and their parents

Variation Sources	*DF*	*Morphine*	*Thebaine*	*Noscapine*	*Codeine*	*Oripavine*	*Papaverine*
				2016			
Repeats	3	0.018	0.018**	0.009**	0.007**	0.0001	0.0005
Genotypes	44	0.288**	0.034**	0.002	0.002**	0.0007**	0.0014**
Error	132	0.033	0.003	0.002	0.0006	0.0000	0.0004
CV (%)		23.97	64.81	98.98	42.65	110.78	117.7
				2017			
Variation Sources	*DF*	*Morphine*	*Thebaine*	*Noscapine*	*Codeine*	*Oripavine*	*Papaverine*
Repeats	3	0.120	0.034*	0.010	0.005	0.0008	0.002
Genotypes	44	0.832**	0.092**	0.086**	0.007**	0.0048**	0.004**
Error	132	0.050	0.011	0.010	0.003	0.0010	0.002
CV (%)		20.66	82.44	52.57	60.43	206.66	157.14

*, **; Significant at P< 0.05 and P<0.01 probability levels, respectively

3.1. Morphine ratio

The morphine ratio in the parents was found to vary between 0.35% and 1.30% in the first year, and it was determined to be 0.67% on average. In the second year, the ratio was determined between 0.40% and 1.85%, and the average was 0.92%. According to the average results of two years, the highest morphine ratio in the parents was found in Ofis 2 and Ofis 1 cultivars, while the lowest proportions were in Bolvadin 95 and Huseyinbey cultivars. The proportion of morphine in F1 hybrid combinations ranged between 0.42% and 1.40% in the first year with average of 0.78%, while it was between 0.46% and 2.03%, and with average of 1.12% in the second year. The highest morphine ratio in hybrid combinations was determined in Ofis NM x Ofis 1 and Ofis 1 x TMO T hybrids, whereas the lowest ratios were in Ofis 96 x Huseyinbey and TMO 1 x Huseyinbey hybrid combinations (Table 3 and 4). Harvest et al. (2009) demonstrated that morphine content correlated with capsule mass and total latex mass. Yadav et al. (2007) stated that positive correlations were determined between the morphine content with the size of the capsule and their weight. Thus, in hybrid combinations, varieties for both high capsule yield and morphine content can be developed. Dubedout (1993) reported that no correlation between the agromorphological characteristics and the content of the morphine. Moreover, the morphine content is often average between the parents values in hybrids. According to the work of Khanna & Shukla (1989) maternal effects had observed for earliness, seed weight, morphine content, plant height, flowering period and number of capsules. Danos (1968) who reported that the quantity of morphine alkaloids in the capsule increases under hot and dry cultivation conditions, while rainy weather after flowering and seed setting reduces the alkaloid content. In this study, it was thought that the precipitation in the first year was higher than the second year and it had a negative effect on the decrease in morphine content. According to the average findings of the two years, morphine content of F1 hybrid combinations (0.95%) was higher than either of the parents (0.80%). Hybrid combinations are higher than their parents shows that has an effect of hybrid vigour.

3.2. Thebaine ratio

The proportion of thebaine in the parents was found to range between 0.01% and 0.42% and the average was determined as 0.09% in the first year, the ratio varied between 0.00% and 0.63% and the average ratio was 0.12% in the second year. The highest thebaine ratio in parents was found in TMO T, while the lowest was in TMO 1 varieties. The rate of thebaine in F1 hybrid combinations was determined as 0.02-0.37%, and average was 0.09% in the first year. In the second year, was determined between 0.00 and 0.61%, and with average of 0.13%. The highest ratio of thebaine in hybrids was in Ofis 2 x TMO T, and the lowest was in TMO 1 x Huseyinbey hybrid combinations (Table 3 and 4). Dubedout (1993) reported that morphine content was positively correlated to the thebaine and codeine contents. Selection would be effective to improve these traits simultaneously. No relation was found between the thebaine content and yield in capsules. The highest thebaine was obtained at the start of flowering (Levy et al. 1986; Levy et al. 1988). Saco & Lopez-Belmonte (1988) was found significant negative correlation between precipitation (mm) and thebaine content.

3.3. Noscapine ratio

The proportion of noscapine in the parents was found to vary between 0.00% and 0.09%, and average was 0.04% in the first year, In the second year, the ratio was determined between 0.02% and 0.53% and 0.16% on average. The highest and the

lowest noscapine ratios in parents were found in Ofis 96 and Celikoglu, respectively. The rate of noscapine in F1 hybrid combinations ranged between 0.01 and 0.12% in the first year, and average was 0.05%. In the second year, ranged between 0.05 and 0.58%, and the average was 0.20%. The highest ratio of noscapine in hybrid combinations was found in Ofis NM x Ofis 1, Ofis 1 x TMO T, while the lowest was determined in Ofis 96 x Celikoglu and TMO 1 x Celikoglu hybrid combinations (Table 3 and 4). Ozgen et al. (2017) in a study reported that noscapine ratio ranged between 0.00% and 1.79% in experiment using 22 lines and one standard variety.

Table 3- Mean values of 2016 and 2017 related to the proportion of alkaloids in the parent (%)

*Parents	Morphine (%)			Thebaine (%)			Noscapine (%)		
	2016	2017	Mean	2016	2017	Mean	2016	2017	Mean
(1)	0.39	0.52	0.45	0.03	0.20	0.11	0.03	0.53	0.28
(2)	0.49	0.62	0.55	0.02	0.00	0.01	0.04	0.07	0.05
(3)	0.46	0.40	0.43	0.06	0.07	0.06	0.07	0.09	0.08
(4)	0.49	0.69	0.59	0.04	0.05	0.04	0.00	0.02	0.01
(5)	0.82	0.95	0.88	0.08	0.05	0.06	0.09	0.11	0.10
(6)	1.30	1.85	1.57	0.10	0.02	0.06	0.04	0.22	0.13
(7)	0.35	0.49	0.42	0.01	0.05	0.03	0.06	0.14	0.10
(8)	0.92	1.72	1.32	0.05	0.03	0.04	0.03	0.08	0.05
(9)	0.84	1.07	0.95	0.42	0.63	0.52	0.08	0.24	0.16
Mean	0.67	0.92	0.80	0.09	0.12	0.10	0.04	0.16	0.10
SE	0.05	0.09		0.02	0.03		0.008	0.02	
Range %	0.28-1.53	0.26-2.23		0-0.58	0-0.85		0-0.19	0-0.71	
	Codeine (%)			Oripavine (%)			Papaverine (%)		
	2016	2017	Mean	2016	2017	Mean	2016	2017	Mean
(1)	0.04	0.12	0.08	0.003	0.000	0.001	0.01	0.02	0.01
(2)	0.04	0.05	0.04	0.001	0.078	0.039	0.01	0.00	0.00
(3)	0.04	0.05	0.04	0.002	0.055	0.028	0.02	0.03	0.02
(4)	0.05	0.08	0.06	0.004	0.000	0.002	0.00	0.00	0.00
(5)	0.08	0.10	0.09	0.009	0.000	0.004	0.03	0.08	0.05
(6)	0.10	0.00	0.05	0.004	0.000	0.002	0.02	0.00	0.01
(7)	0.03	0.08	0.05	0.001	0.019	0.010	0.04	0.03	0.03
(8)	0.07	0.04	0.05	0.001	0.000	0.000	0.01	0.00	0.00
(9)	0.09	0.10	0.09	0.073	0.178	0.125	0.01	0.05	0.03
Mean	0.06	0.06	0.06	0.010	0.036	0.023	0.01	0.02	0.02
SE	0.005	0.01		0.003	0.01		0.004	0.008	
Range %	0-0.15	0-0.21		0-0.08	0-0.35		0-0.12	0-0.26	

*(1); Ofis 96, (2); TMO 1, (3); Huseyinbey, (4); Celikoglu, (5); Ofis NM, (6); Ofis 1, (7); Bolvadin 95, (8); Ofis 2, (9); TMO T, SE; Standard error

3.4. Codeine ratio

Codeine ratio was determined between 0.03% and 0.10% in the first year, 0.06% on average, between 0.00% and 0.12% in the second year, and 0.06% on average in the parents. The highest codeine ratio in parents was found in TMO T, and the lowest was in TMO 1 and Huseyinbey varieties. F1 hybrid combinations, codeine ratio ranged between 0.02 and 0.14%, and 0.06% on average in the first year, In the second year ranged between 0.01 and 0.21%, and 0.10% on average. The highest codeine proportion in hybrids was found in Ofis 1 x TMO T and Celikoglu x Ofis 1 hybrids, while the lowest was in Huseyinbey x Ofis 2 hybrid combinations (Table 3 and 5). Papaver bracteatum L. can be used to be a potential alternative for the higher yields of

codeine from unit area in breeding (Bohm 1981). Laughlin (1980) the highest codeine and morphine was obtained about five weeks after full flowers.

Table 4- Mean values of 2016 and 2017 for morphine, thebaine and noscapine ratios in F1 hybrid combinations (%)

*Crosses	Morphine (%)			Thebaine (%)			Noscapine (%)		
	2016	2017	Mean	2016	2017	Mean	2016	2017	Mean
1x2	0.47	0.76	0.61	0.03	0.01	0.02	0.04	0.11	0.07
1x3	0.44	0.46	0.45	0.04	0.01	0.02	0.04	0.08	0.06
1x4	0.58	0.53	0.55	0.03	0.01	0.02	0.04	0.05	0.04
1x5	0.66	0.85	0.75	0.09	0.19	0.14	0.03	0.13	0.08
1x6	1.02	1.41	1.21	0.08	0.16	0.12	0.08	0.45	0.26
1x7	0.53	0.56	0.54	0.05	0.04	0.04	0.04	0.08	0.06
1x8	0.66	1.04	0.85	0.03	0.02	0.02	0.01	0.12	0.06
1x9	0.67	0.99	0.83	0.11	0.26	0.18	0.04	0.15	0.09
2x3	0.42	0.63	0.52	0.02	0.00	0.01	0.04	0.05	0.04
2x4	0.51	0.70	0.60	0.05	0.03	0.04	0.02	0.06	0.04
2x5	0.66	0.95	0.80	0.03	0.07	0.05	0.01	0.11	0.06
2x6	1.04	1.64	1.34	0.02	0.07	0.04	0.05	0.34	0.19
2x7	0.63	0.95	0.79	0.02	0.08	0.05	0.08	0.14	0.11
2x8	0.84	1.48	1.16	0.02	0.11	0.06	0.03	0.24	0.13
2x9	0.84	1.08	0.96	0.05	0.09	0.07	0.03	0.20	0.11
3x4	0.44	0.64	0.54	0.04	0.09	0.06	0.03	0.11	0.07
3x5	0.60	0.84	0.72	0.08	0.19	0.13	0.03	0.14	0.08
3x6	1.00	1.26	1.13	0.06	0.18	0.12	0.07	0.39	0.23
3x7	0.50	0.59	0.54	0.04	0.08	0.06	0.04	0.09	0.06
3x8	0.83	1.03	0.93	0.03	0.02	0.02	0.04	0.10	0.07
3x9	0.93	0.90	0.91	0.14	0.25	0.19	0.04	0.30	0.17
4x5	0.62	0.96	0.79	0.15	0.07	0.11	0.03	0.12	0.07
4x6	1.19	1.52	1.35	0.12	0.25	0.18	0.03	0.32	0.17
4x7	0.49	0.73	0.61	0.04	0.04	0.04	0.08	0.07	0.07
4x8	0.83	1.27	1.05	0.06	0.07	0.06	0.01	0.08	0.04
4x9	0.64	1.03	0.83	0.29	0.48	0.38	0.02	0.14	0.08
5x6	1.40	1.92	1.66	0.09	0.09	0.09	0.12	0.50	0.31
5x7	0.72	1.09	0.90	0.06	0.05	0.05	0.05	0.15	0.10
5x8	1.08	2.03	1.55	0.13	0.14	0.13	0.07	0.31	0.19
5x9	0.88	1.51	1.19	0.37	0.37	0.37	0.04	0.28	0.16
6x7	1.06	1.47	1.26	0.04	0.13	0.08	0.09	0.23	0.16
6x8	1.05	1.68	1.36	0.06	0.06	0.06	0.09	0.37	0.23
6x9	1.23	1.86	1.54	0.29	0.42	0.35	0.05	0.58	0.31
7x8	0.73	1.02	0.87	0.03	0.02	0.02	0.03	0.11	0.07
7x9	0.79	1.01	0.90	0.16	0.09	0.12	0.06	0.10	0.08
8x9	1.14	1.92	1.53	0.23	0.61	0.42	0.06	0.51	0.28
Mean	0.78	1.12	0.95	0.09	0.13	0.11	0.05	0.20	0.12
LSD (0.05)	0.27	0.30		0.07	0.13		0.05	0.13	
SE	0.02	0.03		0.008	0.01		0.004	0.01	

*(1);Ofis 96, (2); TMO 1, (3);Huseyinbey, (4);Celikoglu, (5);Ofis NM, (6);Ofis 1, (7);Bolvadin 95, (8);Ofis 2, (9);TMO T, SE; Standard error

3.5. Oripavine ratio

The proportion of oripavine in the parents was found between 0.001% and 0.073%, and average was 0.010% in the first year. In the second year the ratio was determined between 0.000% and 0.178%, and with average of 0.036%. The ratio of oripavine in parents was the highest in TMO T, and the lowest was in Ofis 2. The rate of oripavine in F1 hybrid combinations was determined as 0.001-0.044%, and with average of 0.008% in the first year, in the second year 0.000-0.118%, and with average of 0.009%.

The cross Ofis NM x TMO T and Bolvadin 95 x TMO T showed the highest oripavine and the lowest was in Ofis 96 x Bolvadin 95 (Table 3 and 5). Yazici et al. (2017) reported that the rate of oripavine ranged between 0.000% and 0.005%. Yazici & Yılmaz (2017), reported that the rate of oripavine varied from 0.01% to 0.02%, in the cultivated as summer and in cultivated as winter; the rate of oripavine varied from 0.0002% to 0.007 %.

3.6. Papaverine ratio

The ratio of papaverine in the parents was found between 0.00% and 0.04%, and average was 0.01% in the first year and in the second year was determined between 0.00% and 0.08% and 0.02% on average. The highest proportion of papaverine in parents

was found in Ofis NM, and the lowest was in Celikoglu. The ratio of papaverine in F1 hybrid combinations ranged between 0.00 and 0.08%, and average was 0.02%, in the first year, in the second year ranged between 0.00 and 0.12%, and 0.03% on the average. The highest ratio of papaverine in hybrids was in Huseyinbey x Ofis 1 and Ofis NM x Ofis 2 hybrid combinations and the lowest was in TMO 1 x Ofis NM (Table 3 and 5). Karabuk (2012) the rate of papaverine between 0.00 and 0.02%, Yazici et al. (2017) the rate of papaverine was reported to be 0.01-0.09% in varieties and in genotypes was reported to be 0.00-0.21%. Khanna & Shukla (1991) found a relationship between white latex and papaverine content.

Table 5- Mean values of 2016 and 2017 regarding codeine, oripavine and papaverine ratios in F1 hybrid combinations (%)

*Crosses	Codeine (%)			Oripavine (%)			Papaverine (%)		
	2016	2017	Mean	2016	2017	Mean	2016	2017	Mean
1x2	0.04	0.09	0.06	0.003	0.017	0.010	0.00	0.01	0.00
1x3	0.06	0.06	0.06	0.002	0.000	0.001	0.01	0.03	0.02
1x4	0.04	0.05	0.04	0.003	0.000	0.001	0.01	0.00	0.00
1x5	0.07	0.09	0.08	0.004	0.000	0.002	0.02	0.02	0.02
1x6	0.05	0.16	0.10	0.003	0.012	0.007	0.01	0.00	0.00
1x7	0.05	0.08	0.06	0.001	0.000	0.000	0.00	0.01	0.00
1x8	0.03	0.04	0.03	0.002	0.000	0.001	0.00	0.01	0.00
1x9	0.03	0.11	0.07	0.012	0.000	0.006	0.01	0.06	0.03
2x3	0.04	0.07	0.05	0.002	0.042	0.022	0.03	0.02	0.02
2x4	0.05	0.08	0.06	0.002	0.000	0.001	0.00	0.02	0.01
2x5	0.04	0.08	0.06	0.001	0.003	0.002	0.00	0.05	0.02
2x6	0.04	0.13	0.08	0.003	0.008	0.005	0.00	0.00	0.00
2x7	0.05	0.10	0.07	0.003	0.004	0.003	0.01	0.02	0.01
2x8	0.04	0.09	0.06	0.002	0.000	0.001	0.00	0.06	0.03
2x9	0.07	0.08	0.07	0.003	0.007	0.005	0.01	0.00	0.00
3x4	0.05	0.14	0.09	0.002	0.000	0.001	0.03	0.05	0.04
3x5	0.04	0.11	0.07	0.008	0.007	0.007	0.06	0.09	0.07
3x6	0.08	0.13	0.10	0.002	0.000	0.001	0.08	0.11	0.09
3x7	0.05	0.09	0.07	0.002	0.000	0.001	0.03	0.04	0.03
3x8	0.04	0.01	0.02	0.002	0.029	0.015	0.02	0.00	0.01
3x9	0.11	0.14	0.12	0.014	0.009	0.011	0.05	0.07	0.06
4x5	0.07	0.07	0.07	0.021	0.000	0.010	0.00	0.03	0.01
4x6	0.11	0.17	0.14	0.006	0.000	0.003	0.02	0.06	0.04
4x7	0.06	0.10	0.08	0.004	0.012	0.008	0.01	0.02	0.01
4x8	0.05	0.07	0.06	0.002	0.000	0.001	0.01	0.00	0.00
4x9	0.11	0.09	0.10	0.021	0.000	0.010	0.02	0.00	0.01
5x6	0.08	0.17	0.12	0.002	0.026	0.014	0.07	0.05	0.06
5x7	0.07	0.07	0.07	0.006	0.000	0.003	0.01	0.03	0.02
5x8	0.07	0.13	0.10	0.010	0.000	0.005	0.06	0.12	0.09
5x9	0.14	0.12	0.13	0.044	0.118	0.081	0.00	0.10	0.05
6x7	0.06	0.12	0.09	0.003	0.000	0.001	0.02	0.02	0.02
6x8	0.05	0.03	0.04	0.004	0.000	0.002	0.01	0.02	0.01
6x9	0.12	0.21	0.16	0.029	0.000	0.014	0.02	0.00	0.01
7x8	0.02	0.05	0.03	0.002	0.009	0.005	0.01	0.00	0.00
7x9	0.09	0.07	0.08	0.026	0.073	0.049	0.00	0.01	0.00
8x9	0.09	0.14	0.11	0.033	0.000	0.016	0.01	0.00	0.00
Mean	0.06	0.10	0.08	0.008	0.010	0.009	0.02	0.03	0.02
LSD (0.05)	0.03	0.05		0.01	0.03		0.01	0.05	
SE	0.003	0.005		0.001	0.003		0.002	0.004	

*(1); Ofis 96, (2); TMO 1, (3);Huseyinbey, (4); Celikoglu, (5); Ofis NM, (6); Ofis 1, (7); Bolvadin 95, (8); Ofis 2, (9); TMO T, SE; Standard error

4. Conclusions

In this study, were used nine parents and 36 hybrid combinations. The experiment was conducted in 2016 and 2017, alkaloid ratios were examined and appropriate parents and hybrid combinations were determined according to alkaloid ratios. According to the average findings of the two years, the parents Ofis 1, Ofis 2, Ofis NM and TMO T were identified as the highest cultivars in a result of investigated all traits. Therefore they could be considered as promising parents in further breeding studies. The mean performance of F1 hybrids was found higher than either of the parental for all traits exhibiting the role of hybrid vigour. In this study, 15 hybrid combinations were found to be higher than the hybrid average values of two years for morphine content and 13 for thebaine, 13 for noscapine, 12 for codeine, 6 for oripavine, 12 for papaverine. Particularly, the crosses Ofis NM x Ofis 1, Ofis 1 x TMO T, Ofis 2 x TMO T, Ofis NM x Ofis 1, Ofis NM x TMO T were

identified as superior hybrids compared to the others. Hybrid combinations with high alkaloid ratios can be evaluated as suitable hybrids for breeding studies.

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Internal Transcribed Spacer (ITS) Fails Barcoding of the Genus *Neotinea* Rchb.f. (Orchidaceae)

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ABSTRACT

Internal Transcribed Spacer (ITS) is one of the most used barcoding regions for the molecular phylogenetics and barcoding of orchids. Our aim in this study is to test the reliability of ITS on barcoding of closely related *Neotinea* spp., including *Neotinea tridentata*, *Neotinea ustulata* subsp. *ustulata* and *Neotinea ustulata* subsp. *aestivalis*, by comparing it to the *acc*D-*psa*I intergenic spacer of the plastid DNA. Both ITS and *accD-psa*I regions were amplified by specific primer sets and sequenced. Phylogenetic trees were regenerated by using Maximum Parsimony approach. The results showed that ITS separated some *N. tridentata* samples of Turkish, Greek, Hungarian and Croatian samples from the others on the phylogenetic trees due to the incomplete lineage sorting. In contrast to ITS, the *acc*D-*psa*I marker could successfully separate *N. tridentata* and *N. ustulata* samples according to a priori species classification. Our findings refer to a hybridisation story between some *N. tridentata* and *N. ustulata*. We propose not to use ITS sequences directly as a barcode and to reconstruct the phylogeny of the *Neotinea* group. Instead, the inclusion of other nuclear regions such as *LFY*, *ADH*, etc., or utilisation of whole genome sequencing could give better barcoding results.

Keywords: Orchids, *acc*D-*psa*I, DNA barcoding, phylogenetic incongruence.

1. Introduction

The orchid family (Orchidaceae) is the second largest flowering plant family represented by 899 genera and 27801 species (The Plant List 2013). Therefore, it is necessary to develop a reliable molecular identification method. Since the 1990s, using molecular markers on the phylogenetic studies of orchids have been increasing incrementally (Chase et al. 2000; Bateman et al. 2003; Sramko et al. 2014). Plant taxonomists have used short sequences of both ribosomal deoxyribonucleic acid (rDNA) and chloroplast deoxyribonucleic acid (cpDNA) as molecular markers to identify species and to reconstruct phylogeny (Zimmer & Wen 2012). Rapidly evolving DNA regions are needed for species-level barcoding (Sramkó et al. 2011; Zimmer & Wen 2012). The Internal Transcribed Spacer of the nuclear ribosomal 18S-5.8S-26S cistron (ITS) is one of the most extensively used molecular marker in orchid identification and molecular phylogenetic since the 1990s (Baldwin et al. 1995; Pridgeon et al. 1997; Bateman et al. 2003; Álvarez & Wendel 2003; Shipunov et al. 2004; Gulyás et al. 2005; Nieto-Feliner & Rosselló 2007; Hollingsworth 2008; Sramko et al. 2014; Li et al. 2015). The main advantages of ITS are (1) universality of primer sets (White et al. 1990), (2) ease of amplification even from historic specimens (i.e., herbarium specimens) due to multi-copy characteristic, and (3) favourable size of the region (~700 bp) (Baldwin et al. 1995). Some researchers point out the concerted evolution of ITS region (Bailey 2003; Gulyás et al. 2005; Pillon et al. 2007; Nieto-Feliner & Rosselló 2007). Concerted evolution is a process that homogenizes the rDNA array within an organism and reduces intra-individual variation (Liao 2008). *acc*D-*psa*I barcoding region covers partial acetyl-CoA carboxylase enzyme (*accD*) and Photosystem I (*psaI*) coding regions and an intergenic region on the chloroplast genome.

In this study, we would like to test the utilities of ITS and *acc*D-*psa*I regions for DNA barcoding of closely related *Neotinea* (Rchb.f.) taxa; *Neotinea tridentata* (Scop.) R. M. Bateman, Pridgeon & M. W. Chase, *Neotinea ustulata* subsp. *ustulata* (L.) R. M. Bateman, Pridgeon & M. W. Chase and *Neotinea ustulata* (L.) R. M. Bateman, Pridgeon & M. W. Chase subsp. *aestivalis* (Kümpel) Kolník, Vlčko & Dítě by comparing the topology of the phylogenetic trees.

2. Material and Methods

2.1. Plant material and DNA extraction

The field-collected leaf samples placed in silica-gel sachets and stored at room temperature until DNA extraction. The samplings of *N. tridentata*, *N. ustulata* subsp. *ustulata*, *N. ustulata* subsp. *aestivalis*, *Neotinea maculata* (Desf.) Stearn and *Neotinea conica* (Willd.) R. M. Bateman, were obtained from Mediterranean, west sub-Mediterranean, middle sub-Mediterranean, Balkan sub-Mediterranean and east sub-Mediterranean regions (Figure 1: 1A and 1B). Altogether, we analysed 23 samples of *Neotinea* spp. from the field and 9 DNA sequences from the nucleotide collection GenBank (Supplementary Data 1). We chose *Ophrys phrygia* H. Fleischm. & Bornm. (Genbank accession no: MH050859) and *Platanthera dilatata* (Pursh) Lindl. ex L. C. Beck (Genbank accession no: JX484921) as out-groups.

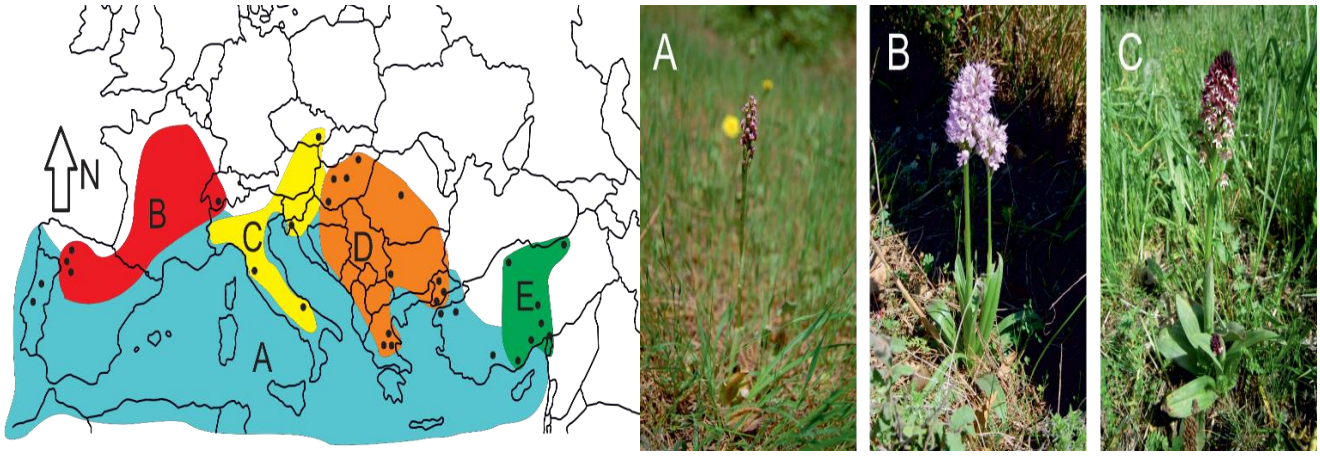

Figure 1- 1A; Sampling map. A: Mediterranean, B; West sub-Mediterranean, C; Mid sub-Mediterranean, D; Balkan sub-Mediterranean and E; East sub-Mediterranean. (*Platanthera dilatata* was excluded). 1B; Images of *Neotinea maculata* (A), *Neotinea tridentata* (B) and *Neotinea ustulata* (C). The image of *N. ustulata* (Vladan Djordjevic).

The DNA extraction was performed according to the cetyltrimethylammonium bromide (CTAB) protocol (Doyle & Doyle 1990). Approximately 10 mg of dried leaves used for the extraction.

2.2. Choice of molecular markers for molecular barcoding

We used ITS as a nuclear-molecular marker to test its molecular barcoding utilities for *Neotinea* spp. which were collected from a wide geographic range. We also added the plastid-encoded *acc*D-*psa*I intergenic region to be compared with ITS. Thus, we would be able to check the hybridisation origin of *N. tridentata*, as well.

2.3. PCR amplification, sequencing and phylogenetic reconstruction

We used angiosperm-specific ITS1A (Gulyás et al. 2005) and universal ITS4 (White et al. 1990) primer pair to amplify the whole ITS region. 25 µL of the PCR reaction mixture consisted of 0.1 volume of 10x High-Fidelity Buffer (Fermentas, USA), 0.5 µL 10 mM of each dNTP (Fermentas, USA), 2 µL 25 mM $MgCl_2$ (Fermentas, USA), 1.25 µL 20 mg mL^{-1} BSA, 0.5 µL 10 µM primers (Thermo Scientific, USA), 0.1 µL 5 Unit High-Fidelity polymerase (Fermentas, USA), 16.65 µL water and 1µL (~5 ng μL^{-1}) genomic DNA. PCR amplification of ITS was performed on BIO-RAD PTC-200 thermal cycler using the following PCR profile: 94 °C for 2 min first denaturation, followed by 33 cycles of denaturation for 20 s at 94 °C, annealing for 30 s at 51 °C and extension for 1 min at 72 °C and finalized by final extension for 10 min at 72 °C. PCR products were then sent to Macrogen Inc. (The Netherlands) for Sanger sequencing (Applied Biosystems 3100 Genetic Analyzer) using ITS1A primer. Double signal peaks and Additive polymorphic sites (APS) were coded according to the International Union of Pure and Applied Chemistry (IUPAC) ambiguity symbols. We annotated the whole ITS region into the parts ITS1, 5.8s and ITS2 according to the sequences retrieved from the GenBank.

We used accD-f and psaI-r primer pair to amplify the *acc*D-*psa*I region (Small et al. 1998). The PCR mixture was the same used for ITS amplification. PCR profile was applied as follows; 94 °C for 3 min first denaturation, followed by 34 cycles of denaturation for 30 s at 94 °C, annealing for 30 s at 55 °C and extension for 1 min at 72 °C and finalized by a final extension for 10 min at 72 °C. Amplified samples were sequenced in both directions using accD-f and psaI-r primers.

For the ITS and *acc*D-*psa*I regions, the sequences were aligned by ClustalW (Larkin et al. 2007) under the bioinformatics software package Geneious R8 (Kearse et al. 2012). We used Maximum Parsimony (MP) heuristic search by PAUP 4.0a (build 159) (Swofford 2003). The MP-heuristic-search settings were: trees obtained from 1000 random replicates holding one tree at each step for stepwise addition, gaps are treated as missing, out-group: *O. phrygia* (ITS) and *P. dilatata* (*acc*D-*psa*I), steepest descent option not in effect, and tree-bisection-reconnection (TBR) was used as branch swapping.

3. Results

3.1. Information obtained from DNA markers

ITS and *acc*D-*psa*I regions were successfully amplified and sent to the sequencing service. The sequence files were imported into Geneious R8 software and checked for quality values. We excluded three samples since sequencing qualities of two samples from Italy, *N. tridentata* from Mt. Gargano and Toskana for ITS and *acc*D-*psa*I, and one sample from Hungary, *N. tridentata* from Tokaj, for ITS were unacceptable for both ITS regions. We detected three interspecific length polymorphisms among five studied taxa; *N. conica* has 613 bp ITS, *N. maculata* has 609 bp ITS length. *N. tridentata*, *N. ustulata* subsp. *ustulata* and *N. ustulata* subsp. *aestivalis* have identical 616 bp ITS length. Whereas those polymorphisms are observed on ITS1 (ranged 234-243 bp) and ITS2 (ranged 217-222 bp) parts, there was no length polymorphism on the coding 5.8S part (Table 1). There was no length polymorphism on the *acc*D-*psa*I region.

Table 1- ITS length polymorphisms on *Neotinea* spp

Sample	*Accession No*	*Collecting site*	*Lengths in base-pair*			
			ITS1	*5.8S*	*ITS2*	*Total Length*
N. conica	AY364880	Caceres-Spain	243	153	217	613
N. maculata	AM711744	Kythera-Greece	234	153	222	609
N. maculata	MH050840	Likouria-Greece	234	153	222	609
N. maculata	AY364873	Tras os Montes-Portugal	234	153	222	609
All *N. tridentata* samples	All Samples	All Samples	242	153	221	616

Table 2- Initial comparison of DNA markers used in the study. *O. phrygia* (ITS) and *P. dilatata* (*acc*D-*psa*I) are treated as out-groups relative to in-group samples

DNA Region	*Origin*	*Length range*	*Number of in-group polymorphic sites*			*Parsimony informative characters*	*Aligned length*	*Variability*			
								Number		*Percentage*	
			ITS1	*5.8S*	*ITS2*			*Out-group*	*In-group*	*Out-group*	*In-group*
ITS	Nucleus	609–623	8 (3.3%)	0 (0%)	3 (0.9%)	37	632	111	44	17.56%	7.0%
*acc*D–*psa*I	Chloroplast	923	-	-	-	8	933	8	3	0.86%	0.32%

Accordingly, the sequence variability of each DNA marker (Table 2) ITS region outperforms the chloroplast region *acc*D-*psa*I in several variable positions (44 to 3 in-group), percentage within the aligned length (7.0% to 0.32% in-group) and parsimony informative characters (37 to 8). This comparison also shows that the ITS1 part of the ITS region has more variable sites (3.3% to 0.9%) than the ITS2 part. The aligned length was calculated as 632 bp for the ITS region and 933 bp for *acc*D-*psa*I region. The protein coding 5.8S part has no variable sites on the studied samples, as expected. We observed APS on the chromatogram data of ITS sequences caused by the multi-copy nature and incomplete concerted evolution feature of the region (Table 3 and Figure 2).

Table 3- Additive Polymorphic Sites (APS) on ITS sequences

Sample	*Number of APS*	*Position (bp)*	*Nucleotides (IUPAC Symbols)*
N. tridentata (Balıkesir-TR)	1	432	G or A (R)
N. tridentata (Çanakkale-TR)	1	513	G or A (R)
N. ustulata subsp. *aestivalis* (Kiralyko-RO)	2	513, 606	G or A (R)
N. ustulata (Velka nad Velickou-CZ)	3	513, 514, 606	G or A (R)
N. tridentata (Adana-TR)	1	555	G or A (R)
N. tridentata (Trabzon-TR)	1	555	G or A (R)
N. tridentata (Antalya-TR)	1	555	G or A (R)

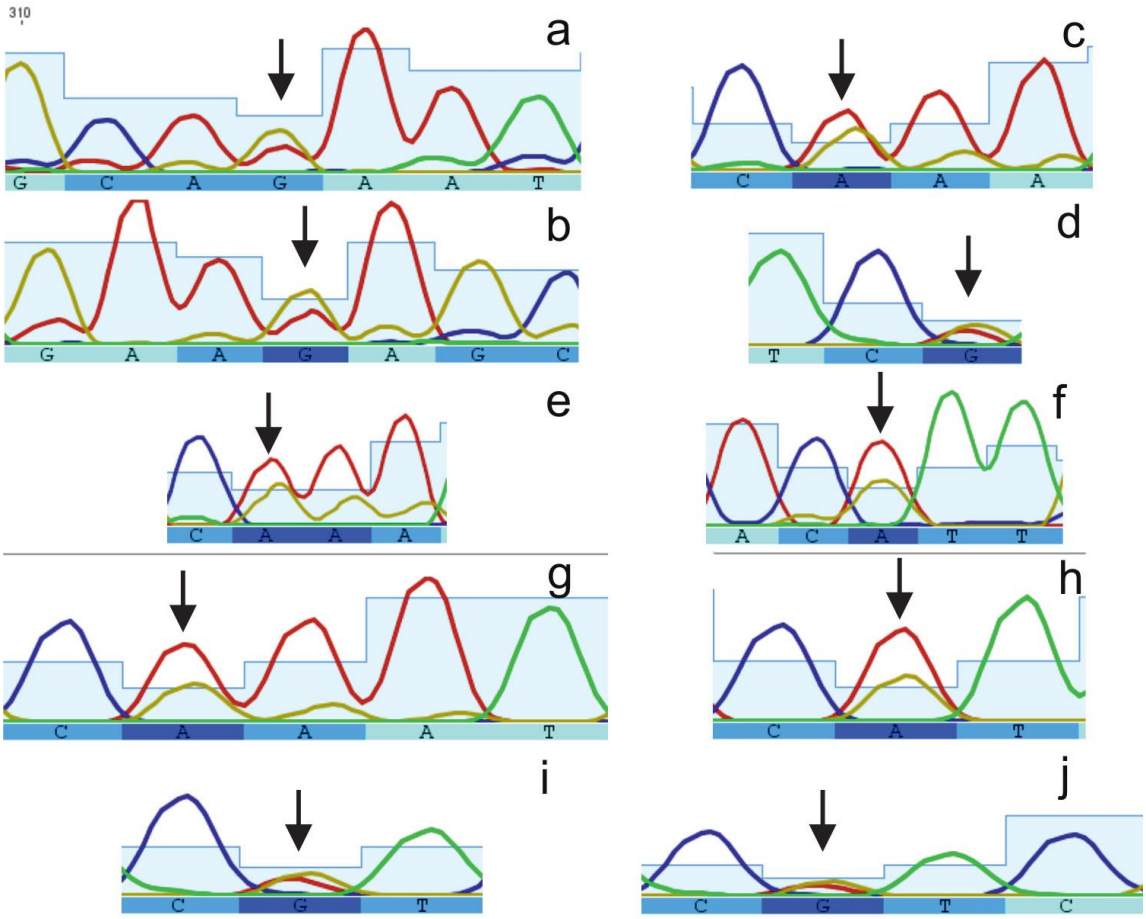

Figure 2- Additive Polymorphic Sites (APS) of ITS sequences

3.2. Phylogenetic reconstruction

The MP phylogenetic analysis of the ITS region used 37 parsimony-informative characters and retained one most-parsimonious tree in 8028 rearrangement trials. The score of the best tree was 119. The MP tree showed moderate to high bootstrap support values (75 to 100).

On the ITS MP tree, *O. phrygia* was designated as the out-group and main clades consisted of *N. maculata* and *N. tridentata*/*N. ustulata* block. *N. tridentata* samples from Turkey (Adana, Antalya and Trabzon), Hungary (Siklos) and Croatia (Ucka) were apart from the main *N. tridentata*/*N. ustulata* clade. ITS sequences of those samples showed differences from other *N. tridentata* samples on the alignment as well since having APS. *N. tridentata* samples collected from Adana, Antalya and

Trabzon samples have APS on the 559th position of the alignment (555th position on the sequence file), and on those positions, the nucleotides were either Adenine or Guanine. *N. tridentata* samples from Hungary (Siklos) and Croatia (Ucka) have different nucleotide (Thymine instead of Cytosine) on the 593rd position of the alignment (589th position on the sequence file). *N. tridentata* sample from Greece (Pelion) had one nucleotide difference (Cytosine instead Thymine) on the 229th position of the alignment (226th position on the sequence file) than other *N. tridentata* samples. There was no APS on the sequence of those samples.

According to the ITS MP tree, *N. tridentata* samples collected from Adana, Trabzon, Antalya (Turkey), Siklos (Hungary), Ucka (Croatia) and Pelion (Greece) are sister to other *N. tridentata* samples. This results in polytomy among *N. tridentata*.

The MP-phylogenetic analysis of the *acc*D-*psa*I region used 8 parsimony-informative characters and retained one most-parsimonious tree in 28776 rearrangement trials. The score of the best tree was 381. On the phylogram of *acc*D-*psa*I sequences, *N. tridentata* samples placed together on one clade did not separate from each other as on the ITS tree (Figure 3). Three *N. ustulata* samples from Bulgaria, Czech Republic and Romania were separated on the tree, and this separation was highly supported by bootstrap. However, *N. ustulata* subsp. *ustulata* from Hungary was grouped with the *N. tridentata* samples. *N. tridentata* showed polytomy on the tree and, therefore, *N. ustulata* and *N. tridentata* samples were placed on the same branch.

4. Discussion

ITS region of nuclear ribosomal DNA is the most popular region for molecular phylogenetic studies for diverse plant groups including orchids, although it has some conspicuous drawbacks such as APS due to incomplete concerted evolution (Nieto-Feliner 2003). APS are observed when two nucleotides involved in a polymorphic site and those are very common when a hybridisation event on the background (Fuertes Aguilar & Nieto-Feliner 2003). Concerted evolution concept described as a genetic process by which repetitive DNA sequences are homogenized among the genome of different species, and it is known that the ITS has concerted evolution characteristic (Liao 2008). APS are indicative of incomplete concerted evolution due to a hybridisation event has not been homogenized within the genome (Bailey 2003). Those polymorphic ITS copies have been reported by authors in the literature (Mayol & Rosselló 2001, Won & Renner 2005). Our sequencing results revealed that the ITS has APS on the sequence chromatogram data (Figure 2). This finding refers to a hybridisation event and incomplete concerted evolution on *Neotinea* spp. particularly distributed in Asia Minor. These circumstances had an impact on the resolution power and caused sample misplacements on the phylogenetic trees. Additionally, incomplete concerted evolution of ITS marker on *Neotinea* spp. is in contradiction with the DNA barcode concept, since double nucleotide peaks are common throughout the sequence.

Our two phylogenetic trees have provided contrasting results for closely related *Neotinea* spp. On the ITS tree, *N. tridentata* samples from Adana, Trabzon, Antalya (Turkey), Siklos (Hungary), Ucka (Croatia) and Pelion (Greece) were separated from other *N. tridentata* samples due to having APS which comes from different copies of the ITS sequence in the genome. On the phylogram (Figure 3) it is also seen that *N. tridentata* samples from Adana, Trabzon and Antalya separated from other samples collected from Turkey. The issue with ITS region was discussed extensively in the study of Nieto-Feliner & Rosselló (2007). In the study, the authors listed the drawbacks of the ITS region and stressed its multi-copy nature which causes less confident results on phylogenetic studies. On the other study on which ITS was used, neither direct sequencing nor cloning efforts provided sufficient data for the phylogeny of *Himantoglossum* W.D.J. Koch *s.l.* (Orchidaceae) (Sramkó et al. 2011). In our study, in contrast to the study of (Pridgeon et al. 1997), the resolution power of the ITS region was insufficient to separate closely related *N. tridentata* and *N. ustulata* species that we sampled.

Our second molecular phylogenetic tool, *acc*D-*psa*I region, performed better than ITS for the topology of the phylogenetic tree. On the tree, *N. tridentata* and *N. ustulata* species were separated except one *N. ustulata* sample from Varpalota (Hungary). When we compared the topologies of our two phylogenetic trees side-by-side, the incongruence is clear. While *acc*D-*psa*I separate *N. tridentata* and *N. ustulata*, ITS does not. The contrasting incongruences on the two differently inherited molecular markers, i.e. cpDNA and ITS, refer to a hybridisation (Rieseberg et al. 1996; Gulyás et al. 2005; Sramkó et al. 2008; Kim & Donoghue 2008; Sramko et al. 2014). In our results, the clear incongruence refers to the complex history of *Neotinea* genus and possible hybridisation between *N. tridentata* and *N. ustulata*, also. ITS region failed at the genus *Neotinea* since incomplete concerted evolution and hybridization background causes double-peaks and additive polymorphic sites on the sequence.

According to the statistics, in the 66% of papers published between 1998 and 2002, the ITS was used and 34% of them used ITS as the only molecular marker (Álvarez & Wendel 2003). This statistic shows the popularity of the ITS marker in the beginning and the improvement stage of the molecular phylogenetic studies. Later, after 2005 to date, the rate of ITS usage as the only molecular marker was decreased to 15.7%, and other molecular markers, i.e. mitochondrial or chloroplast sequences, were included to support the data gathered from the ITS (Nieto-Feliner & Rosselló 2007) that indicated the doubts on the ITS and attempts to find new molecular markers.

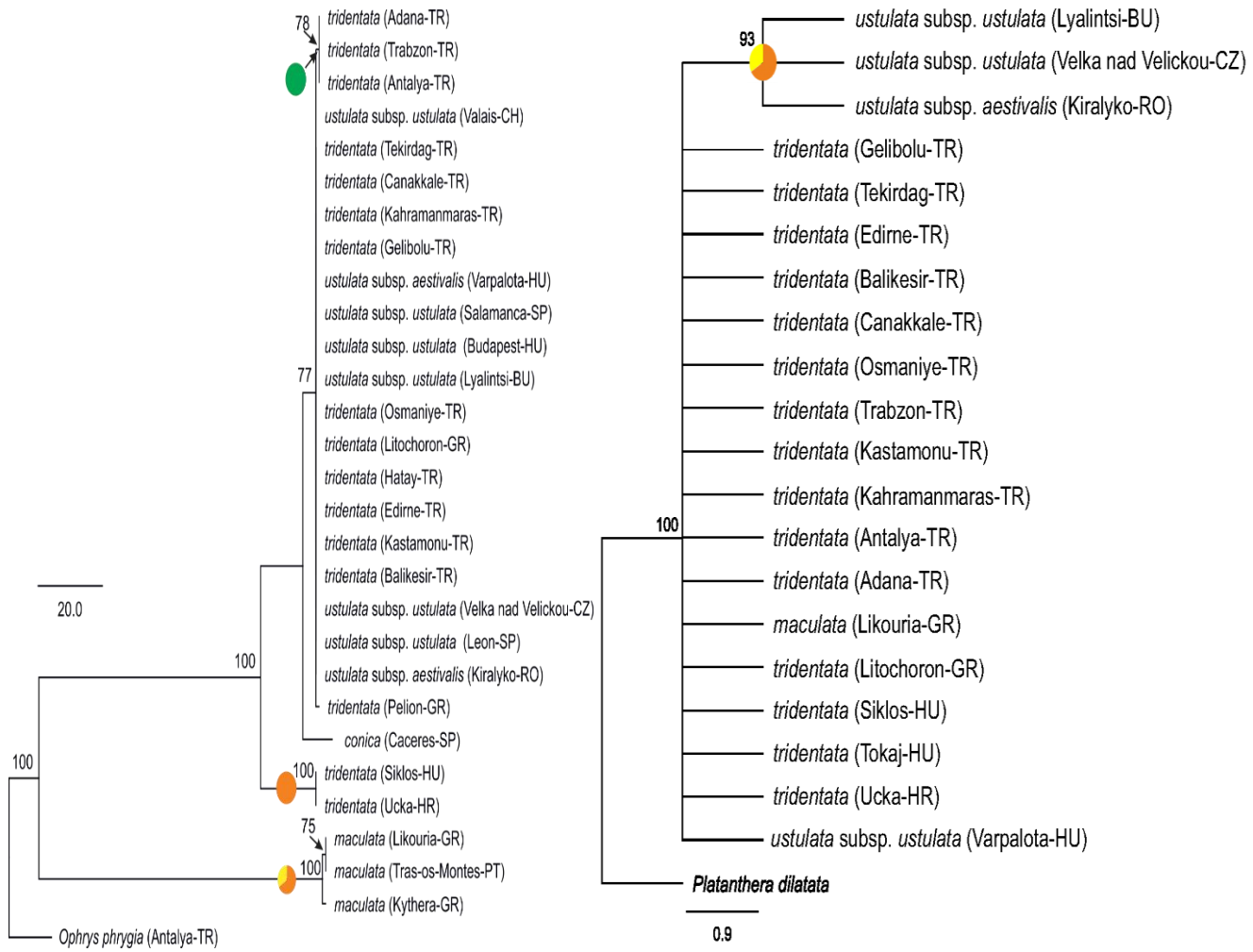

Figure 3- A: MP Phylogram of *Neotinea* spp. based on ITS sequences. The regions of origin are shown in parenthesis as country codes. *O. phrygia* is selected as the out-group. B: MP Cladogram of *Neotinea* spp. based on *acc*D-*psa*I sequence. The regions of origin are shown in parenthesis as country codes. Bootstrap-support values are shown on nods. *P. dilatata* is selected as the out-group. The pie chart represents the regions where differentiated samples collected from

5. Conclusions

On the history of molecular markers, the ITS region is still popular. It is not possible to abandon ITS as a molecular marker from the recent phylogenetic studies, particularly on orchids. However, researchers who study closely related species such as *Neotinea* spp., must consider the drawback of the marker. The strong conflicts between ITS and *acc*D-*psa*I point out a possible hybridisation story. Fortunately, there are new technological ways of securing correct reconstruction of molecular phylogenetic trees and barcoding the closely related species, e.g. utilization of Next Generation Sequencing (NGS) techniques like RAD-seq.

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Supplementary Table

The whole list of the specimens sampled is available on Table S1 with the GenBank-accession numbers.

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The Effect of Maize (*Zea mays* L.) / Soybean (*Glycine max* (L.) Merr.) Intercropping and Biofertilizer (Azotobacter) on Yield, Leaf Area Index and Land Equivalent Ratio

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ABSTRACT
This study was conducted to determine the effects of the different intercropping design of maize/soybean compared with sole cropping, in combination with Vitormone (biofertilizer; Azotobacter) during 2012 and 2013. There were four different intercropping designs were used in the experiment: *i*) sole maize and soybean, *ii*) 1 maize + 1 soybean in alternate rows, *iii*) 1 maize + 2 soybeans in alternate rows and *iv*) 1 maize + 2 soybeans in intra rows under control with the Vitormone application. The experiment was laid out in a randomized complete block design with two factors and three replications. The results showed that the seed/grain yields and the Leaf area index of sole soybean and sole maize outperformed the intercropping. The highest yields for both maize (8.0 t ha^{-1}) and soybean (3.26 t ha^{-1}) and land equivalent ratio (1.26) were recorded at 1 maize + 2 soybeans in alternate rows among the intercropping. The effect of Vitormone was significantly positive for all cropping system except 1 maize + 1 soybean in alternate rows. This suggests that 1 maize + 2 soybeans in alternate rows can intercrop and combine with the Vitormone. The intercropping of cereal/legume has the potential to improve the utilization of resources in monocropped lands.

Keywords: Alternate row and intra row, Intercropping, LAI (leaf area index), LER (land equivalent ratio), the Vitormone

1. Introduction

Intercropping can be defined as a multiple cropping system that two or more crops planted in a field during a growing season (Yong et al. 2015). Intercropping is a way to increase diversity in an agricultural ecosystem. The most benefit of intercropping is optimum utilization of plant resources such as nitrogen in Gramineae (or Poaceae)/Legumes (or Fabaceae) intercropping (Nasri et al. 2014). As known, Legume crop is used as soil N supply. When the crop with large canopy intercropped with the small crops, such as maize and soybean intercropping, soybean yield could decrease due to interspecific light competition (Liu et al. 2017). Thus, different intercropping designs such as alternate rows and intra rows were studied in many types of research (Ijoyah & Fanen 2012; Mandal et al. 2014). The optimum inter-row and intra-row distances are the most important to produce a high yield in maize/soybean intercropping (Kim et al. 2018). The LER has been recommended to evaluate the yield advantage of intercropping compared to monocropping (Mahallati et al. 2014). It was reported that the LER values above 1 determined in maize/soybean intercropping (Dolijanvic et al. 2009; Tsujimoto et al. 2015; Kamara et al. 2017).

The Büyük Menderes Basin has a typical Mediterranean climatic characteristic. Although monocropped cotton grown in many areas with saline and alkali soils, agricultural production is intensive, with winter wheat or forage crops–summer cotton or maize rotation. Yavas & Unay (2016 a, b) stated that poor nodulation of soybean and cowpea with maize intercropping occurred in the west of Büyük Menderes Basin. Nodulation failure is caused by soil compaction, flooding, and high pH levels. Vitormone is one of the foliar liquid bio-fertilizers containing *Azotobacter chroococcum* which species responsible for atmospheric nitrogen fixation (Mohiuddin et al. 2000; Ramteke et al. 2016). In many studies, it concluded that the Vitormone promoted the plant growth (Ghosh & Dayal 1998; Gaikwad et al. 2008).

There are few published reports on the poor nodulating of legume in grain/legume intercropping and interaction of bio-fertilizer (the Vitormone) and different intercropping design. Therefore, we conducted the present study to (a) determine the yield performance in maize and soybean intercropping under the Vitormone application; (b) explore the optimum intercropping design.

2. Material and Methods

2.1. Field layout and plant materials

This study was carried out at the field of research and application (37° 45' North, 27° 45' East) of the Field Crops Department of the Agricultural Faculty of Aydın Adnan Menderes University during the summer of the 2012 and 2013. The area is affected by a Mediterranean type of climate with hot and dry summer (Sensoy et al. 2007). The mean temperatures from April to September show that July and August exceed the long term mean temperatures (Figure 1) (provided by the Turkish State Meteorological Service).

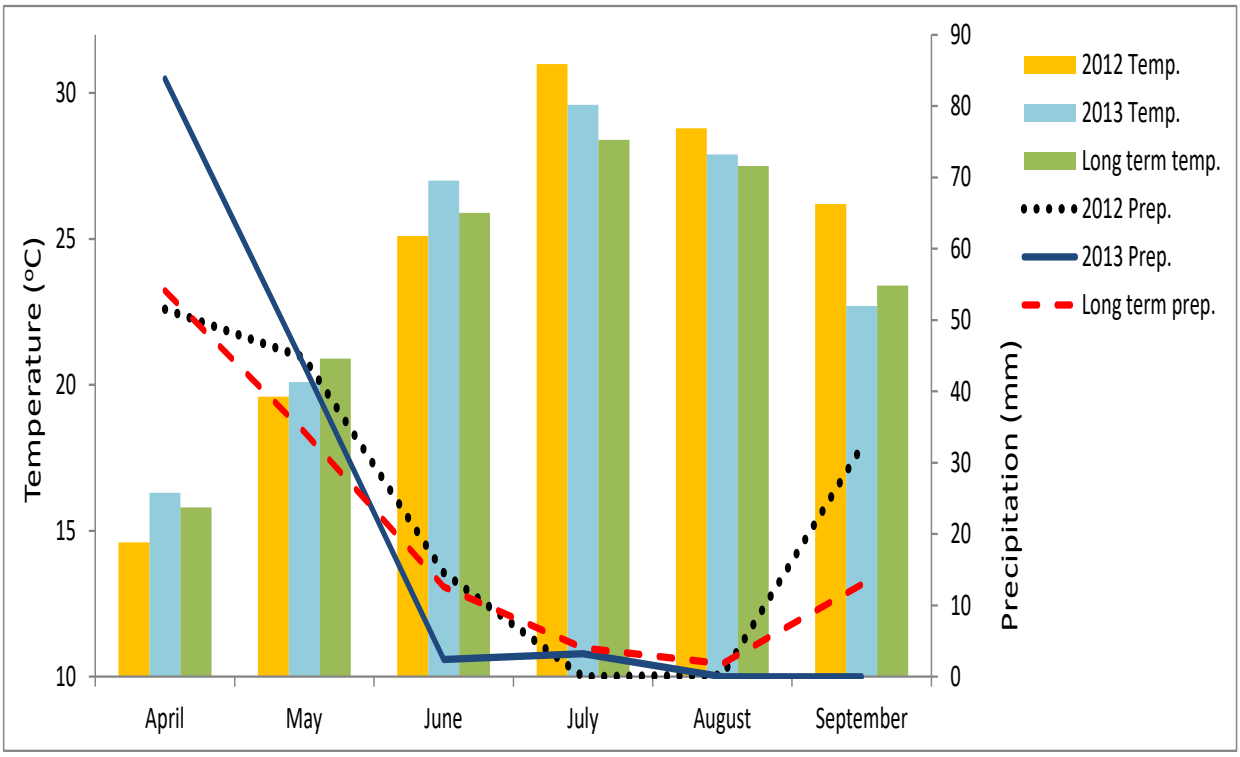

Figure 1- Precipitation and temperature during the cropping season in 2012 and 2013 with a long period

The experiment was sown on loamy sand soil having pH 7.60 (slight alkali), organic matter level is 1.20% (insufficient), total nitrogen 0.095% (medium), available P_2O_5 level is 2.08 mg kg^{-1} (insufficient) and available K_2O level is 1528 mg kg^{-1} (medium) (provided by Aegean University soil analysis laboratory).

P31G98 hybrid maize (*Zea mays* L.) and Umut soybean cultivar (*Glycine max* (L.) Merr.) were used as plant material. The soil characteristics where the experiment is carried out are alkali and poor in organic matter due to monoculture cotton growing. For this reason, it was difficult the nodulation of soybean. Therefore, Vitormone has been used as a factor in the application of biological fertilizer. The Vitormone provides the optimal environment for activating leaf surfaces by organisms and promotes a rich flora formation on the leaf surface (Golenberg & West 2013; Uddin et al. 2014).

In the study, "Randomized Complete Blocks Design with Two Factors" was used to simultaneously investigate the effects of intercropping and biofertilizer (the Vitormone) application (Cochran & Cox 1950). Sowings were carried out in 3 replications on May 1, 2012, and May 7, 2013. As a second factor, maize and soybean sowings were arranged as alternate rows, intra row and sole. The list of crops design in intercropping is given in Table 1 and schematized in Figure 2.

Sole maize (M_B) was planted at 0.70 x 0.15 m and soybean (S_B) planted at 0.70 x 0.05 m sowing standards. Each parcel consists of 12 rows (42 m^2) with a length of 5 m. The alternate rows were planted with 1 row of maize and 1 row of soybean and 35 cm rows and 15 cm and 5 cm rows respectively in the sowing areas (MS_A). There are approximately 90,000 plants ha^{-1} maize and 285,500 plants ha^{-1} soybean in the areas of plain seed and alternate seeding with soybean cultivation. These parcels consist of 24 rows with a length of 5 m (42 m^2). Another alternate sequence was 1 maize + 2 soybean cultivars (MS_D), and 1.05 m x 0.15 m sowing norm for maize and 0.35 m x 0.05 m sowing norm for soybeans were applied. In this regard, the plant densities were 63,490 plants ha^{-1} for maize and 380,940 plants ha^{-1} for soybean. The parcels consist of 24 rows of 5 m length (42 m^2). In the sowing on the same row (MS_C) parcels, two soybeans with 0.05 m space were 0.25 m between two maize. The plant densities were 57,140 plants ha^{-1} for maize and 114,280 plants ha^{-1} for soybean.

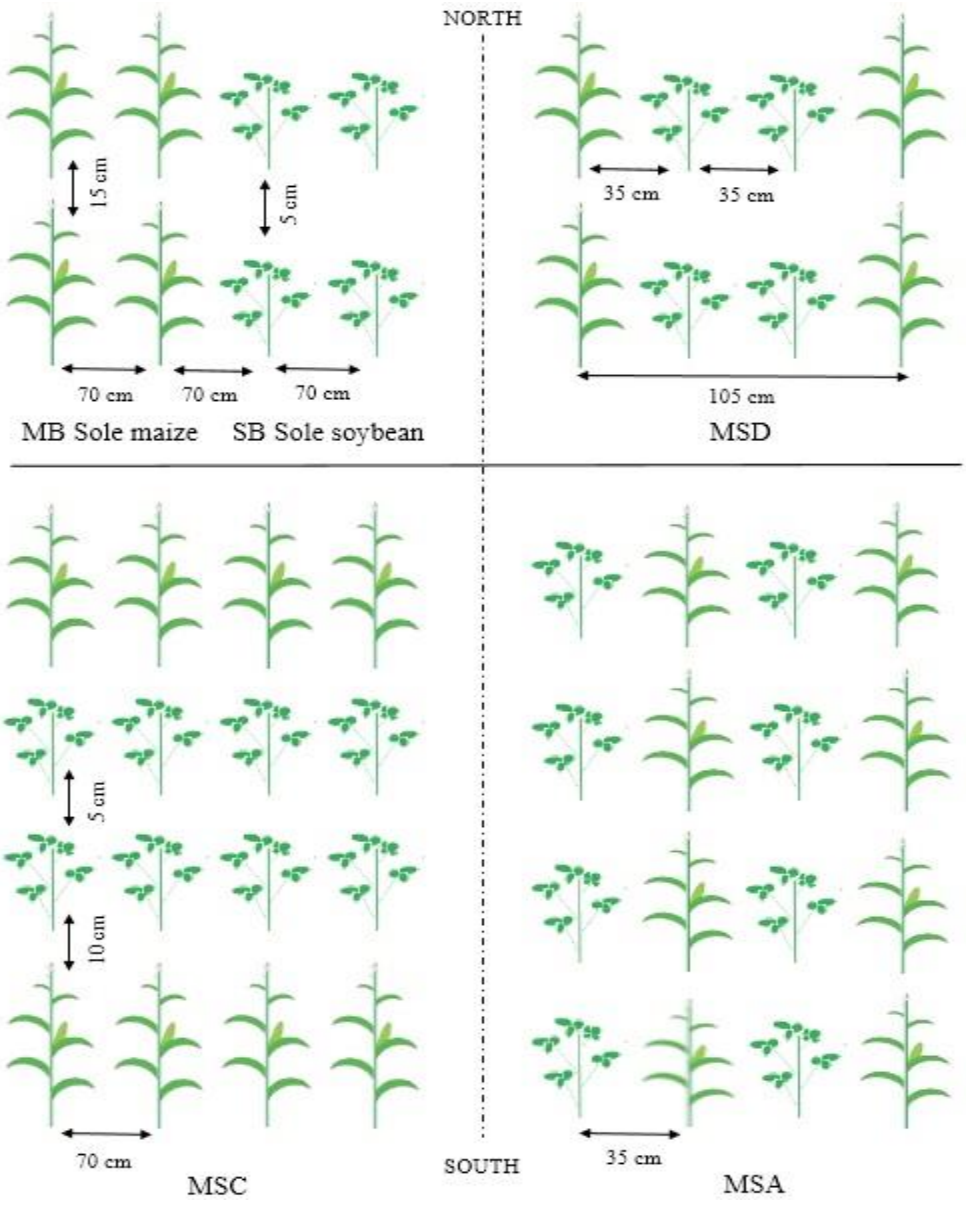

Figure 2- Diagrammatic sketch of different maize-soybean intercropping system

Table 1- The list of crops design in the cropping system

Crop	*Crop Design in intercropping*	*Symbol*
Maize	Sole	M_BV^-
Soybean	Sole	S_BV^-
Maize	Sole + Vitormone	M_BV^+
Soybean	Sole + Vitormone	S_BV^+
1 Maize + 1 Soybean	Alternate Rows	MS_AV^-
1 Maize + 1 Soybean	Alternate Rows + Vitormone	MS_AV^+
1 Maize + 2 Soybean	Alternate Rows	MS_DV^-
1 Maize + 2 Soybean	Alternate Rows + Vitormone	MS_DV^+
1 Maize + 2 Soybean	Intra row	MS_CV^-
1 Maize + 2 Soybean	Intra row + Vitormone	MS_CV^+

For all parcels, the recommended fertilizer rate of 180:80:80 of NPK was applied using a compound fertilizer (NPK 15:15:15) to supply 80 kg each of NPK ha^{-1} before sowing as basal application. Urea (46% N) to supply the remaining dose (100 kg ha^{-1}) of nitrogen was applied before first irrigation for all parcels except sole soybean. Three hoeings were applied for weeding. The Vitormone (Biological fertilizer) application, which is a factor in our study, prepared the Vitormone application (1 mL Vitormone + 1-L water which is the recommended the dose of Vitormone solution). It was applied twice with the mechanical back-sprayer at the flowering stage and 1 week after flowering in soybean.

2.2. Yield

For the yield of maize and soybean; 20 tagged plants were randomly selected from two rows of each plot leaving the outside rows as borders, and all obtained data were corrected to 14% grain moisture for both crops (Nyoki & Ndakidemi 2018).

2.3. Leaf area index (LAI)

For the LAI, data were collected from twenty plants at two the middle rows of each parcel at the stage of silking/tasseling for maize and peak flowering for soybean (Liu et al. 2017). The samples were separated into leaves. Leaf width and length measured in all leaves maize and multiplied to a coefficient factor of 0.75. Single leaf area for soybean was measured using an LI-3100 (LI-COR, Lincoln, NE) leaf area meter device. The LAI (m^2 m^{-2}) was determined by taking into account the number of plants in the unit area.

2.4. Land equivalent ratio (LER)

The LER is widely used to evaluate the land productivity of intercropping and was calculated according to the following formula (Ofori & Stern 1987);

LER= [Intercropping maize yield /Sole maize yield] + [Intercropping soybean yield /Sole soybean yield]

LER > 1 was evaluated as an effective intercropping system in terms of land utilization.

2.5. Statistical analysis

The data of yield and LAI were statistically analyzed using TARIST statistical Package Program (Acikgoz et al. 1994). The differences between the means were compared by the least significant difference (LSD) at the 5% level.

3. Results and Discussion

The results from the present study indicated that interactive effects of intercropping design and Vitormone application for maize yield were significant in the 2012 and 2013 cropping seasons. The high maize yields recorded in sole maize with the Vitormone (16.2 and 15.3 t ha^{-1}) and the non-Vitormone plots (14.9 and 14.3 t ha^{-1}) compared to intercropping plots (Table 2). In both years, MS_DV^+ (8.0 t ha^{-1}) and MS_CV^+ (7.0 and 7.6 t ha^{-1}) parcels produced the yields of the second group. In the parcels (MS_A) which alternate rows were planted with 1 row of maize and 1 row of soybean, maize yields of the non-Vitormone applications were higher than that of the Vitormone applications in both years.

Table 2- The results of variance analysis for the yield

Source of variation	*df*	*Yield*			
		Maize		*Soybean*	
		2012	*2013*	*2012*	*2013*
Block	2	14091.56	3826.54	66.14	332.65
Vitormone (A)	1	321.20	2795.04*	15595.80**	14113.50**
Crop Design (B)	3	1041140.43**	849598.15**	164448.33**	133185.98**
Ax B	3	80637.43**	11888.26**	3885.59**	682.18
Error	14	4540.51	515.83	126.93	620.48

*, **; significant at 5% and 1% probability level, respectively

The interaction of intercropping design and the Vitormone application was significant for soybean yield in 2012 whereas the differences of intercropping designs and the Vitormone applications were found significant in 2013. The yields of sole soybean parcels were higher than intercropping parcels in the Vitormone (4.8 t ha^{-1}) and the non-Vitormone (3.7 t ha^{-1}) parcels (Table 3). These values followed by MS_DV^+ (3.4 t ha^{-1}) and MS_DV^- (2.8 t ha^{-1}). The yields of MS_C and MS_A extensively decreased compared to sole cropping and MS_D. In 2013, sole soybean had the highest yield (4.3 t ha^{-1}) and was followed by MS_D (3.2 t ha^{-1}). Also, the yield of soybean in the Vitormone application recorded higher yield of 2.8 t ha^{-1} compared to the non-Vitormone (2.3 t ha^{-1}). Several studies (Ijoyah & Fanen 2012; Khan et al. 2012; Undie et al. 2012; Verdelli et al. 2012; Hirpa 2013; Ijoyah et al. 2013; Osang et al. 2014) suggested that the maize and soybean yield of intercropping over sole cropping decreased due to interspecific competition. In intercropping systems, yield advantage for maize and soybean could be found, which does not support findings of Li et al. (2001), Ngwira et al. (2012). Also, it was documented that the Vitormone application resulted with higher yield in

the soybean (Tahir et al. 2009; Subowo et al. 2010; Koushal & Singh 2011; Ngalamu et al. 2013; Salih et al. 2014), faba bean (*Vicia faba* L.) (Osman et al. 2010), chickpea (*Cicer arietinum* L.) (Uddin et al. 2014). The additional Vitormone application could be enhanced the yields of maize and soybean in especially poor nodulating conditions.

Table 3- Mean yield of the intercropping system and Vitormone application in 2012 and 2013

	Maize (t ha^{-1})				*Soybean (t ha^{-1})*		
	2012		*2013*		*2012*		*2013*
	V^+	V^-	V^+	V^-	V^+	V^-	
A	6.2 e	9.6 c	6.2 f	7.3 e	0.8 e	0.9 e	1.3 c
B	16.2 a	14.9 b	15.3 a	14.3 b	4.8 a	3.7 b	4.3 a
C	7.0 de	6.4 e	7.6 d	7.2 e	1.3 d	0.9 e	1.2 c
D	8.0 d	6.4 e	8.0 c	7.5 de	3.4 b	2.8 c	3.2 b
LSD $_{(0.05)}$	1.18		0.39		0.20		0.30
V^+							2.8 a
V^-							2.3 b
LSD $_{(0.05)}$							0.2

A; 1 M + 1 S (Alternate Rows), B; Sole, C; 1 M + 2 S (intra row), D; 1 M + 2 S (Alternate Rows) , V; Vitormone. Within each column, values with the same letter do not significantly differ at 5%

The LAI is a positive indicator to improve the yield and to minimize the evaporation (Kubota et al. 2015). Also, it is probably the most important factor in the competition for light and critical to maintaining high yield in intercropping (Kamara et al. 2017). The effects of interaction between the intercropping system and Vitormone application on the LAI were found to be significant for the maize in the 2012 and 2013; for the soybean in 2013 (Table 4). The differences in the intercropping system and the Vitormone application for the soybean in 2012 were significant. The LAI values of sole maize with Vitormone (6.00 $m^2 m^{-2}$) or without the Vitormone (5.90 $m^2 m^{-2}$) were significantly higher than intercropping parcels in 2012 (Table 5). MS_A plots significantly exhibited the highest LAI values (4.41 $m^2 m^{-2}$ and 4.72 $m^2 m^{-2}$) compared to other intercropping parcels, MS_D and MS_C, respectively. The LAI values of 2013 growing season were similar to 2012 values for maize. In terms of the soybean's LAI, the sole soybean (7.28) had a significantly higher LAI value than that of intercropping systems followed by MS_D (6.40) in 2012. Similar ranging among the LAI values of intercropping systems in both V^+ and V^- parcels were recorded for soybean. However, it should be noted that the sum of maize and soybean LAI in intercropping was always superior to sole maize and soybean (Walker & Ogindo 2003; Kubota et al. 2015).

The LER provides an accurate evaluation of the competition in the intercropping system. Also, the LER of more than 1.0 reveals the yield advantage of intercropping. The LERs of different intercropping systems varied from 0.55 (MS_AV^+) to 1.32 (MS_DV^+). In contrast to many published literature, there was no strong the LER above 1.50 in maize/soybean intercropping (Rahman et al. 2017; Chen et al. 2018). The yield capacities of mentioned studies were 5.63-8.53 t ha^{-1} for the maize and 1.59-2.22 t ha^{-1} for soybean (Chen et al. 2018), 4.9-10.8 t ha^{-1} for the maize and 0.11-1.85 t ha^{-1} for soybean (Rahman et al. 2017), whereas yield values were 6.2-16.2 t ha^{-1} for the maize and 0.8-4.8 t ha^{-1} for the soybean in our study. It can be concluded that yield capacity should be considered when evaluating the LER, especially in the fertile areas. The MS_D, 1 Maize + 2 Soybean in alternate rows, produced greater than other intercropping systems (Table 6). The all LER values of Vitormone application parcels except MSA were found to be higher than the non-Vitormone application. In previous studies, it was documented that the highest LER value (1.26) was found in 1 maize + 2 soybeans in alternate rows (Addo-Quaye et al. 2011; Ijoyah & Fanen 2012; Mandal et al. 2014). Especially, the yield losses of MS_A and MS_C resulted from the continuous shading of the maize, and soybean yield decreased (Lv et al. 2014; Liu et al. 2017).

Table 4- The results of variance analysis for the LAI

Source of variation	*df*	*LAI*			
		Maize		*Soybean*	
		2012	*2013*	*2012*	*2013*
Block	2	0.01	0.00	0.02	0.00
Vitormone (A)	1	0.01	0.00	0.11*	0.11**
Crop Design (B)	3	8.15**	8.61**	14.39**	14.82**
A x B	3	0.05**	0.02**	0.04	0.02**
Error	14	0.00	0.00	0.01	0.00

*, **; significant at 5% and 1% probability level, respectively. LAI; leaf area index

Table 5- Mean LAI of the intercropping system and Vitormone application in 2012 and 2013

	Maize (m^2 m^{-2})				*Soybean (m^2 m^{-2})*		
	2012		*2013*		*2012*	*2013*	
	V+	*V-*	*V+*	*V-*		*V+*	*V-*
A	4.41 c	4.72 b	4.38 c	4.51 b	4.72 c	4.81 e	4.78 e
B	6.00 a	5.90 a	5.94 a	5.90 a	7.28 a	7.33 a	7.27 b
C	3.24 e	3.19 e	3.41 d	3.28 e	3.84 d	3.96 f	3.73 g
D	3.93 e	3.90 d	3.47 d	3.40 d	6.40 b	6.60 c	6.38 d
LSD (0.05)	0.105		0.071		0.139	0.046	
V+					5.62 a		
V-					5.48 b		
LSD (0.05)					0.098		

A; 1 M + 1 S (Alternate Rows), B; Sole, C; 1 M + 2 S (intra row), D; 1 M + 2 S (Alternate Rows) , V; Vitormone.
Within each column, values with the same letter do not significantly differ at 5%.

Table 6- The LER values for intercropping system and Vitormone application

	LER		
Intercropping System	*2012*	*2013*	*Mean*
MS_AV^-	0.88	0.76	0.82
MS_AV^+	0.55	0.70	0.63
MS_CV^-	0.67	0.72	0.69
MS_CV^+	0.70	0.85	0.78
MS_DV^-	1.19	1.21	1.20
MS_DV^+	1.20	1.32	1.26

LER; Land equivalence ratio

4. Conclusions

The interaction of the intercropping design and the Vitormone were significant for yield and the LAI in both years. The Sole maize and the sole soybean were shown to have a significantly higher yield and the LAI values than the intercropping system. The results of LER confirmed that the 1 maize + 2 soybeans in the alternate rows were preferable among the all intercropped yields. Finally, it can be concluded that the combination of intercropping and Vitormone was found suitable for the poor nodulating conditions in the monocropped agricultural system.

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A Study on Milk Compositions of Hair Goat and Saanen x Hair Goat Crossbreed (F_1) under Semi-Intensive Conditions

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ABSTRACT

This study was carried out to evaluate compositions of milk from Hair goat and Saanen x Hair goat crossbreed (F_1) under semi-intensive conditions. The research was conducted on 20 Hair goats and 20 Saanen x Hair goat crossbreed (F_1) at Farm for Research and Application of Van Yuzuncu Yil University in Turkey. All the experimental goats were 3 years old and were raised under semi-intensive conditions. In the current study, the average fat, non-fat solid, protein, lactose, total solids, lactic acid, pH values of Hair and crossbreed goat milks were 3.98%, 3.80%; 9.54%, 9.24%; 3.59%, 3.48%; 5.24%, 5.08%; 13.51%, 13.04%; 0.24%, 0.25%; 6.62, 6.64, respectively. Lactation stage had a significant influence ($P<0.05$; $P<0.001$) on milk fat, non-fat solid, protein, lactose, total solids contents and lactic acid value in Hair and crossbreed goats' milk. Also, genotype had a significant influence ($P<0.001$) on non-fat solid, protein, lactose and total solids contents of the milk. In conclusion, Hair goats and crossbreed goats produce milk with a variable milk fat, non-fat solid, protein, lactose, total solids contents at the different stages of lactation. The mean values of milk non-fat solid, protein, lactose and total solids in Hair goats' milk were higher than those of crossbreed goats. Also, milk fat content, lactic acid and pH values in Hair and crossbreed goats' milk were similar.

Keywords: Hair goat, Saanen x Hair goat (F_1) crossbreed, Milk composition, Semi-intensive conditions

1. Introduction

Goat breeding is an important livestock activity for underdeveloped and developing countries due to the low cost of production. Turkey has favorable geographical characteristics and socio-economic structure for goat breeding. The goats that grown in a large part of Turkey has been contributed significantly to the agricultural economy (Turkstat 2017). Goat population are 10 922 427 in Turkey and 98.11% of its are Hair goat. The annual amount of milk obtained from indigenous goat herds is approximately 561 826 tons (Turkstat 2018).

The energy and nutrients that form basis of a healthy life need to be consumed in quantities needed by consumed foods. Adequate and balanced nutrition plays an important role in protection of growth, development and health of the individual. One of the four basic food groups that need to be taken every meal is milk and dairy products. Milk which is a good food in terms of other nutrients except for Vitamin C and iron is necessary for every period of human life (Çak & Demirel 2018).

Goat milk consumption supplies well and balanced nutrition for the children. The symptoms of allergies to cow milk may disappear with goat milk consumption for the children (Merin et al. 1988). The chemical composition of milk of animal species differs (Rezaei et al. 2016). Components like fat and protein in milk are significant both the dairy industry and human nutrition. The nutritional value of milk is tightly interested in its composition, which is influenced by factors such as breed, feeding, lactation stage, season, etc. (Abdelsalam et al. 2000). Variability of milk production is largely affected by nutrition (Min et al. 2005). One of the major factors that affect milk yield and composition is the stage of lactation. There are three main stages of lactation namely early, mid and late lactation (Idowu & Adewumi 2017). Mioč et al. (2008) reported that Saanen breed had higher daily and lactation milk yield but lower percentage of milk constituents than Alpine breed. Fat and protein increased with advancing stage of lactation, while lactose had a reverse trend. Ciappesoni et al. (2004) found that milk protein of Czech White Shorthaired Goats was significantly influenced by effect of stage of lactation.

There has been an increasing interest towards goats' milk and its products in Turkey in recent years. However, studies on milk compositions of pure and crossbreed goats, in Turkey and similar agroecological zones, are limited. Therefore, further researches are needed to evaluate milk components of pure and crossbreed goats in these zones.

This research was carried out to evaluate compositions of milk from Hair goat and Saanen x Hair goat crossbreed (F_1) under semi-intensive conditions.

2. Material and Methods

2.1. Goats, shelter and management

The research was carried out 20 Hair goats and 20 Saanen x Hair goat crossbreed (F_1) at Farm for Research and Application of Van Yuzuncu Yil University in Turkey in 2016. Van is located between 42° 40' and 44° 30' east longitudes, 37° 43' and 39° 26' north latitudes. The climatic situation of Van is hot and dried in summer, and cold and snowy in winter. In 2016, the average annual ambient temperature and rainfall of Van were 9.5 °C and 393.5 mm, respectively. All the experimental goats were 3 years old. Goats were housed in semi-open sheds under semi-intensive conditions. Goats in both groups were fed 600 g/day of concentrate (16% crude protein and 2500 kcal metabolizable energy per kg dry matter) and grazed on pasture during lactation.

2.2. The analysis of milk samples

Physical and chemical compositions of milk were defined in the 1st, 2nd, 3rd, 4th and 5th months of lactation. First of milk samples were collected in March 2016. Milk samples were taken once a day at 9-10 hours in the morning and were carried on quickly to the laboratory for analysis in the cold chain. Total solids, fat content, total protein content, lactose content and lactic acidity of milk were analyzed Gravimetric method, Gerber method, Kjeldahl method, Photometric method, and Titration method respectively Kurt et al. (2003). pH was detected by a pH meter (Kosikowski 1982).

2.3. Statistical analysis

Descriptive statistics for studied variables (characteristics) were presented as mean and standard error of mean. Data were analyzed by two factors experiments with repeated measurements on one factor levels in GLM procedure by the following statistical model:

$$Y_{ijm} = \mu + \alpha_i + \pi_{m(i)} + \beta_j + \alpha\beta_{ij} + \beta\pi_{jm(i)} + e_{1(ijm)}$$

(i=1,..,k; k=2; j=1,..,p; p=5 ve m=1,..,n_i; n=40)

Where;

Y_{ijm}: milk fat, non-fat solid, protein, lactose, total solids, lactic acid and pH values measurements on each goat

μ: overall mean,

α_i: effect of the i^{th} goat genotype [Hair, Saanen x Hair goat (F_1)],

$\pi_{m(i)}$: effect of the i^{th} goat genotype m^{th} random effect of the animal (error 1)

β_j : effect of the j^{th} lactation stages

$\alpha\beta_{ij}$: interactions between genotypes and lactation stages,

$\beta\pi_{jm(i)}$: interactions between the animal and lactation stages in the level of the i^{th} goat genotype,

$e_{1(ijm)}$: random error.

After ANOVA, Tukey multiple comparison test was also used to identify different lactation stages. Statistical significance level was considered as 5% and SPSS (ver. 13) statistical program was used for all statistical computations (SPSS 2006).

3. Results and Discussion

Effects of lactation stage, genotype and interaction between lactation stage with genotype on milk components of Hair goat and Saanen x Hair goat crossbreed (F_1) are presented in Table 1. The results of the study showed that lactation stage had a significant influence ($P<0.05$ and $P<0.001$) on milk fat, non-fat solid, protein, lactose, total solids contents and lactic acid value in Hair and

crossbreed goats' milk. Genotype had a significant influence (P<0.001) on non-fat solid, protein, lactose and total solids contents of milk in the 4th month of lactation. Non-fat solid, protein, lactose and total solids contents of milk in the 4th month of lactation had in favor of Hair goats. Also, when the overall means of milk contents in Hair goats' milk compared with those of crossbreed goats, Hair goats' milk had a higher mean value of non-fat solid, protein, lactose and total solids contents. In addition, there was significant (P<0.05) interaction between genotype and lactation stage on total solids content of the milk.

Milk components in lactation stages of Hair and crossbreed goats are presented in Figure 1. It is seen that considerable changes occurred according to lactation stages of milk contents in Hair and crossbreed goats' milk.

Table 1- Effect of lactation stage, genotype and interaction between lactation stage with genotype on milk components of Hair goat and Saanen x Hair goat crossbreed (F_1)

Milk components	*Genotype (G)*	*n*	*Lactation Stages (LS)*					*Significance of LS*	*G x LS*	*Overall means*
			1st month	*2nd month*	*3rd month*	*4th month*	*5th month*			
			Mean ±SEM	*Mean ± SEM*	*Mean ± SEM*	*Mean ± SEM*	*Mean ± SEM*			Mean ± SEM
Fat (%)	Hair goat	20	4.02±0.12^{ab}	3.90±0.11^{b}	4.32±0.08^{a}	3.83±0.16^{b}	3.80±0.19^{b}	*	ns	3.98±0.06
	Saanen x Hair goat	20	3.95±0.15^{a}	4.08±0.12^{a}	4.35±0.07^{a}	3.34±0.21^{b}	3.30±0.19^{b}	***		3.80±0.08
Non-fat solid (%)	Hair goat	20	9.31±0.09^{b}	9.93±0.08^{a}	9.26±0.07^{b}	9.84±0.09$^{a\,\#}$	9.35±0.20^{b}	***	ns	9.54±0.06$^{\#}$
	Saanen x Hair goat	20	9.12±0.11^{b}	9.77±0.10^{a}	8.96±0.17^{b}	9.11±0.07^{b}	9.24±0.12^{b}	***		9.24±0.06
Protein (%)	Hair goat	20	3.52±0.04^{b}	3.73±0.03^{a}	3.49±0.03^{b}	3.71±0.03$^{a\,\#}$	3.53±0.07^{b}	***	ns	3.59±0.02$^{\#}$
	Saanen x Hair goat	20	3.44±0.04^{b}	3.69±0.05^{a}	3.38±0.06^{b}	3.43±0.02^{b}	3.48±0.04^{b}	***		3.48±0.02
Lactose (%)	Hair goat	20	5.10±0.05^{b}	5.49±0.05^{a}	5.08±0.04^{b}	5.39±0.05$^{a\,\#}$	5.14±0.10^{b}	***	ns	5.24±0.03$^{\#}$
	Saanen x Hair goat	20	4.98±0.06^{b}	5.42±0.07^{a}	4.92±0.09^{b}	5.00±0.03^{b}	5.07±0.06^{b}	***		5.08±0.03
Total solids (%)	Hair goat	20	13.33±0.15^{ab}	13.83±0.12^{a}	13.58±0.07^{ab}	13.67±0.21$^{a\,\#}$	13.15±0.23^{b}	*	*	13.51±0.08$^{\#}$
	Saanen x Hair goat	20	13.07±0.15^{bc}	13.85±0.18^{a}	13.31±0.18^{ab}	12.45±0.23^{d}	12.54±0.22^{cd}	***		13.04±0.10
Lactic acid (%)	Hair goat	20	0.21±0.00^{c}	0.23±0.01^{b}	0.22±0.00^{c}	0.22±0.01^{c}	0.33±0.00^{a}	***	ns	0.24±0.01
	Saanen x Hair goat	20	0.22±0.01^{c}	0.24±0.00^{b}	0.22±0.01^{c}	0.22±0.00^{c}	0.34±0.01^{a}	***		0.25±0.00
pH	Hair goat	20	6.62±0.02	6.62±0.02	6.63±0.02	6.61±0.02	6.65±0.01	ns	ns	6.62±0.01
	Saanen x Hair goat	20	6.56±0.03^{b}	6.56±0.02^{b}	6.68±0.02^{a}	6.70±0.01^{a}	6.70±0.01^{a}	***		6.64±0.01

^{a,b,c,d}; values indicated with lowercase letters are different from other lactation stages within the same row, *; P<0.05, ***; P<0.001, ns; non-significant
Values indicated with '#' within the same column is in favor of Hair goats (P<0.001), SEM; Standard error of mean

The fat content in milk varies depending on factors such as lactation period, genotype and nutrition (Haenlein 2004; Kondyli et al. 2012). In this study, it was observed that the most variable compound in the milk of both goat genotypes was fat. Throughout lactation, the fat content of Hair goat milk varied from 3.80 to 4.32%; the fat content of crossbreed goat milk varied from 3.30 to 4.35%. The average fat contents of Hair and crossbreed goats were 3.98 and 3.80%, respectively. Fat content of Hair goat milk in this study is consistent with the value (3.93%) reported for Hair goats by (Cak et al. 2017). Milk fat contents obtained for both goat genotypes in this study were higher than the values (2.84 and 3.72%) reported for the Czech White Shorthaired goats by Ciappesoni et al. (2004), however, were lower than milk fat contents (4.02 and 4.70%) reported for Damascus and Boer goats and by Güler et al. (2007) and Mestawet et al. (2012). These differences in milk fat content were probably due to genotype, feeding, management, climate, etc. conditions. Cak et al. (2017) reported that lactation stage had a significant influence on milk fat. Results which reported correspond with the results of this study. Milk fat content for both goat genotypes in the present study was lowest at the end of lactation stage. This finding is inconsistent with finding reported for Hair goats by Cak et al. (2017).

Goat milk proteins have very important differences in amino acid compositions when compared milk proteins of other mammalian species, in addition to this, the relative proportions of the various milk proteins differ in goat and cow milk (Monaci et al. 2006). The protein content of milk is very important in cheese production. In this study, throughout lactation, the protein content of Hair goat milk varied from 3.49 to 3.73%; the protein content of crossbreed goat milk varied from 3.38 to 3.69%. The protein content of both goat genotypes was significantly influenced by the lactation stage. The average protein contents of Hair and crossbreed goats were 3.59 and 3.48%, respectively. Protein contents of Hair and crossbreed goat milks in this research were higher than the value (3.45%) notified for Hair goats by Cak et al. (2017), however, were lower than the values (4.05%) notified

for Boer goats and by Mestawet et al. (2012). These differences in milk protein content were probably due to genotype, feeding, management, climate, etc. conditions.

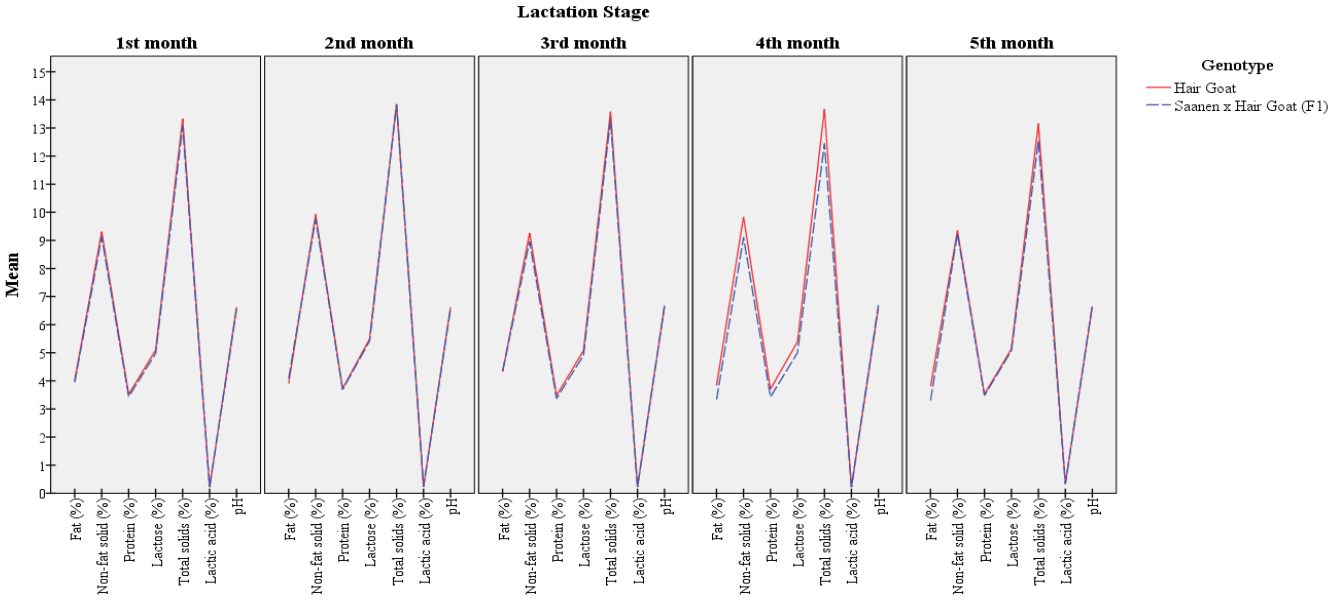

Figure 1- Changes in milk components according to lactation stages in goat genotypes

Total solids are important for nutritional value of milk and processing of products. In the current study, throughout lactation, the total solids content of Hair goat milk varied from 13.15 to 13.83; the total solids content of crossbreed goat milk varied from 12.45 to 13.85%. The content of total solids in milk of both goat genotypes statistically differed in lactation stages. However, the effect of lactation stage on total solids in the milk found the non-significant by Addass et al. (2013). The mean total solids contents of Hair and crossbreed goat milks were 13.51 and 13.04%, respectively. The content of total solids obtained for Hair goat milk in the present study was higher than the values (13.07 and 12.2%) reported for Hair and Damascus goats (Keskin et al. 2004; Cak et al. 2017). The content of total solids in crossbreed goat milk was similar to the value reported for Hair goats by Cak et al. (2017), however, was higher than the value notified for Damascus goats by Keskin et al. (2004).

In this study, lactose content was different in the early and late lactation stages of both Saanen x Hair goat and Hair goat genotype. Conversely, Cak et al. (2017) reported that it was similarity in the early and late lactation stages. Also, Mestawet et al. (2012) and Prasad et al. (2005) notified that lactose content in the early lactation stage and the late lactation stage was highest, lowest respectively. Lactose contents of Hair and crossbreed goat milks in our study were similar to the finding (5.15%) reported by Cak et al. (2017), were higher than the result (4.66%) notified by Kanwal et al. (2004).

In the current study, the effect of lactation stage season on pH of Hair goat milk was statistically non-important, however, crossbreed goat milk was statistically significant. Addass et al. (2013) notified that the pH was not influenced by the lactation stage season.

4. Conclusions

The findings of the current study showed that Hair goats and crossbreed goats produce milk with a variable milk fat, non-fat solid, protein, lactose, total solids contents at the different stages of lactation. The mean values of milk non-fat solid, protein, lactose and total solids in Hair goats' milk were higher than those of crossbreed goats. These results also indicated that fat content, lactic acid and pH values in Hair and crossbreed goats' milk were similar.

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Evaluation of some groundwater quality parameters using geostatistics in the urban coastal aquifer of Bosaso plain, Somalia

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ABSTRACT

Groundwater is a major drinking water resource in arid coastal regions. The groundwater quality of Bosaso city experienced degradation due to rapid urbanization and industrialization. This study was carried out to delineate the spatial distribution of groundwater quality parameters and evaluate groundwater suitability for drinking and irrigation uses. The groundwater samples were collected from Bosaso Plain to determine the pH, electrical conductivity (EC), total dissolved solids (TDS), and sodium and chloride concentrations. To categorize water quality for irrigation purposes, sodium adsorption ratio (SAR) was calculated. Ordinary kriging procedure was performed in order to map the spatial distribution of groundwater quality parameters. The interpretation of laboratory analysis results revealed that the most of groundwater wells in the study area is unsafe for drinking purposes due to high salinity, except for the central area (Biyo Kulule). In terms of irrigation uses, the minor area may be under the risk of alkalinity or sodium hazard. However, all groundwater supply points are not suitable for irrigation due to the salinization risk and can be only used to irrigate high salt-tolerant crops. The final maps show that the groundwater quality decreases from southeast to the north of the plain. This indicates that the groundwater is probably subjected to the seawater intrusion. In this regard, the implementation of a groundwater monitoring program is necessary to achieve concrete results. Nevertheless, the most suitable groundwater quality is found to be at the central part of the Bosaso plain.

Keywords: *Groundwater quality, Irrigation water, Geostatistics, GIS, Bosaso Plain, Somalia*

1. Introduction

Coastal areas in arid and semi-arid regions highly depend on groundwater resources due to the lack of surface water. Bosaso is the major city of Puntland state, and groundwater is the main water source. The city faces degradation in water quality and water supply shortage due to the rapid population growth in recent years and effects of climate change and droughts (Said et al. 2019). Although groundwater monitoring is necessary for taking effective measures, currently there is no groundwater monitoring program in place. Proper management of groundwater resource is very important to meet the increasing demand for water. Furthermore, understanding the spatial distribution of groundwater quality helps authorities to develop optimal management strategy of groundwater resources in order to ensure sustainable development of the society.

Mapping groundwater quality parameters through using geostatistics has become well known. There are numerous studies on groundwater quality evaluation using geostatistical techniques all around the world (Nur et al. 2012; Shamsudduha 2007; Goovaerts et al. 2005; Zehtabian et al. 2013). Sarath Prasanth et al. (2012) conducted a study to assess the spatial distribution of groundwater quality in the coastal city of Alappuzha in India. The results revealed the groundwater is entirely suitable for drinking water supply. Additionally, it presents that the groundwater is appropriate for irrigation purposes excluding a few sites. Arslan (2012) used ordinary kriging procedures to analyze the spatial distribution of groundwater salinity in the Bafra plain of Turkey. Based on the research results, it was shown that groundwater salinity levels were in a decreasing trend behavior. Nas & Berktay (2008) used geostatistical techniques to map and evaluate groundwater in Konya province in Turkey. The results revealed that the groundwater quality decreased from south to north. Although geostatistical techniques have been made widely applicable in the hydro-sciences all over the world since the early 1970s, literature review leads us to believe that application of kriging procedures to the water science needs to be further popularized in Somalia.

The objectives of the study are two-fold: a) to delineate the spatial distribution of some groundwater quality parameters in a coastal aquifer of Bosaso plain, Somalia, through using geostatistical approach, b) to make preliminary evaluations whether the groundwater resource unique in the area is suitable for drinking and irrigation uses.

2. Material and Methods

2.1. Study area

Formerly known as Bandar Qasim, Bosaso is located Puntland state of Somalia on the Gulf of Aden coast, lies between Latitude 11° 17′N and Longitude 49° 11′ E (Figure 1). Bosaso is the third largest city in the country and its population is estimated about 430,000 residents. Based on the Köppen-Geiger climate classification, the climate of the study area is arid type and has a hot desert climate (Rubel & Kottek 2010). Furthermore, the city annually receives very little rainfall, on average, less than 50 mm year^{-1}. Groundwater is the primary source of drinking water for the services sector and irrigation water for agricultural sector.

Bosaso plain is a wide plain (approximately 70 km^2 area) having a triangular shape. Depths of groundwater wells are subject to change, varying between 1 to 100 m. The deepest water table, i.e. maximum depth of groundwater from soil surface, is 51.6 m in the south-eastern part of the plain. However, depth-to-watertable is minimum (3 m) in the northern coastline and zero at the interface between land and ocean surface. General groundwater flow direction is from south to north, towards the ocean.

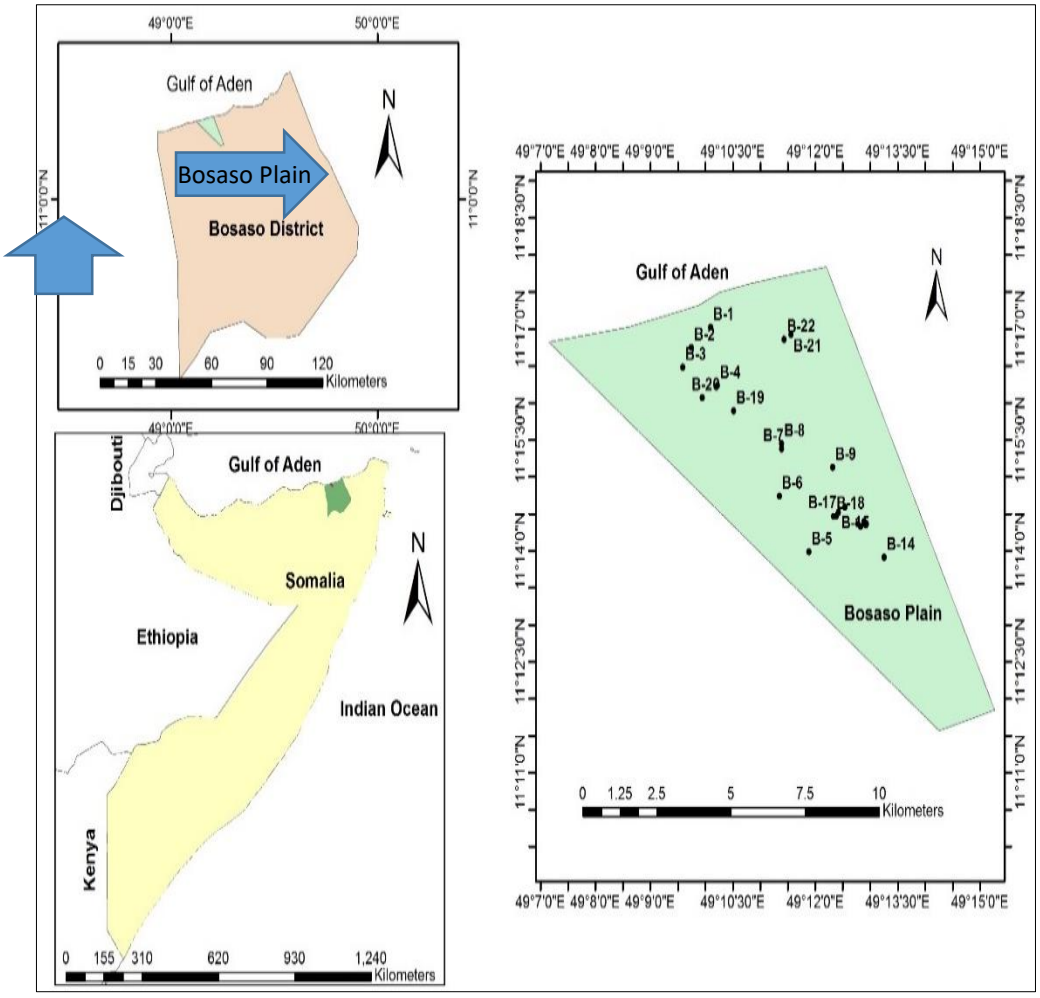

Figure 1- Location map of the study area and spatial distribution of groundwater wells (sampling points)

2.2. Data

Groundwater sampling locations were spatially distributed to cover the entire study area. Water samples were collected from groundwater wells in the study area in July of 2018. A total of 22 groundwater wells was visited. Location of sampling sites was determined by utilizing a hand-held Garmin GPS device. The collected water samples were returned to the water quality laboratory of Department of Agricultural Structures and Irrigation in Adana, Turkey, within four days. Groundwater quality parameters such as pH, electrical conductivity (EC, dS m^{-1}), total dissolved solids (TDS, mg L^{-1}), sodium (Na, meq L^{-1}), chloride (Cl, meq L^{-1}), calcium (Ca, meq L^{-1}) and magnesium (Mg, meq L^{-1}) were determined in the lab. Based on Ayers & Westcot (1994), TDS was calculated by using chemical analysis results of Na, K, Ca, Mg, Cl, CO_3, HCO_3, SO_4 ions in the unit of mg L^{-1}. Additionally, following the procedures given in Fetter et al. (2017), sodium adsorption ratio (SAR) was calculated for each water sampling location by using Na, Ca and Mg analysis results in meq L^{-1} unit.

2.3. Geostatistical methods

In this study, we adopted semivariogram models for determining the spatial dependence structure of groundwater parameters and ordinary kriging (OK) estimation technique for mapping the variables considered. Kriging interpolation is known as the best linear unbiased estimation technique (BLUE). In this regard, it is an objective mapping technique by allowing the user to make optimal spatial interpolations at unsampled, i.e. unvisited, locations (Cetin & Kirda 2003). The OK techniques are well documented in the latest literature (Masoud 2014; Sheikhy Narany et al. 2014). One of the main benefits of kriging estimation method is that it might be able to provide the user with the interpolation error, i.e. kriging estimation error, of the values of the regionalized variable where there are no initial measurements. This feature provides a measure of the estimation accuracy and reliability of the spatial distribution of the variable (Yunsel et al. 2002; Theodossiou & Latinopoulos 2006). The step-by-step procedure for ordinary kriging application is given in Figure 2.

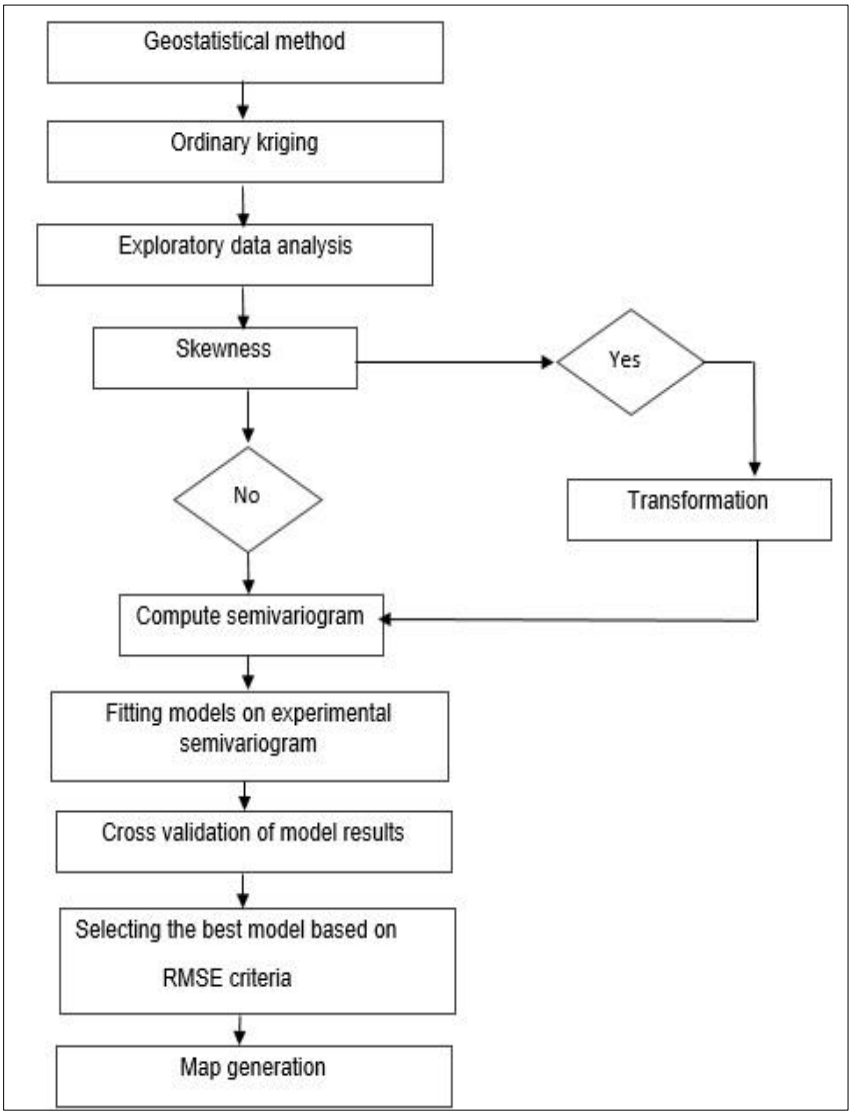

Figure 2- Flow chart of the procedure for ordinary kriging analaysis

The geostatistical modelling approach of kriging known as spatial interpolation technique (Clark & Harper 2007; Cetin & Kirda 2003) was adopted and, in turn, the semivariance structure of each water quality parameter was obtained by calculating experimental semivariogram values (Equation 1).

$$\hat{\gamma}(h) = \frac{1}{2N_h}\sum_{i=1}^{N_h}(g_i - g_{i+1})^2 \quad (1)$$

Where; h stands for the separation vector, i.e. distance between pairs of observations; $\hat{\gamma}(h)$ is the calculated value of the semivariogram, i.e. semi-variance, for h; N_h is the total number of pairs separated by h; g_i *and* g_{i+1} are values of the variable "g" at the point x_i and at a point of distance h from the point x_{i+1}. Theoretical semivariogram models and respective parameters were determined by applying methodology given in Figure 2.

3. Results and Discussion

In this study, some of the water quality parameters were used to evaluate if groundwater is suitable for drinking and irrigation uses, or not. Groundwater samples have been classified based on drinking water quality standards given by WHO (2008). Due to the fact that groundwater wells have been utilized for irrigation in the region, suitability of groundwaters for irrigation purposes have been assessed on the basis of SAR and EC by adapting the standard procedures given in Richards (1954), Ayers & Westcot (1994). Therefore, groundwater quality parameters consisting of pH, EC, TDS, Na, Cl, and SAR were obtained from the laboratory analysis. Practical information derived from the interpretation of results was, in turn, discussed below.

3.1. Geostatistical analysis results

Conventional statistical analysis has been carried out to check the normality of data because the kriging interpolation method gives best prediction when data are normally distributed. As seen from the descriptive statistics given in Table 1, only pH data agree with the assumption of normality, and all the other parameters are characterized by a right-skewed distribution. Distribution of skewed data might be rendered symmetrical by using a 3-parameter lognormal (LN3) probability distribution function. The descriptive statistics of data sets and the transformation method was given in Table 1.

Table 1- Descriptive statistics of groundwater quality parameters

Statistics	pH	EC (dS m^{-1})	TDS (mg L^{-1})	SAR (meq $L^{-1})^{0.5}$	Cl (mg L^{-1})	Na (mg L^{-1})
N	22	22	22	22	22	22
Min	7.03	1.44	1035.3	1.42	149.74	82.22
Max	8.00	16.50	10083.5	15.40	4502.40	2172.1
Mean	7.42	3.91	2665.4	4.09	775.51	404.69
Median	7.33	2.02	1427.3	2.07	273.65	145.56
SD	0.25	3.67	2357.0	3.98	1022.50	533.32
CV (%)	3	94	88	97	132	132
Skewness	0.86	2.22	1.87	1.74	2.61	2.18
Kurtosis	0.25	5.79	3.53	2.35	8.09	4.98
Transformation	Normal	3-parameter lognormal				

Semivariogram analysis was carried out in the ArcGIS platform by using *"Geostatistical Analyst Tool"*. In order to obtain experimental semivariograms for each variable, Euclidian distances between observation pairs were calculated at the very beginning of the geostatistical analysis. Consequently, geographical coordinates of observation wells were transferred to Universal Transverse Mercator (UTM) coordinate system for rendering possible distance calculations. Then, experimental semivariograms have been obtained for each variable; hence, seven candidate experimental semivariogram models (*Gaussian, Spherical, Circular, Exponential, Tetraspherical, Pentaspherical, Hole effect*) and their parameters were determined and goodness-of-fit tests of each model have performed accordingly. The best fitted theoretical semivariogram model for each variable or groundwater quality parameter was chosen based on the lowest value of RMSE. Figure 3 shows omni-directional experimental semivariogram data points (semivariogram cloud) around the theoretical semivariogram model (solid line in blue color). Table 2 illustrates best-fitted theoretical semivariogram models and their fixed model parameters after conducting the cross-validation checks. After fixing the theoretical model and its parameters for each variable of interest, kriging maps of groundwater quality variables were generated by performing ordinary kriging interpolation technique. Based on the semivariogram analysis results, our conclusion was that all groundwater quality parameters were spatial dependent; therefore, range of influence varied between 1100 and 1950 m as seen in Table 2 as well as in Figure 3.

Table 2- Best-fitted semivariogram models and their estimated parameters for the transformed data, i.e. LN3, except for pH

Parameters	Semivariogram Model	Nugget (C_0)	Sill (C_0 + C)	Range (m)	RMSE
pH	Hole effect	0.0002	0.0005	1131.5	0.242
EC (dS m^{-1})	Exponential	0.0000	0.2803	1893.9	3.539
TDS (mg L^{-1})	Exponential	0.0000	0.2800	1948.9	2272.495
SAR (meq $L^{-1})^{0.5}$	Exponential	0.0000	0.3159	1840.0	3.654
Cl (mg L^{-1})	Hole effect	0.0646	0.4580	1678.1	1013.851
Na (mg L^{-1})	Exponential	0.0000	0.5835	1858.1	520.058

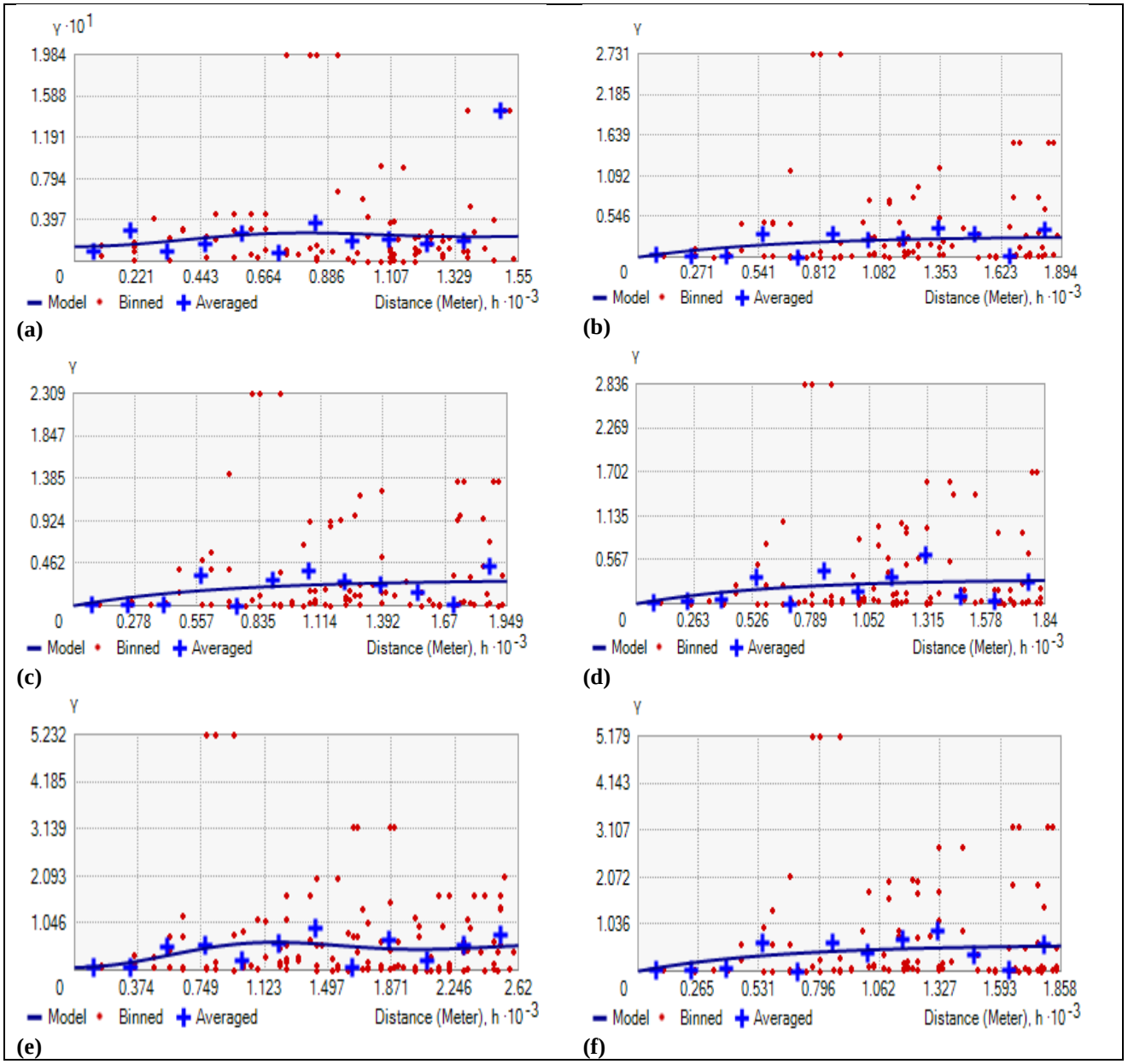

Figure 3- Best fitted semivariogram models; (a) pH, (b) electrical conductivity (EC), (c) total dissolved solids (TDS), (d) sodium adsorption ratio (SAR), (e) chloride (Cl), and (f) sodium (Na). Easting and northing stand for distance between sample pairs and semivariance value of the variable considered, respectively

3.2. Groundwater quality assesment for drinking water

3.2.1. Changes in pH

pH is an important indicator of water that is changing chemically. Not only does the pH of a stream affect organisms living in the water, but also a changing pH in a stream can be an indicator of increasing pollution or some other environmental factors. This is also true for groundwater. In this study, pH values of groundwater samples ranged from 7.03 to 8.00 as shown Figure 4a, indicating alkaline conditions in the groundwater system. Geology of the Bosaso plain corroborates alkaline character of the aquifer system. Additionally, pH data for the variables clearly revealed that the groundwater in Bosaso plain was within limits of pH range 6.5-8.5 as specified by WHO (2008). Based on WHO standards, it could be concluded that groundwater in the study site met the drinking water quality requirements with regard to pH.

3.2.2. Electrical conductivity

Electrical conductivity (EC) is a measure of water potential to deliver an electric current. The higher the salinity concentrations of water are, the higher EC is expected if measured. Pursuant thereto, EC values were found to be higher than 1.5 dS m^{-1} except for three samples, and groundwater was classified as saline water according to Langenegger (1990). In line with WHO

standards for drinking water, the maximum allowable limit of EC is 1.5 dS m^{-1}. Spatial distribution map of EC (Figure 4b) shows that EC is increasing from south-east inland to the northern coastline of the study area. According to WHO standards, most of the groundwater samples are above the permissible EC limit with the highest value of 16.51 dS m^{-1}. As seen in Figure 4b, EC of groundwater is remarkably increasing while approaching the shoreline. Substantial rises in EC values shorewards may be a clear indication of seawater intrusion into coastal aquifers of the study area. It could be concluded that areas stretching along the shore are potentially subject to severe salinization risk.

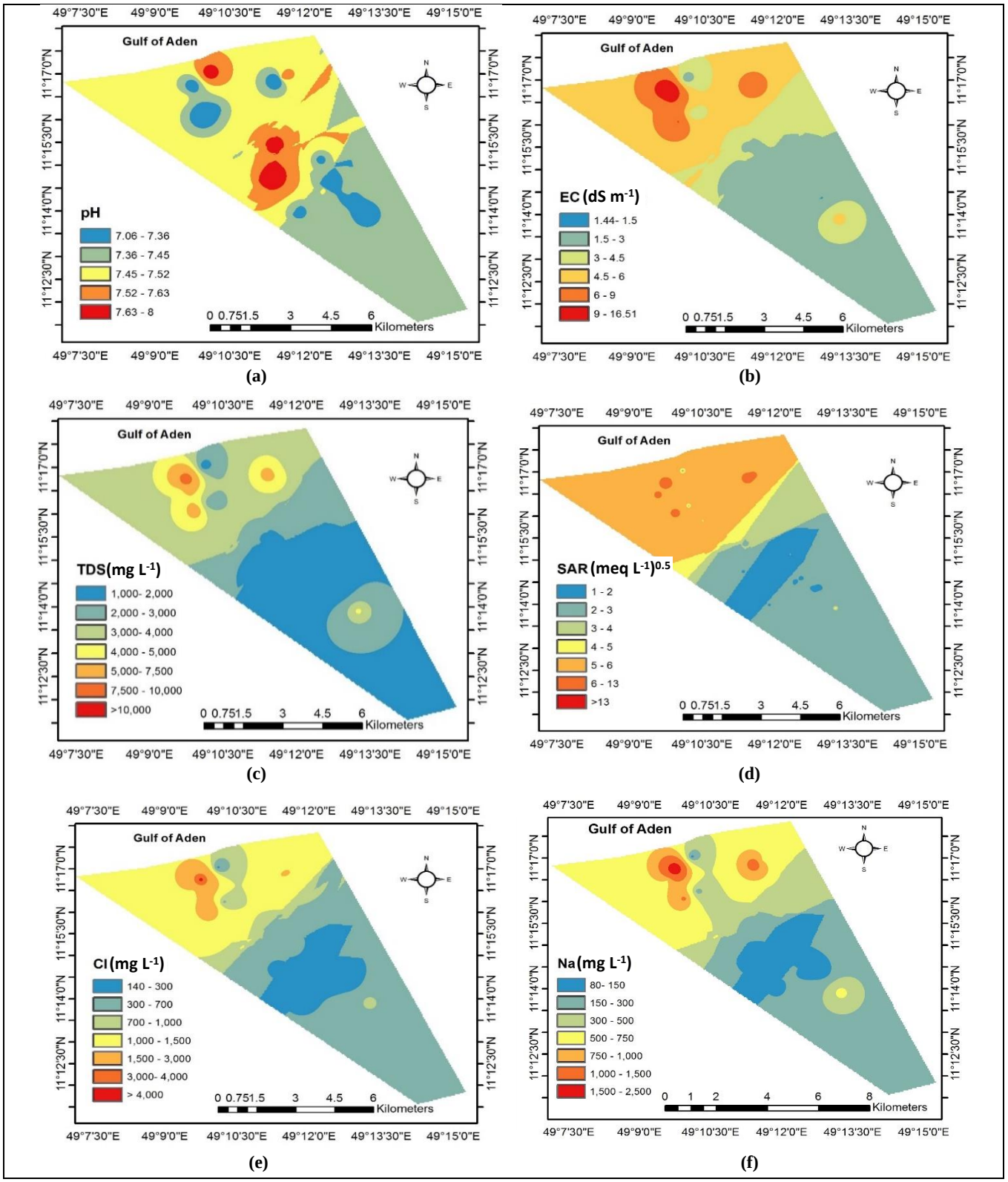

Figure 4- Spatial distribution map of analyzed groundwater parameters in Bosaso plain: (a) pH, (b) electrical conductivity (EC), (c) total dissolved solids (TDS), (d) sodium adsorption ratio (SAR), (e) chloride (Cl), and (f) sodium (Na)

3.2.3. Total dissolved solids

Total dissolved solids (TDS) contain inorganic salts such as potassium, magnesium, calcium, sodium, bicarbonates, sulfates and chlorides and some small quantities of organic matter. The values of TDS in groundwater in the study area varied from 1035.3 mg L^{-1} to 10,083.5 mg L^{-1}, revealing a very high and dominant salinization problem in some specific locations in the study area. As stated by WHO standards, the acceptable level limit of TDS for drinking water is 500 mg L^{-1}. In this respect, all TDS concentrations in the study area exceed the acceptable limit, indicating the salinization pressure. On the other hand, based on WHO standards, the maximum allowable amount of TDS is 1500 mg L^{-1}. TDS map in Figure 4c shows that total dissolved solids in the central part of the study area are within the maximum allowable amount. But in the north, TDS is above the maximum allowable amount. It is globally postulated that coastal aquifers, like Bosaso Plain, are under the risk of seawater intrusion, i.e. water quality deterioration. However, seawater intrusion was corroborated with the EC, SAR, TDS, Cl and Na distribution maps. It is important to emphasize that the relationship between EC and TDS is rather high as seen in Figure 4b and 4c. On the other hand, regression analysis revealed that the relationship was in the form of linear [*TDS (mg* L^{-1}*)= 659.72*EC(dS* m^{-1}*)*] and statistically significant (R^2*=0.972*). Our conclusion was that this type of mathematical relationship can be used confidently in practice for estimation of TDS from precise EC measurements in the field.

3.2.4. Chloride

The source of chloride in groundwater is both natural and anthropogenic, such as run-off containing road deicing salts, the use of inorganic fertilizers, landfill leachates, septic tank effluents, animal feeds, industrial effluents, irrigation and drainage waters, and seawater intrusion in coastal areas (Karanth 1987). Chloride concentration increases when mineral content increases (Sawyer et al. 2002). The chloride ion concentration in fresh waters is fairly low and typically less than 100 mg L^{-1}, except for brackish or saline waters (Fetter et al. 2017). Higher chloride concentrations in drinking water may cause cardiovascular disease and blood pressure (McCallum et al. 2015). Therefore, the chloride concentrations of groundwaters in the urban coastal aquifer of Bosaso plain needs determining due to the fact that the aquifer is a source of drinking water for the local community. Chloride concentrations of groundwater samples collected in this study varied from 149.74 to 4502.4 mg L^{-1} as seen in Table 1. The highest concentration of chloride is witnessed to be in the north along the coastline. Cl concentrations are higher than the maximum allowable limits by WHO standards (250 mg L^{-1}) as shown in Figure 4e. The closer to the sea is, the higher Cl concentration is in the groundwater body. The increasing trend in Cl concentration towards the sea may be an apparent indication of seawater intrusion to the coastal aquifer in use. Likewise, the spatial distribution of Na concentration supported the postulate of seawater intrusion in the study site.

3.2.5. Sodium

All groundwater samples contain sodium element to some extent because most rocks and soils naturally contain sodium compounds. The origin of sodium in groundwater may be from varied sources such as the erosion of salt deposits and sodium bearing rock minerals. As a matter of fact, over-exploitation of the coastal aquifer can lead a lateral movement of seawater into freshwater body (Hem 1985), causing to increase of sodium concentrations remarkably. High sodium contamination in drinking waters is quite risky, for high sodium in drinking water can cause higher systolic and diastolic blood pressure (Hallenback et al. 1981). In this study, the aquifer is contiguous to the sea, and the area is almost flat with a slope of less than 0.6%, on the average. Sodium concentration map in Figure 4f shows an increasing trend in the concentrations from the southeast to the north. Sodium concentrations in the northern parts of the study area exceed the maximum permissible limit of 250 mg L^{-1} indicated by WHO and groundwater with Na concentrations higher than 250 mg L^{-1} is not safe for drinking purposes. It is important to emphasize that the variability in sodium concentrations are extremely high (CV>130%), revealing the evidence of seawater intrusion into the aquifer. On the other hand, spatial distribution of EC, TDS, SAR, Cl and Na shows that interpolated values by the shore increase remarkably. This might be attributed to the effect of seawater intrusion, too. Therefore, groundwater abstraction strategy should consider the seawater intrusion issue and degradation in groundwater quality, which jeopardize the sustainable use of urban coastal groundwater aquifer in the future.

3.3. Groundwater quality assessment for irrigation water supply

Electrical conductivity (EC) and the sodium adsorption ration (SAR) of any source of water are among the most widely used irrigation water quality parameters (Appelo & Postma 2005). However, Ayers & Westcot (1994) points out that some specific ionic composition of water is of great importance regarding irrigation water quality standards. On the other hand, SAR indicates the effect of relative cation concentration on sodium hazard and its accumulation in the soil. SAR is a more reliable method (Richards 1954) for determining the suitability of water for irrigation purposes. SAR gives a very good assessment of water quality of irrigation uses with respect to sodium hazard, since it is more closely related to exchangeable sodium percentages (Tiwari & Manzoor 1988)**.** Hence, SAR is calculated using the following formula (Equation 2):

$$SAR = \frac{Na}{\sqrt{\frac{Ca+Mg}{2}}} \quad (2)$$

Where; concentrations are in the unit of meq L^{-1}.

Some descriptive statistics of SAR values were given in Table 1. As seen from Table 1, SAR values were characterized with a right-skewed distribution and rather high variability (CV=97%). Therefore, SAR values in the study area varied from 1.42 to 15.40 (meq L^{-1})$^{0.5}$ as shown in the Figure 4d. Nevertheless, SAR values of all samples were found to be less than 10 (meq L^{-1})$^{0.5}$ except two samples and were classified as excellent in terms of irrigation water quality standards according to Todd (1980) and Richards (1954). However, the permissible value of SAR in irrigation waters is 13 (meq L^{-1})$^{0.5}$. If the permissible level of SAR is considered, it is clear from the Figure 4d that some parts of the study area contiguous to the sea is under the risk of sodium hazard. Farmers leaving in those areas should pay attention to sodium hazard risk to the crops and soils as well. On the other hand, as seen clearly from Table 1, minimum, mean and maximum EC of groundwater is 1.44, 3.91 and 16.5 dS m^{-1}, respectively. The salinity of groundwater samples in the area was found to be beyond the usual rage, i.e. 0<EC<3 dS m^{-1} and 0<TDS<2000 mg L^{-1} (Ayers & Westcot 1994) in irrigation waters. Based on permissible salinity levels in terms of irrigation, we have enough evidence to conclude that majority of groundwater wells in the study area are rather risky to the agricultural crops based on the irrigation water quality criteria given in Ayers & Westcot (1994).

Richards (1954), Todd (1980) and many others, e.g. Appelo & Postma (2005), noted several advantages of joint evaluations of SAR and EC values on a graph, apart from individual assessments. And consequently, groundwaters in the study area were assessed according to the US Salinity Laboratory (USSL) classification (Richards 1954) by plotting groundwater salinity (EC) data against SAR values (Figure 5). Surprisingly, graphical results indicated that 68% of water samples fell within the C3S1 and C4S1 categories/classes -low risk of sodium hazard associated with high and very high salinity hazard, respectively-and only 9% fell into the C4S2 class–appreciable sodium hazard particularly in fine textured soils associated with very high salinity hazard, i.e. not suitable for irrigation under ordinary conditions, albeit used presently- as shown in Figure 5. This groundwater of poor quality is in turn suitable only for high salt-tolerant crops. Results further indicated that 23% of water samples, belonging to category C4S3 and C4S4, were generally not suitable for irrigation due to poor water quality associated with high salinity as well as high sodium hazard risk. Therefore, farmers in the area must restrict themselves to adopt modern irrigation methods such as drip irrigation and subsurface irrigation in the area in order to prevent crops and soils from salinization risk or salinity hazard as well as sodium injury for sustainable agriculture.

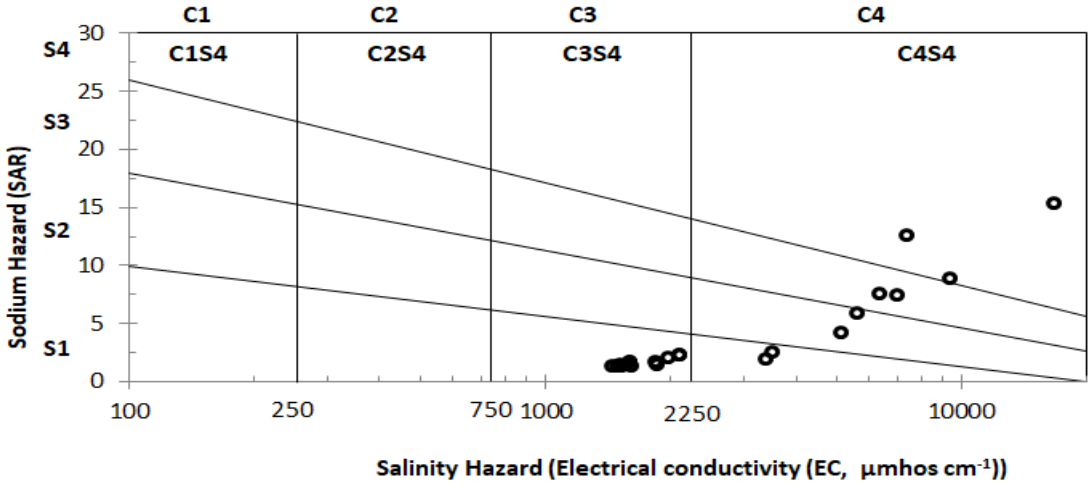

Figure 5- Classification of groundwater samples by US Salinity Laboratory diagram

4. Conclusions

Groundwater resources are essential for drinking water supply of Bosaso city. Ordinary kriging was utilized to map the spatial distribution of groundwater quality parameters. The study illustrated that the kriging method was a useful tool for delineating the spatial distribution of the groundwater quality parameters. According to the drinking water standards, results showed that the most of groundwaters in the study area was unsafe for drinking purposes, except in the central area (Biyo Kulule) where municipal water supply was located. As regards to irrigation uses, the very minor area may be under the risk of alkalinity or sodium hazard. However, half of the study area is under the salinization risk, not suitable for irrigation, except for high salt-tolerant crops/vegetation. Spatial distribution of groundwater quality maps reveals that quality decreases from the southeast to the north (coastline) of the plain. Research findings indicated that the Bosaso aquifer has been affected by seawater intrusion due to the over groundwater extraction. Therefore, we are recommending to be closed all wells located in the contaminated areas especially in the northwest (Balade) to avoid further groundwater degradation by seawater intrusion. Currently, the groundwater quality in the study area has not been monitored. Therefore, this study will assist the water authority for taking appropriate measures in order to maintain efficient groundwater exploitation and management plans in Bosaso plain. Our final

concrete conclusion is that further research is needed in this area to figure out the extent of seawater intrusion and develop seawater intrusion model. In turn, monitoring of groundwater depths and quality parameters has been highly recommended for the sake of reliable future studies.

Acknowledgements

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Reproductive Characteristics of Chios Ram Lambs During the First Year of Life in Rural Farm Conditions

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ABSTRACT
This study was conducted to determine some of the reproductive characteristics of young Chios rams. For the purposes of this study, the data was obtained and used from 30 Chios ram lambs born in 2009 between the ages of 90-360 days. The minimum and maximum values of the testicular diameter (cm) and length (cm), scrotal circumference (cm), scrotal length (cm), testicular volume (cm^3) of the Chios ram lambs were 1.59-7.30, 3.05-14.23, 12.00-37.50, 7.00-32.00, 17.39-771.44, respectively. Phenotypic correlations between all testis characteristics were found to be statistically significant ($P<0.01$). The average testosterone hormone in Chios ram lambs was observed to be 7.05 ± 0.31 ng x ml^{-1}. The ejaculate volume, sperm density, progressive motility ratio, immotility ratio, general averages of the ratio of dead spermatozoa and mass movement- consistency of sperm values were examined on sperm of ram lambs at 240, 270, 300 and 330 age of days. Mean values of these traits were obtained as 1.37 mL; 3.95 x 10^9 mL^{-1}, 82.20 %, 7.07 %, 10.73 % and 4.40-4.09, respectively. Moreover, progressive motility, the effect of live weight and age in other sperm characteristics was found statistically nonsignificant ($P>0.05$). The testicular diameter and volume, scrotal circumference and scrotal length enlarged related with positively the rate of the sperm motility. As a result, estimating the rate of live spermatozoa of the ram lambs by taking morphological measurements at an early age can be useful. Therefore, this information was vulnerable in indirect selection programs.

Keywords: *Chios; Ram Lamb; Testicular Characteristics; Testosterone Hormone; Sperm Characteristics*

1. Introduction

One of the most important indicators of successful livestock is reproductive performance. Good reproductive management of domestic animals requires detailed information about the onset of puberty and sexual maturity (Ake-Lopez et al. 2016; Al-Kawmani et al. 2018). This is also used as important criteria for the selection of ram lambs in a breed (Kridli & Al-Yacoub 2006; Hassanin et al. 2013). In addition, although most sheep breeds are season-dependent, it is known that puberty varies between one breed to another in rams (Hassanin et al. 2013). The capacity of reproductive yields for male animals can be predicted in short time by using some measurements and this information might be used to reveal for reproductive fertility of relative females by indirect selection (Odabaşıoğlu et al. 1992; Aygün & Karaca 1995; Taşkın & Kaymakçı 1996; Rege et al. 2000; Toe et al. 2000; Kaymakçı 2016). In the selection of superior rams in terms of fertility, the use of certain properties alone or in combination and the resulting effect levels are important (Kridli & Said 1999; Price et al. 2000). The efficiency of selection programs depends on these characteristics.

Chios sheep is one of the most important native and prolific breeds in Turkey. In this study, variables related to reproductive characteristics of Chios rams during the first year of life have been revealed in rural farm conditions. Also, investigation of the relationship between testis characteristics, serum testosterone level, sperm yield, semen quality and studied reproductive characteristics of Chios ram lambs were aimed. This research, moreover, can help the early and correct selection of Chios ram lamb and will provide important scientific information in the literature.

2. Material and Methods

2.1 Animal material

A total of 30 Chios ram lambs were used as an animal material for the study. Lambs were raised with their mothers until the

weaning period, and they were separated from their mothers after 3 months of age. The lambs were randomly selected from those with a maximum age difference of 15 days, 2-years old mother, and twin-born. They were raised using natural methods until weaning age. The animals were fed according to the existing feeding program. Data were obtained monthly from lambs between 90 - 360 days of age.

2.2 Examined characteristics

In order to determine the testis characteristics, the diameter and length were measured with an electronic caliper, testicular volume was calculated by 0.0396 x Mean Testicular Length x (Scrotal Circumference)^2, scrotal circumference and scrotal length were measured using a flexible tape (Kırk 2001; Yılmaz & Cengiz 2006; Kaymakçı 2016). Blood samples were collected monthly from the jugular vein of each ram in 10 mL heparinized vacutainer tubes, between the ages of 3 and 12 months at 8.30 - 10.00 am. Bloods were centrifuged at 5000 rpm for 10 minutes in a cooled centrifuge set at + 4 °C. Serum samples for each ram lamb were collected in 2 mL eppendorf tubes and stored in the freezer at -20 °C until hormone was determined (Tietz 1987). Testosterone hormone determination, using Testosterone Sheep Kit, was made by Radioimmunoassay (RIA) method (Delgadillo et al. 1991; Wilson 1999; Yılmaz 1999; Cusabio 2011). The semen characteristics of the animals were determined with taking the average of monthly measurements by artificial vagina. The amount of sperm (mL), consistency of sperm (0-5) and sperm mass movement (0-5) were examined (Tekin 1990; Taşkın 1995). A chemical preparation was used as a diluent (Minitube 2013). Sperm vision has proven to be reliable in the field and has been developed for the purpose of determining the spermatological properties with practical and recent technology. Sperm vision can be analyzed with a computer-connected microscope, was used to calculate total live, progressive motility and immotility ratios (%) (Tsakmakidis 2010; Minitube 2013). Sperm density (109 mL^{-1}) was measured with the photometer.

2.3 Analysis of data

In the study, normality test was applied to the testosterone hormone, testis and sperm characteristics. As a result of this test, it was determined that the data of testosterone hormone was not valid to normal distribution assumptions and square root transformation was applied. The effect of age on testosterone hormone, testis and semen properties was determined by covariance analysis (Model 1) (Düzgüneş et al. 1987; İkiz et al. 1996). The least square means of age effect for all levels were estimated and difference of the means were compared by Duncan multiple comparison test (Düzgüneş et al. 1987). In addition, the correlations between testosterone hormone, testis and sperm characteristics were investigated. Phenotypic correlation coefficients and their significance levels were discussed. However, the tendency of testosterone hormone, testis and sperm characteristics according to age and live weights were modelled by regression analysis (Düzgüneş et al. 1987; İkiz et al. 1996) (Model 2). Ordinal logistic regression analysis was performed for semen movement and sperm consistency (İkiz et al. 1996). All statistics were performed by using JMP 5.0.1.2 and IBM SPSS 25v statistical package programs (JMP 2003; IBM SPSS 2017).

$$Y_{ij} = \mu + a_i + b_1 (X_{ij} - \overline{X}) + e_{ij} \quad (1)$$

Where the parameters denote; Y_{ij}: Characteristics of testis, sperm or testosterone hormone level, μ: Population mean, a_i: i. age effect, b_1: The regression coefficient related with live weight, X_{ij}: The covariate at i. age and j. ram lamb, $\overline{x}$: Mean live weight of ram lamb and e_{ij}: Random error term, respectively.

$$Y_{ijk} = b_0 + b_i X_i + b_j X_j + e_{ijk} \quad (2)$$

Where the parameters denote; Y_{ijk}: Characteristics of testis or sperm, b_0: The constant term of Y axis, b_i: The regression coefficient for live weight of ram lamb, b_j: The regression coefficient for age, X_i: Live weight of ram lamb, X_j: The age effect and e_{ijk}: Random error term, respectively.

3. Results and Discussion

The least square means of the testis characteristics of Chios ram lambs are given in Table 1. It was observed that testicular diameter and length increased continuously in the 90-360 days of age groups. The age effect on testicular diameter, length and volume were found statistically significant (P<0.01), however the age effect was not varied after the age of 210 days. Moore & Sanford (1987) also found similar results in their study of Suffolk and ½ Dorset x ¼ Leicester x ¼ Suffolk crossbreed rams. Scrotal length changed significantly at 90, 210 and 360 days; it steadily increased until August and then rapidly declined. It was thought that this difference appeared with live weight and age and then was affected by temperature and season in other periods. Kaymakçı et al. (1988) found that the impact of age on the scrotal circumference was non-significant (P>0.05), although Moraes et al. (1992), Odabaşıoğlu et al. (1992), Aygün & Karaca (1995) reported that the effect of live weight was significant (P<0.05). Age and live weight had significant effect on all testis characteristics (P<0.01). Some researchers have also reported similar findings (Nowakowski & Cwikla 1994; Aygün & Karaca 1995; Taşkın & Kaymakçı 1996; Salhaba et al. 2001; Özdemir & Altın 2002; Yılmaz & Aygün 2002; İnce & Karaca 2009).

Table 1- Least square means and standard deviations of testis characteristics in Chios ram lambs and regression analysis results

		TD, cm	*TL, cm*	*SC, cm*	*SL, cm*	*TV, cm³*
Effect	n	$\overline{x} \pm S_{\overline{x}}$	$\overline{x} \pm S_{\overline{x}}$	$\overline{x} \pm S_{\overline{x}}$	$\overline{x} \pm S_{\overline{x}}$	$\overline{x} \pm S_{\overline{x}}$
Overall Mean	298	4.94±0.09	9.73±0.16	28.88±0.41	21.34±0.35	377.01±11.96
Age (Day)						
90	30	3.48±0.17^{d}	6.51±0,25^{e}	21.35±0.74^{f}	15.89±0.59^{e}	246.05±21.03^{d}
120	30	3.90±0.13^{c}	7.80±0.21^{d}	25.04±0.65^{e}	18.18±0.51^{cd}	268.78±18.24^{d}
150	30	4.24±0.09^{c}	8.78±0.17^{c}	27.95±0.49^{d}	18.07±0.39^{d}	298.65±13.89^{cd}
180	30	4.67±0.09^{b}	9.68±0.15^{b}	29.82±0.45^{cd}	19.87±0.35^{c}	349.79±12.64^{bc}
210	30	5.22±0.08^{a}	10.39±0.14^{a}	31.83±0.43^{ab}	24.33±0.33^{ab}	419.55±12.04^{a}
240	30	5.47±0.08^{a}	10.67±0.15^{a}	32.28±0.43^{a}	25.17±0.34^{a}	452.13±12.22^{a}
270	30	5.56±0.09^{a}	10.74±0.16^{a}	31.64±0.47^{abc}	25.04±0.37^{a}	450.44±13.23^{a}
300	30	5.59±0.10^{a}	10.79±0.18^{a}	30.89±0.52^{abc}	23.82±0.41^{ab}	445.06±14.77^{a}
330	29	5.64±0.12^{a}	10.96±0.20^{a}	29.83±0.60^{bcd}	22.43±0.47^{b}	436.00±16.99^{a}
360	29	5.64±0.13^{a}	11.03±0.24^{a}	28.19±0.70^{de}	20.61±0.55^{c}	406.55±19.21^{ab}
Regression	(linear)					
Live Weight		0.04±0.001**	0.08±0.007**	0.24±0.03**	0.19±0.02**	7.82±0.65**

**a,b,c,d,e,f; Mean with different letters in the same column indicate significant differences (P<0.01), TD: Testicular Diameter, TL: Testicular Length, SC: Scrotal Circumference, SL: Scrotal Length, TV: Testicular Volume

The regression equations related to testis characteristics of Chios ram lambs are shown in Table 2. Since the age and live weight in ram lambs led to high variability in testis characteristics, all testis characteristics in the study were affected by age and live weight. This finding was consistent with that reported by Salhaba et al. (2001) and Al-Kawmani et al. (2018). Testicular size may be useful as a selection criterion to increase the reproductive capacity of male lambs (Toe et al. 2000; Hassanin et al. 2013; Ake-Lopez et al. 2016; Al-Kawmani et al. 2018). When the change of testosterone hormone level of Chios ram lambs was examined by age, it was observed that testosterone levels started to decrease in spring but raised back to the initial levels until the end of summer and decreased again at the end of August. However, the effect of age and live weight on testosterone hormone was not statistically significant (P>0.05) therefore any the table information was not given. The total secretion of testosterone was determined as 7.05 ± 0.31 ng x mL^{-1} during the all months. This value was lower than values reported in Ile de France, Herdwick, Norfolk, Shetland and Wildshire breeds (Gonzalez et al. 1988; Lincoln et al. 1990), and higher than Canadian, Outaouais, Rideau and Finnish Landrace, İvesi, Muflon and Soay x Merino, Akkaraman, Konya Merino, Iran Moghani breeds (Lincoln et al. 1990; Langford et al. 1998; Gündoğan 1999; Kaya et al. 1999; Kırk 2001; Zamiria et al. 2010). Nevertheless, this value agreed with the reports of Lincoln et al. (1990) on Blackface and Portland, Ismaeel (2018) on Nuaimie and Aguirre et al. (2007) on Pelibuey rams. In this study, the increase in the amount of testosterone with the sexual maturity (Al-Kawmani et al. 2018), but the decrease for the amount of testosterone at the end of mating season and by cooling weather was found to be consistent with Courot & Ortavant (1981) and Gündoğan & Demirci (2003).

Table 2- Regression equations related with testis characteristics on Chios ram lambs

Parameter	*Regression Equations*	$S_{\overline{x}}$	*F*	R^2
Testicular diameter	Y=1.003+0.007 age+0.050 LW	0.09	<0.001	0.88*
Testicular length	Y=2.586+0.012 age+0.098 LW	0.17	<0.001	0.88*
Scrotal circumference	Y=11.802+0.009 age+0.327 LW	0.59	<0.001	0.76*
Scrotal length	Y=6.737+0.018 age+0.231 LW	0.51	<0.001	0.76*
Testicular volume	Y=-155.423+0.615 age+8.573 LW	13.14	<0.001	0.86*

*P<0.05, LW: Live Weight

The least square means for the sperm characteristics of Chios ram lambs are given on Table 3. The volume of ejaculate measured in Chios ram lambs was consistent with Iranian Moghani, Corriedale, Akkaraman and Çine Çaparı breeds (Gülyüz & Yıldız 1995; İnce & Karaca 2009; Zamiria et al. 2010). However, it was lower than the values reported for Karya (İnce & Karaca 2009), Santa Inês (Souza et al. 2010), and Chios and Friesian breeds (Karagiannidis et al. 2000). The sperm density was similar to rams of Pelibuey, Norduz, Karayaka, Konya Merino, Dağlıç, Akkaraman breeds (Gündoğan et al. 1997; Kaya et al. 1999; Yılmaz & Cengiz 2006; Aguirre et al. 2007; Gündoğan 2007; Kulaksız et al. 2010). In terms of sperm motility ratio for German Black Head, Awl, Iranian Moghani, Hamdane, Karayaka, Akkaraman breeds (Soylu et al. 1991; Karaca et al. 1998; Gündoğan et al. 2002; Sönmez & Demirci 2003; Kulaksız et al. 2010; Zamiria et al. 2010) and the immotility ratio (Aksoy et al. 1994; Karakuş & Cengiz 2007) was consistent with domestic, local and culture breeds such as Akkaraman and Karakaş breeds. The ratio of dead spermatozoa in Chios ram lambs were lower than the levels reported for Çine Çaparı (İnce & Karaca 2009), Karayaka (Kulaksız et al. 2010), and Karya and Chios breeds (Yılmaz & Karaca 2004); and higher than Karakaş, Norduz and Hamdane breeds (Karaca et al. 1998; Karakuş & Cengiz 2007). The ejaculate volume, sperm density, and sperm motility values obtained in this study were higher than those of prolific breeds such as Chios, Barki, Dorset Down, German Black Head, Border

Leicester, Rambouillet and Romanov (Soylu et al. 1991; Gülyüz & Yıldız, 1995; Martin et al. 1999; Taha et al. 2000; Aguirre et al. 2007; Gündoğan 2007).

Table 3- Least square means and standard deviations of some sperm characteristics in Chios ram lambs and regression analysis results

Traits		*EV, mL*	*SD x 10^9 mL^{-1}*	*PMR, %*	*IMR, %*	*DSO, %*
Effect	n	$\bar{x} \pm S_{\bar{x}}$	$\bar{x} \pm S_{\bar{x}}$	$\bar{x} \pm S_{\bar{x}}$	$\bar{x} \pm S_{\bar{x}}$	$\bar{x} \pm S_{\bar{x}}$
Overall Mean	35	1.37±0.05	3.95±0.18	82.20±0.99	7.07±0.58	10.73±0.55
Age (Day)						
240	3	1.44±0.23	4.89±0.71	81.32±3.75	9.38±2.14	9.29±2.23
270	7	1.21±0.13	3.95±0.41	82.81±2.13	6.30±1.22	10.86±1.27
300	12	1.47±0.09	4.13±0.29	84.80±150	5.31±0.86	9.88±0.89
330	13	1.36±0.11	3.56±0.34	79.68±1.77	8.57±1.01	11.77±1.05
Regression	(linear)					
Live Weight		-0.01±0.01	-0.03±0.04	0.51±0.22*	-0.23±0.12	-0.28±0.13*

*P<0.05, EV: Ejaculate Volume, SD: Sperm Density, PMR: Progressive Motility Ratio, IMR: Immotility Ratio, DSO: Dead Spermatozoa Ratio

While the effect of age on spermatological characteristics was not significant, the effect of live weight on the ratio of motile spermatozoa and dead spermatozoa ratio was significant (P<0.05). Salhaba et al. (2003) obtained that age did not have a significant effect on spermatozoa viability between 11-20 months of age. According to Gündoğan et al. (2003b), the change of sperm density with age was not significant, but Souza et al. (2010) found that it was important. Several studies on rams emphasized that the change in ejaculate volume with age was significant (P<0.05) (Rege et al. 2000; Gündoğan et al. 2003a; Salhaba et al. 2003). Öztürkler et al. (1997) found that photoperiodic effect greatly changed the sperm characteristics, Maxwell (1986) and Yılmaz & Karaca (2004) also indicated that the quality of sperm was better during the mating season. As expected, there was an increase in the ratio of motile spermatozoa with age, and a decrease in abnormal spermatozoa rates when sperm was taken on the 240th and 300th days. Similar results have been observed in other studies (Alexopoulos et al. 1991; Taşkın 1995; Kırk 2001; Gündoğan et al. 2003a, Souza et al. 2010). However, these increases and decreases in the rate of spermatozoa were not statistically significant in these studies. Salhaba et al. (2003) also reported that the live sperm ratio was not affected by age and season. Nevertheless, Dufour et al. (1984) found that the highest rate of live spermatozoa was in October to November, and İnce & Karaca (2009) found the highest rate of spermatozoa in the autumn and the lowest in the summer. These results were consistent with the findings in this study. It was determined that the sperm of Chios lambs had the same mass movement and cream color on the analysis days (Table 4).

Table 4- Descriptive statistics of sperm mass movement and consistency of sperm in Chios ram lambs

Traits		*Mass Movement*	*Consistency of Sperm*
Effect	n	$\bar{x} \pm S_{\bar{x}}$	$\bar{x} \pm S_{\bar{x}}$
Overall Mean	35	4.40±0.07	4.09±0.08
Age (Day)			
240	3	4.17±0.44	3.50±0.50
270	7	4.36±1.57	4.07±0.06
300	12	4.53±0.88	4.29±0.13
330	13	4.36±0.09	4.06±0.13

For Chios ram lambs, the live weight and age were found to be nonsignificant (P>0.05) in predicting sperm characteristics (Table 5), but it had a significant effect on the ratio of live spermatozoa in one direction (P<0.05). Apart from age and live weight, other environmental and some genetic factors are thought to play a role on these characteristics.

Table 5- Regression equations related with sperm characteristics in Chios ram lambs

Parameter	Regression Equations	$S_{\bar{x}}$	F	R^2
Ejaculate volume	Y=1.805+0.001 age-0.011 LW	0.62	0.65	0,03
Sperm density	Y=9.532-0.010 age-0.039 LW	1.86	0.06	0,12
Progressive motility ratio, %	Y=59.637-0.032 age+0.504 LW	10.30	0.04	0.19*
Immotility ratio, %	Y=18.369+0.013 age-0.237 LW	6.24	0.13	0.13
Dead spermatozoa ratio, %	Y=21.849+0.020 age-0.268 LW	5.80	0.11	0.11

*P<0.05, LW: Live Weight

Phenotypic correlations between reproductive traits in Chios ram lambs are given in Table 6. The correlations between all testis characteristics were found to be significant (P<0.01). This result is consistent with some studies (Kaymakçı et al. 1988; Odabaşıoğlu et al. 1992; Taşkın 1995; Kaya et al. 1999; Karagiannidis et al. 2000; Rege et al. 2000; Yılmaz & Aygün 2002; Gündoğan et al. 2003b; Kulaksız et al. 2010). The ejaculate volume had significant correlations with mass movement (P<0.05), sperm density (P<0.05), sperm consistency (P<0.01), and progressive motility ratio (P<0.01). The obtained result is similar with some studies (Kaymakçı et al. 1988; Odabaşıoğlu et al. 1992; Taşkın 1995; Kaya et al. 1999; Karagiannidis et al. 2000; Rege et al. 2000; Gündoğan et al. 2003b; Yılmaz & Karaca 2004; Yılmaz 2006; Kulaksız et al. 2010). Whilst there was a positive correlation between testicular diameter, ejaculate volume and progressive motility ratio in mass motility; the relationships between the testicular diameter and scrotal circumference with the dead spermatozoa ratio, and between scrotal circumference and testicular volume with immotility ratio had negative significant (P<0.01). Toe et al. (1994) and Al-Kawmani et al. (2018) stated that sperm quality would increase as testis develops. The increase or decrease in the testosterone hormone levels of Chios ram lambs was statistically insignificant. Some researchers mentioned the importance of the features related to the testosterone hormone (Langford et al. 1990; Lincoln et al. 1990; Fernandez et al. 1993; Taşkın 1995; Gündoğan 1999; Aygün & Karaca 2000; Rege et al. 2000; Gündoğan et al. 2003b), yet others have emphasized that testosterone levels alone were not sufficient in determining the libidos of the rams and could be affected by environmental factors (Dufour et al. 1984). In this study, the positive correlation was determined between the live weight and age of the Chios ram lambs; and there was also a significant positive correlation between testicular diameter, testicular length, testicular volume, scrotal circumference and length with live weight and age (P<0.05; P<0.01). The live weight and age had significant and negative correlations only with sperm density from sperm characteristics.

Table 6- Correlations between reproduction traits in Chios ram lambs

	TL	*SC*	*SL*	*TV*	*EV*	*SD*	*PMR*	*IMR*	*DSO*	*MM*	*CS*	*T*	*LW*	*A*
TD	0.9525**	0.9310**	0.9067**	0.9583**	0.3195	-0.1114	0.3669**	-0.1570	-0.4900**	0.3209**	0.3860*	-0.2723	0.926**	0.916**
TL		0.9339**	0.8899**	0.9586**	-0.0851	-0.3425	-0.0280	-0.0819	0.1385	-0.2810	-0.1554	-0.2780	0.924**	0.908**
SC			0.9117**	0.9583**	0.2763	0.1764	0.4255**	-0.3572**	-0.3857**	0.1805	0.4993**	0.0786	0.872**	0.827**
SL				0.9120**	0.1150	-0.0456	0.4745**	-0.4599**	-0.3673	0.3078	0.3358	0.0720	0.866*	0.840**
TV					0.1650	-0.0539	0.3170**	-0.3202**	-0.2294	-0.0129	0.3094**	-0.0985	0.925**	0.895**
EV						0.3797*	0.2199**	-0.1451	-0.2649	0.3084*	0.4862**	0.0394	-0.1894	-0.1130
SD							0.1299	-0.0966	-0.1454	0.0504	0.3098	-0.0882	-0.4906**	-0.5331**
PMR								-0.9290**	-0.8643**	0.7264**	0.6293**	0.0434	0.2990	0.0792
IMR									0.7117**	-0.4740**	-0.4924**	-0.0729	-0.291	-0.0278
DSO										-0.8038**	-0.6078**	-0.0026	-0.3187	-0.1128
MM											0.6559**	-0.0223	0.2627	0.0770
CS												0.0634	0.1988	0.1691
T													-0.2304	-0.3406
LW														0.932**

TD; Testicular Diameter TL; Testicular Length, SC; Scrotal Circumference, SL; Scrotal Length, TV; Testicular Volume, EV: Ejaculate Volume, SD; Sperm Density, PMR; Progressive Motility Ratio, IMR; Immotility Ratio, DSO; Dead Spermatozoa Ratio, MM; Mass Movement, CS; Consistency of Sperm, T; Testosterone, LW; Live Weight, A; Age *P<0.05, **P<0.01

4. Conclusions

In this study, testis, sperm characteristics and testosterone hormone levels of Chios ram lambs were determined from 3rd month to 12th months of age. The effect of live weight and age on these reproductive traits was examined. In spite of the changes in the live weight and age, the changes in these properties are given by regression equations. It is known that testis characteristics of rams differ with breeds and the growth rates of some testis characteristics are different from each other. According to the findings of this study, all testis characteristics can be defined for a specific age and live weight can be determined from the regression equations based on the significant effect of age and live weight. As the testicular diameter and volume, scrotal circumference and length enlarged, the rate of the sperm motility increased. This result can be used to estimate the rate of live spermatozoa of the ram lambs by taking morphological measurements at an early age.

In conclusions, because of the higher inheritance level of the testis characteristics they can be used as indirect selection criterion. As a result, the degree of success in the selection programs for ram lambs will be increase. This study was the first to examine the relationships among the reproductive characteristics of the Chios sheep breed in Turkey. It will contribute to the future investigations on reproductive studies and these results must be incorporated with genomic studies by several genes related by twins and reproduction.

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Historic Landscape Characterization in Protected Areas; A Case Study Kazdagı National Park

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ABSTRACT
Landscapes, whose characters are the result of the action and interaction of natural and/or human factors, are dynamic. Proper understanding of today's landscapes is only possible through the knowledge of their historical dimension, in other words, changes that have occurred in time-slices or time-depth. Historic Landscape Characterization (HLC) is an approach to the management and understanding the present historic environment as a product of past changes and as the basis for future change.

HLC was applied to Kazdağı National Park which has very important ecosystems in terms of the future of Turkey especially Asia and Europe. In this research, Kazdağı National Park Historic Landscape Character Types were mapped and relevant attributes were assigned them in 127 years of time depth with ArcGIS 10.1 software. HLC which was completed on the computer has been confirmed by field study and 'Direction of change', 'Rarity', 'Vulnerability' analysis was carried out. Finally, the relevant assessments were made in order to contribute protection, management and planning of National Park.

Keywords: *Historic landscape characterization; Time depth; vulnerability; Rarity; direction of change; Kazdağı national park*

1. Introduction

The concept of landscape first was defined as 'all the characteristics of a piece of land' about 200 years ago by the German geographer Alexander von Humboldt (1769-1859) (Antrop 2013).

In the case of the general provisions section of the European Landscape Convention, which opened for signature on 20.10.2000 and entered into force on 01.03.2004, the definition of *landscape* is '..... an area, as perceived by people, whose character is the result of the action and interaction of natural and/or human factors' (Council of Europe 2000). This definition has four aspects: (1) physical (an area), (2) holistic, (3) temporal (the result of the action and interaction of natural and/or human factors, developed through time), and (4) subjective-cultural (peoples' perceptions of the landscape) (Franch- Pardo et al. 2017).

Briefly, Landscape reflects the relationship between people and place. It is a product of the interaction of the natural, cultural, perceptual and aesthetic components of our environment (Tudor 2014). The changes that occur in this process have continuous and reached the dimensions that can cause the rapid loss of the characteristics of cultural landscapes with their different results (Antrop 2005; Tudor 2014).

According to European Landscape Convention, each member country is responsible 'to identify its own landscapes throughout its territory', 'to analyze their characteristics and the forces, pressures transforming them', and 'to take note of changes' (Council of Europe 2000). Landscape protection, management and planning as the three main tools are including in the European Landscape Convention, and in this context, various approaches have been developed to define the landscapes that is the basis of these processes.

Landscape Character Assessment (LCA) is one of these methods and an assessment technique originally developed in the 1990s in the UK to identify the combination of biophysical and socio-cultural elements that define a given landscape

and distinguish it from others (Swanwick 2002; Tudor 2014; Franch-Pardo et al. 2017). Landscape character assessment is also a tool to help understand what the landscape is like today, how it has come to be like that, and how it may change in the future. In this context, The Landscape Character Analysis method outlines the necessity of knowledge of past landscapes in terms of shaping future landscapes in the light of understanding past landscape/historic landscape (Fairclough 2010).

The concept of historic landscape first was used by archaeologists who suggested the historic depths of modern landscapes to the planners and managers at the beginning of the 90s (Rippon & Turner 1993; Rippon 2012).

In this context, a proper understanding of today's landscapes is only possible through the knowledge of their historic dimension and changes that have occurred in time-slices or time-depth. In other words, it is possible with the identifying and perception of historic landscapes (Sengur 2018). There are many techniques such as *Landscape biography*, *Historic ecology*, and *Historic geography* evaluating the perception of the landscape on the basis of landscape character in the scope of changes that occurred in a particular or long time. Also, Historic Landscape Character Analysis/Characterization (HLC) method approach which allows the comparison of today's landscape that we perceive with the values of the past with providing historic dimension to Landscape character analysis was developed by the Institute of English Heritage with the new name Institute of Historic England (Swanwick 2002; Aldred & Fairclough 2003; Fairclough & Macinnes 2003; Clark et al. 2004; Austin et al. 2007; Herring 2009). Program with the same purpose was named as Historic Land-use Assessment-HLA by the Institute of Historic Scotland in Scotland (Dixon et al. 1999; Fairclough & Maccines 2003).

Historic Landscape Characterisation is a Geographic Information Systems (GIS) based approach defining visible historic landscape character that occurred in a time depth in the modern landscape, and a detailed analysis with the current status of the landscape can be performed. First in 1994, it was implemented as a 'Cornwall Historic Landscape Characterisation Project' in the Cornwall region by the Institute of Historic England with the support of Cornwall Council and the Countryside Commission. With this Project, the main principles of the method have been identified (Aldred & Fairclough 2003; Winterburn 2008; Rippon 2012).

The development of the method has gained momentum with the implementation of similar areas as a region in the United Kingdom, Implementation of Historic Landscape Characterisation as a Programme was supported by the Institute of Historic England and local authorities. Several projects, especially for protected areas, have been developed, usually covering whole counties and occasionally extending beyond county boundaries (Aldred & Fairclough 2003; Herlin & Fairclough 2013). Historic Landscape Characterisation projects were applied to over 95% of United Kingdom.

International projects that were performed by academics and experts who are working on this topic in the United Kingdom and the Institute of Historic England have been effective in implementing and recognizing the historic landscape characterisation method in other countries. By defining historic landscape character types and areas in Silivri and its environment (Thrace region, Turkey), which is located to the east of the Mediterranean and The Island of Naxos (Aegean Sea, Greece), changes in rural landscape have been assessed in approximately 1500 years, including medieval and modern periods in two different areas with the research Project "Unlocking Historic Landscapes in the Eastern Mediterranean" within the scope of 'Landscape and Environment Programme', which was supported by the United Kingdom Art and Humanities Research Council in 2006-2007 (Crow & Turner 2009; Crow & Turner 2010; Crow et al. 2011).

In this research, HLC method approach which has been in accepted in general context and has application examples were implemented in Kazdağı (Ida) National park which is one of the protected areas in Turkey with its natural, cultural, archeological and historical values. The method was conducted in a time depth of 127 years, covering 1890-1920, 1940-1970 and 2000-Present periods. The application of the HLC method, which is practiced in European countries, especially in the UK, to the protected area scale for the first time in Turkey is the original value of this research. In addition, the research is also important according to be one of the first works carried out using the HLC method.

2. Material and Methods

The research material is all the necessary materials related to the research method and the study area. We conducted the HLC method in Kazdağı (Ida) National Park which is located between 39º41'25.00 "north latitude and 26º55'34.55 " east longitude in Balıkesir province, Edremit county (Figure 1). Kazdağı National Park has been selected as a research area with respect to its natural, cultural, historical and archaeological features and its protected area status.

Spatial and non-spatial data were used in the determination process of the Historic Landscape Characterization of the area. The other tools and equipment were also used to creation and evaluation of these data. The HLC method and other approaches in this context evaluate landscape from a long time to the present. However, the lack of a spatial database (historic maps or aerial photos) in our country makes the time depth more limited. So, the periodically varying materials were used in three different time periods with a time depth of 127 years.

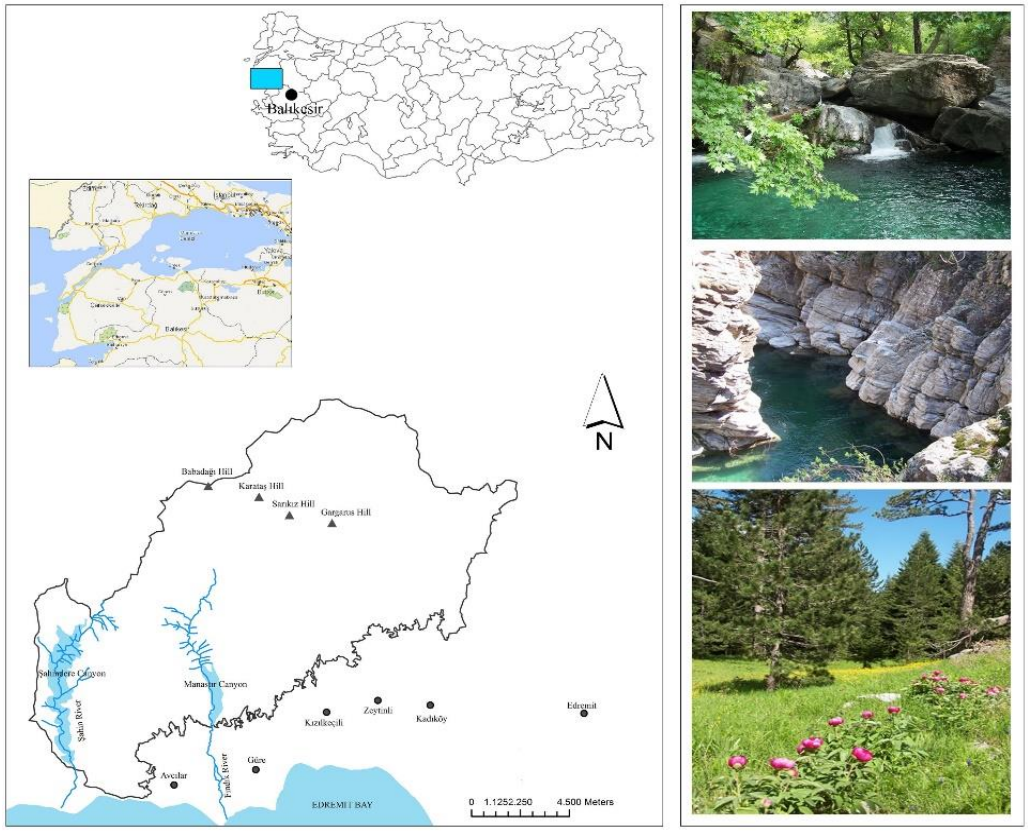

Figure 1- Location of Study Area

For the first period: Historic maps covering research area from 1890 to 1920 have been used as the main source. Among the maps of this period are H. Kiepert (1890) (1/250 000), V. Cuinet (1891) (1/500 000), R. Kiepert (1911) (1/400 000) and the Ottoman map (1919) (1/200 000).

For the second period: Topographical map (Edremit sheet) (1950) (1/200 000), Edremit-Çanakkale map (1944) (1/200 000) and the morphological map of Biga Peninsula Southwest Section (1/500 000) (1960) (Bilgin 1969) which cover the research area have been used as the main source. Also, (1953) (1/40 000) aerial photos with the roll number R 23 (108-109-110-111-112-113-114), R-26 (116-117-118-119-120) and R-83 (188-189-190-191-192-193-194) have been investigated stereoscopically.

For the third period: For the 3rd period which is covering the years from the 2000s to today, 1/25 000 scale topographic maps with sheet number I17d2, I17d3, I17c1, I17c2, I17c3, I17c4 have been used as the main source. Also, ArcGIS Basemap 2016 has been used as the current satellite image.

In addition to, Kazdağı National Park Management Plan and 1/50 000 scale Kazdağı National Park Natural and Cultural Values Map, 1/50 000 scale Tree Species Map and 1/50 000 Tree Age Class Map which produced under this plan are other sources benefited for three periods.

One of the Geographic Information System software ArcGIS 10.1 has been used in the process of creation and analysis of data sets, determination of historic landscape character types and analysis of the direction of change, vulnerability and rarity.

The 'Historic Landscape Characterization' process, which is carried out for the purpose and objectives of the research, consists of four phases. These steps are; Determination of historic landscape character types (Preparation of data set, Analysis of data set, Creation of polygons of historic landscape character types, Morphological analysis and assignment of historic landscape character types), Verification of historic landscape character types by field study, The analysis of direction of change, vulnerability and rarity and Overall evaluation.

In the process of determining the historic landscape characters, firstly the preparation and analysis of the data set have been carried out. During the preparation of data set, all the historic and modern maps periodically used in the research were geographically corrected with 0.00001 error rate using ArcGIS 10.1 software in a UTM (Universal Transverse Mercator) projection system and used as a baseline map during the creation of data layers.

The most important stage of HLC is the identification of polygons as a historic landscape character type which reflects their common features from the past to today. There are basic historic landscape character types that are used commonly (Enclosed land, Unenclosed land, Woodland, Industrial land, Military, Ornamental and recreational areas, Settlements, Orchards, Communication, Water and valley floor) (Clark et al. 2003). Historic landscape character types are varied depending on the scope of work, situation of landscape and the aim of the analysis. So, we have determined the polygons as a common historic landscape character type as an Enclosed land (Open spaces with little/no vegetation, Woodland, Maquies, Agricultural areas) and Water bodies (River and canyon) in the Kazdağı National Park. In the definition process of polygons; each polygon must reflect the same historic landscape character type, pattern (regular or irregular) and dominant boundary morphology (straight, curved, variable). According to this, we have identified attributes that comprise the historic landscape character types and we have assigned these attributes to the polygons specified on the map as common historic landscape character types with ArcGIS 10.1. These attributes for Kazdağı National Park are Period, Area, Place name, Confidence, Landscape pattern, Boundary morphology, Endemic plant assets, Native plant assets, important plant assets, Monumental tree assets, Cultural Heritage assets and Archaeological Heritage assets. Thus, historic landscape character types have been identified for three periods and mapping studies have been completed.

Historic landscape character types obtained for the three-time period were confirmed by the field studies carried out with landscape architects, archaeologists and botanical experts in this process. In addition, evaluations of physical geography experts and knowledge of local people were also utilized during the study period.

The next stage of the study is analyses of the direction of change, rarity and vulnerability phase. Firstly, the direction of change analysis has been carried out in order to reveal how the landscape has developed and where or when the great threats and pressures occurred on the landscape. The evaluation of historic landscape character types at a specific time depth is an effective method to understand the continuity and stability of these character types. The analysis was carried out to measure the change in the process from the first period (1890-1920) to present day was carried out to give an indication of those HLC types which have decreased most rapidly, those which are stable and those which are increasing or new in the third period (present day). The direction of the change map obtained by comparing the 1st-period historic landscape character types map and 3rd-period historic landscape character types map. The difference in area was converted to a percentage and the scale of gain and loss defined in the following ranges: decreasing critically (>50% loss), decreasing rapidly (21-49% loss), decreasing slowly (1-20% loss), stable, increasing slowly (1-20% gain), increasing rapidly (21-69% gain), increasing significantly (>70% gain), and new (Williams 2008).

In the second stage, rarity analysis has been conducted to determine whether historic landscape character types were common, frequent or rare. Rarity map obtained by calculating the total area of each HLC type as a percentage of the research area. All the HLC types have been assessed against each other according to the area that they cover to produce a scale of occurrence, or rarity. The area of each type was converted to a percentage and this figure plotted on a logarithmic scale in Excel. This enabled breaks and jumps in the range to be identified and six categories were developed: extremely rare (<0.01%), very rare (0.01-0.09%), rare (0.1-0.3%), occasional (0.35-1.0%), frequent (1.0-5.8%), and common (>5.9%) (Williams 2008).

Analysis of rarity and direction of change give information about the common historic landscape character types but cannot provide information about how these characters have changed and their vulnerabilities to future changes. However, the analysis of rarity and direction of change can be considered together to give a general idea about the determination of vulnerabilities (e.g. a rare and decreasing rapidly historic landscape character type will be highly vulnerable to change). The vulnerability of each historic landscape character type has been calculated using the direction of change and rarity scores. The logarithmic values of each have been added together to give a range of scores between 200 and minus 99 which have been divided into the ranges low (minus 99-1), medium (9-90), and high (100-200) (Williams 2008).

The river historic landscape character types were also included to analysis process and the areas affected by the rivers in the study area were determined by creating a 100 m buffer zone through ArcGIS 10.1 software (Mayer et al. 2005). According to the values mentioned above, the direction of change, vulnerability and rarity analysis of historic landscape character types of Kazdağı National Park have been carried out.

3. Results

As a result of the research, historic landscape character types maps of the three periods have been created. According to these maps, spatial distributions of historic landscape character types have been analyzed and the following results were obtained (Figure 2).

For the first period, as a woodland historic landscape character type, 1401 ha *Pinus brutia-Quercus* sp.-*Pinus nigra*, 1145 ha *Pinus nigra-Abies equi trojani*, 46 ha *Pinus nigra* woodland and as a maqui 45 ha *Pinus brutia-Quercus* sp. community which still exist today have been detected in Kazdağı National Park between 1890 and 1920.

In the second period (1940-1970), 3083 ha *Pinus brutia-Quercus* sp.-*Pinus nigra*, 2132 ha *Pinus nigra-Quercus* sp., 1602 ha *Pinus nigra-Quercus* sp.-*Castanea* sp.- *Abies equi trojani*,543 ha *Pinus nigra*, 166 ha *Pinus nigra-Pinus brutia*, 151 ha *Pinus nigra-Abies equi trojani*, 101ha *Pinus brutia* have been detected as a woodland historic landscape character type. For maquis historic landscape character type, 150 ha *Pinus brutia-Quercus* sp. community have been determined. Also in this period, 273 ha Olive field has been detected as an agricultural area historic landscape character type. Besides this 656 ha canyon, 758 ha bare rock and 273 ha olive field historic landscape character type has been found.

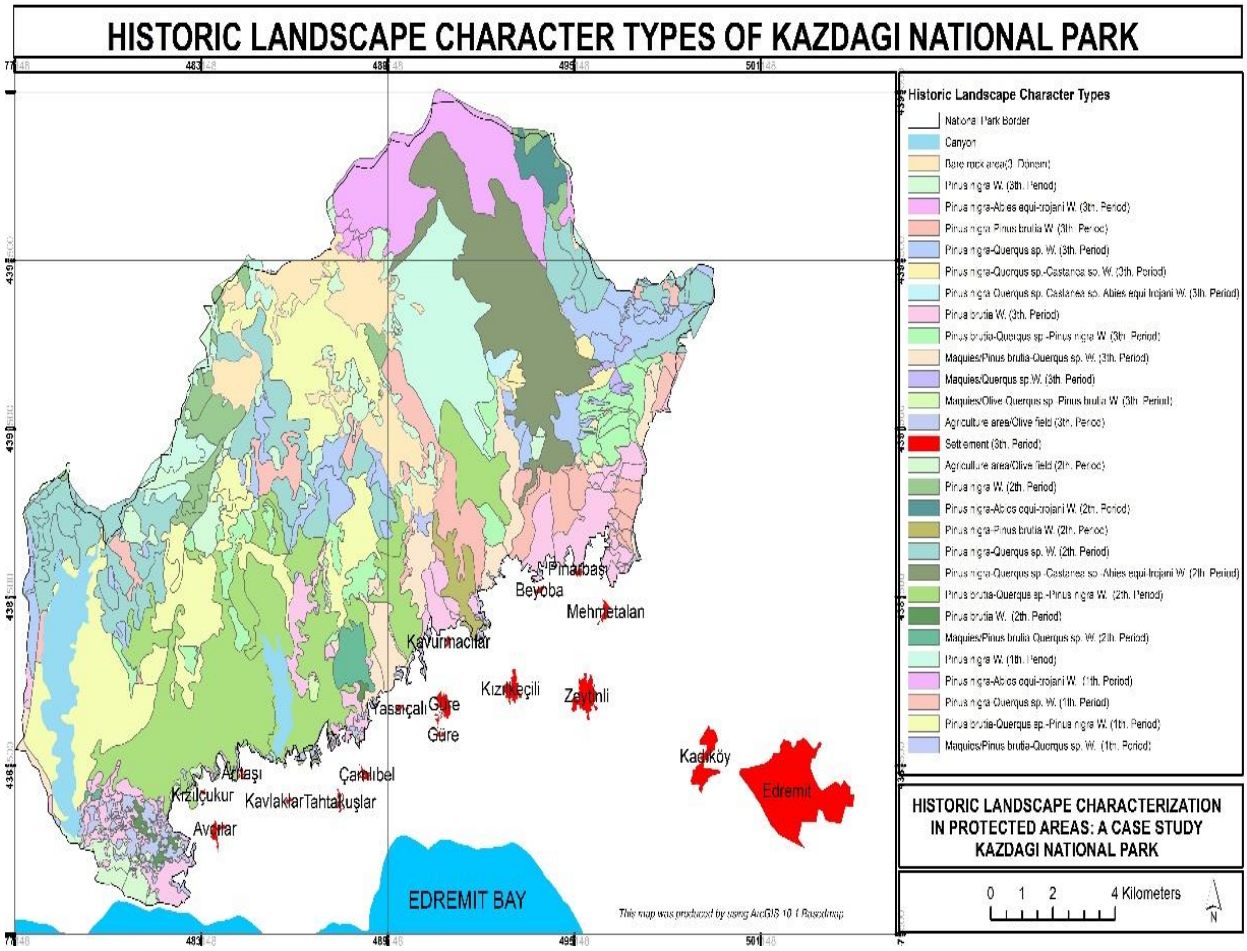

Figure 2- Historic Landscape Character Types Maps of Three Periods

Finally, in the third period (2000-present), 1223 ha *Pinus brutia*, 1077 ha *Pinus nigra-Quercus* sp., 814 ha *Pinus brutia- Quercus* sp.-*Pinus nigra*, 769 ha *Pinus nigra*, 675 ha *Pinus nigra-Pinus brutia*, 107 ha *Pinus nigra-Abies equi trojani*, 102 ha *Pinus nigra-Quercus* sp.-*Castanea* sp., 46 ha *Pinus nigra-Quercus* sp.-*Castanea* sp.-*Abies equi trojani* woodland historic landscape character type and 729 ha *Pinus brutia-Quercus* sp., 48 ha *Quercus* sp.,16 ha *Olive-Quercus* sp.-*Pinus brutia* maquis historic landscape character type, 506 ha olive field historic landscape character type, 1097 ha bare rock and 692 ha Canyon historic landscape character type have been determined. The area affected by water bodies for river historic landscape character type has been determined by creating a 100 m buffer zone in ArcGIS 10.1. Periodically these values are for the first period 226 ha, second period 598 ha and the third period 575 ha. The canyon historic landscape character type for the water surface has been determined in the second and third periods and it could not be evaluated as spatial due to the inadequate scale of the maps in the first period.

The historic landscape characterization methodology identifies past landscapes spatially, as well as determines their perceptions and uses in the past with interpretations through information obtained from historic and antic sources. In this context, Kazdağı National Park Historic landscape character types have been assessed in terms of spatial analysis as well as their perceptions and characters in the past. After identifying the historic landscape character types and defining the perception, usage and characteristics of these landscapes in the past periods, the analysis of rarity and vulnerability was carried out and maps of rarity and vulnerability for three periods of the Kazdağı National Park were obtained (Figures 3 and 4).

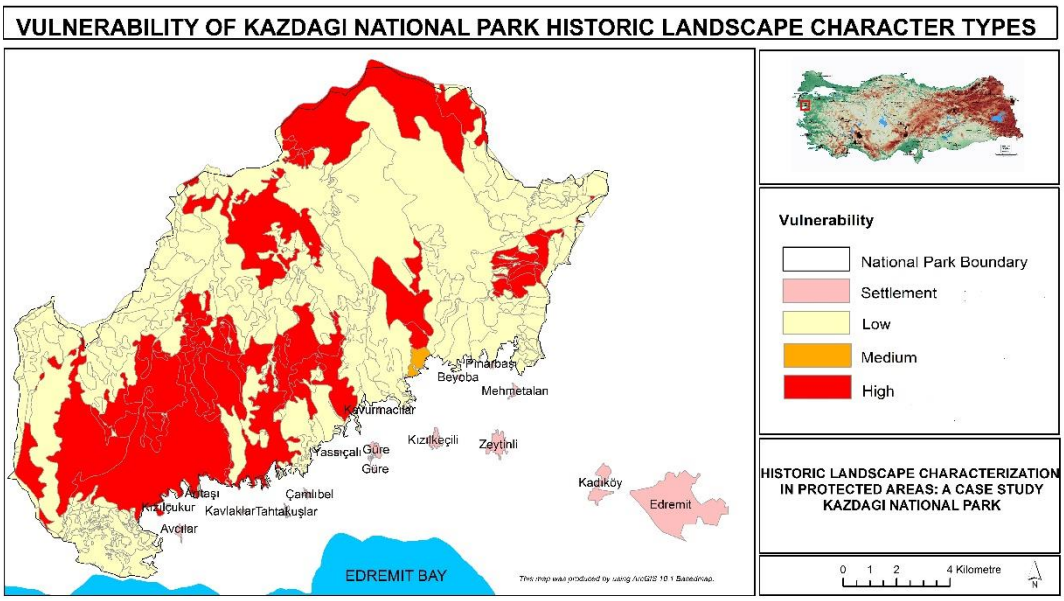

Figure 3- Vulnerability of Historic Landscape Character Types of Kazdağı National Park

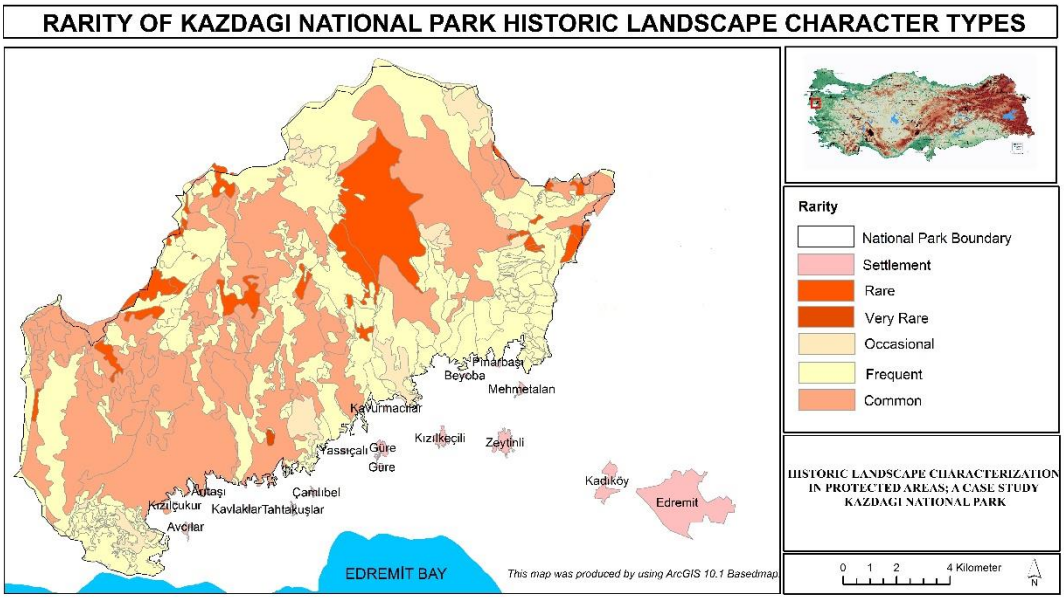

Figure 4- Rarity of Historic Landscape Character Types of Kazdağı National Park

When Kazdağı National Park is evaluated in this context, 13915 ha low, 55 ha medium and 8853 ha high fragile historic landscapes have been determined. The findings show that about 40% of the Kazdağı National Park has a high degree of vulnerability.

Increasing environmental problems and population, with changing economic conditions and consumption habits increase pressures that occur on the landscape. In this context, landscape management studies are becoming increasingly important (Sengur 2018).

There are four potential uses of historic landscape characterization: Landscape Management, Spatial Planning, Learning-Presentation (Research and Development Studies) and Landscape Character Analysis and Strategies (Clark et al. 2004).

Historic landscape characterization studies integrated with landscape character analysis will contribute development plans, long-term development plans, and environmental plans. It will also be a guide to many professional disciplines, such as landscape architects, urban and regional planners, architects and archaeologists.

A key factor in securing the future of the National Parks' heritage is the statutory management plan each National Park Authority is required to produce. The protection and promotion of the historic environment is a key feature in these management plans, which address the implications of a wide variety of environmental pressures affecting the heritage (Tunnicliffe 2006).

With this study, an up to date database that provides information about the past and present landscape of the Kazdağları National Park has been created, and important information has been presented to the National Park Management Plan through the analysis studies performed.

In this context, The Historic landscape characterization method is GIS-based and uses classifications of landscape types or character types as attributes on which to base the analysis. It is fundamentally a subjective process and interpretative (by archaeologists with specialist skills) but uses actual data derived from cartographic and archaeological sources, allowing objectivity and transparency to be built into the characterization process. Also, one useful application of Historic landscape characterization is the creation of baseline data that are area based and comprehensive and which are used to measure, monitor and assess the impact of developments and changes to the (historic) landscape. This is important for cultural heritage and its role in the planning process (Aldred 2005). Another important contribution of The Historic landscape characterization studies revealing the historic values of the national parks is to enable people to understand the heritage values of national parks and to contribute to the protection and development of these areas by people.

The research area is largely natural area as well as its historic and cultural heritage value. In this context, one of the characteristic features of historic landscapes of Kazdağı National Park is the existence of endemic plants. In the study, the existence of endemic plants in the past periods has been revealed by using the books of Flora of Turkey Series by P. H. Davis. The method can be carried out with a much longer time relative to the purpose of the study, research area and sufficiency of the data to be used. In this context, researches about Historic landscape characterization carried out with a long time depth in natural areas should be evaluated more comprehensively, supported by the science of Palynology. With all these values and a 40% fragility level, the Kazdağı National Park is an internationally important region that needs to be quickly taken action in terms of management and protection of the landscape. According to Green (2009), there are four different management strategies for historic landscapes on a large scale. These; 'Conserve and Restore', 'Conserve', 'Selective Conservation' and 'Mitigate or Restore'. According to its vulnerability level, Kazdağı National Park should be evaluated in the 'Conserve and Restore' (historic landscapes of medium to high significance which have declined rapidly or critically. Surviving examples of these landscapes need protection whilst opportunities and projects to restore significant lost sites would be desirable in most instances.) must be protected with a new system. According to Sengur (2010), the Kazdağları Region has been evaluated as a World Heritage, and it was determined that it has similar characteristics with the 6^{th}, 7^{th}, 9^{th}, and 10^{th} criteria of UNESCO World Heritage. The findings of the research support this finding. The value of resources in our globalizing world has been exceeded the limits such as region or country, this situation has brought the need for international responsibility and protection.

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