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Research Article / Araştırma Makalesi

Effect of Medium Composition on *in vitro* Seed Germination and Plant Development in Kentucky Bluegrass (*Poa pratensis* L. cv. Evora)

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Abstract: In the current study, the effects of De Greef & Jacobs (DG), Linsmaier & Skoog (LS), Murashige & Skoog (MS), and Schenk & Hildebrandt (SH) media were tested on seed germination and plant development in *Poa pratensis* cv. Evora. The highest germination rate (83±2.74%) was found on SH medium, whereas LS medium gave the lowest (46±4.18%) germination rate. The statistically same leaf numbers were recorded from SH (2.10±0.27) and DG (2.12±0.18) media. SH and DG media gave 4.28±0.28 cm and 4.16±0.31 cm mean leaf lengths, respectively. SH medium gave the maximum mean root number (3.09±0.26). However, the LS medium gave the lowest mean root number (1.84±0.10). The longest roots (1.43±0.19 cm) were observed in the plants grown in SH medium. However, DG medium had the minimum mean root length (0.81±0.08 cm). In conclusion, SH medium should be preferred over the other medium tested in *in vitro* tissue culture studies on the species to increase the biomass production yield. The efficacy of SH medium in the *in vitro* propagation of *P. pratensis* could be increased using growth promoters.

Keywords: Macroelements; microelements; tissue culture; turfgrass; vitamins.

Besiyeri Bileşiminin Çayır Salkımotunda (*Poa pratensis* L. cv. Evora) *in vitro* Tohum Çimlenmesi ve Bitki Gelişimi Üzerindeki Etkisi

Öz: Bu çalışmada, *Poa pratensis* cv. Evora'da De Greef & Jacobs (DG), Linsmaier & Skoog (LS), Murashige & Skoog (MS) ve Schenk & Hildebrandt (SH) besiyerlerinin tohum çimlenmesi ve bitki gelişimi üzerindeki etkileri test edilmiştir. En yüksek çimlenme oranı (%83±2.74) SH ortamında bulunurken, LS ortamı en düşük (%46±4.18) çimlenme oranını vermiştir. Ortalama yaprak sayıları SH (2.10±0.27) ve DG (2.12±0.18) ortamlarından istatistiksel olarak aynı kaydedilmiştir. Ortalama yaprak uzunlukları SH ve DG ortamlarında sırasıyla 4.28±0.28 cm ve 4.16±0.31 cm olarak ölçülmüştür. Maksimum ortalama kök sayısı (3.09±0.26) SH ortamından elde edilmiştir. Bununla birlikte, en düşük ortalama kök sayısı (1.84±0.10) LS ortamında kaydedilmiştir. En uzun kökler (1.43±0.19 cm) SH ortamında yetiştirilen bitkilerde gözlenmiştir. Bununla birlikte, DG ortamı minimum ortalama kök uzunluğunu (0.81±0.08 cm) vermiştir. Sonuç olarak, biyokütle üretimini artırmak için bu tür üzerinde yapılan *in vitro* doku kültürü çalışmalarında test edilen diğer besiyerlerine kıyasla SH besiyeri tercih edilmelidir. SH ortamının *P. pratensis*'in *in vitro* gelişimindeki etkinliği büyüme destekleyicileri kullanılarak artırılabilir.

Anahtar kelimeler: Makroelementler; mikroelementler; doku kültürü; çim; vitaminler.

1. Introduction

Kentucky bluegrass (*Poa pratensis* L.) is a perennial and dense turf-producing cool-season grass, which is generally used after getting mixed with perennial ryegrass (*Lolium perenne* L.) to obtain a more stabilized soil and disease-resistant turf with better color and quality (Walker et al., 2007). The species is native to Europe and Asia but has been cultivated for sports and ornamental use in many regions with a temperate climate (Casler, 2006). It grows well on limestone-originated, medium-textured, and well-drained soils. However, it can adapt to poorly-drained and heavy-textured soils. The species can also tolerate severe droughts; although, it prefers humid areas with temperatures between 15 and 32°C (Bush, 2002). These advantages of *P. pratensis* make it an attractive option among other grass species used for forage and lawn production. Like other turfgrasses, the primary propagation method of *P. pratensis* is seed sowing since the vegetative propagation from rhizomes is labor-intensive for such plants.

Plant tissue culture is primarily used to process *in vitro* plant material utilizing meristems and organs that can be differentiated under controlled environmental conditions. The technique is used alongside other plant biotechnology applications such as molecular breeding and disease-resistant plants' production through genetic engineering applications. The optimization of tissue culture medium for *in vitro* plant development plays a significant role in forage and turfgrass biotechnology (Esmaeili et al., 2018). Components in tissue culture media have a vital function in the germination of plant seeds. Every plant tissue culture medium has specific formulations consisted of macro- and microelements, vitamins, and other components such as amino acids at different ratios (Acemi et al., 2018). The success of the *in vitro* propagation study is highly dependent on tissue culture medium composition. Therefore, different medium compositions should be tested to select the most favorable culture medium that meets the plant's macro- and microelement requirements in *in vitro* culture. The seeds' poor germination, the comparatively modest

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growth rate of the seedlings, and the lowest yield of *P. pratensis* among other *Poa* species pose difficulties in the turf establishment using the species (Giolo et al., 2017; Akdeniz et al., 2018). Thus, this study aims to determine the plant's requirements for its efficient seed germination and development. In addition, a better understanding of the plant's developmental physiology is provided by exploring the optimum *in vitro* seed germination medium for *P. pratensis*.

2. Material and Methods

2.1. Seed source and disinfection

The seeds of commercially available *P. pratensis* L. cv. Evora (DLF Seeds Ltd., Denmark) were supplied by a local dealer (Sekoya Tohumculuk Ziraat San. & Tic. A. Ş.) in Turkey and kept in a dry and dark place. Seeds' disinfection and sowing were performed in a laminar flow cabinet. One hundred seeds were placed into pouches (4×4 cm) made from filter papers and were disinfected by gently shaking for 8 min in 1% (w/v) sodium hypochlorite (NaOCl) solution. The NaOCl residues on the seeds were removed by soaking the pouch into sterile distilled water several times. The pouch with seeds was then placed onto a sterile platform made of filter papers to take excess water on the seeds.

2.2. Media preparation, seeds' sowing, and culture conditions

Four culture media with different formulations were employed to test their effects on seeds' germination and development of the species. The media described by De Greef & Jacobs (1979) (DG), Linsmaier & Skoog (1965) (LS), Murashige & Skoog (1962) (MS), and Schenk & Hildebrandt (1972) (SH) were prepared and supplemented with 30 g L^{-1} sucrose and 7 g L^{-1} agar and their pH was set to 5.7 using 1 N NaOH or 1 N HCl before autoclaving at 121°C under a pressure of 118 kPa for 20 min. The media formulations are given in Table 1. The paper pouches were opened with a sterile blade and forceps and the disinfected seeds were then sown onto the media. The cultures were incubated in a plant growth chamber with a 16-h photoperiod at 23±1°C and under the illumination of 60 µmol m^{-2} s^{-1} photosynthetic photon flux density.

Table 1. Comparison of the compositions of the culture media tested on *in vitro* development of *P. pratensis*.

Content	Quantity in medium (mg L^{-1})			
Macroelements	**DG**	**LS**	**MS**	**SH**
$CaCl_2$	226.50	332.02	332.02	151.00
KNO_3	2000.00	1900.00	1900.00	2500.00
$MgSO_4$	244.33	180.54	180.54	195.05
NaH_2PO_4	250.00			
KH_2PO_4		170.00	170.00	
$(NH_4)H_2PO_4$				300.00
$(NH_4)_2SO_4$	400.00			
NH_4NO_3		1650.00	1650.00	
KCl	600.00			
Microelements				
$CoCl_2.6H_2O$	0.0025	0.025	0.025	0.10
$CuSO_4.5H_2O$	0.0025	0.025	0.025	0.20
FeNaEDTA	36.7	36.7	36.7	19.8
H_3BO_3	10.62	6.2	6.2	5.00
KI	1.58	0.83	0.83	1.00
$MnSO_4.H_2O$	1.68	16.9	16.9	10.00
$Na_2MoO_4.2H_2O$	0.0025	0.25	0.25	0.10
$ZnSO_4.7H_2O$	1.06	8.6	8.6	1.00
Vitamins				
myo-inositol	100.0	100.0	100.0	1000.0
Nicotinic acid	1.0		0.5	5.0
Pyridoxine HCl	1.0		0.5	0.5
Thiamine HCl	10.0	0.4	0.1	5.0
Amino acids				
Glycine			2.0	

2.3. Data collection and statistical analysis

One hundred seeds (20 seeds per vessel) were sown onto each medium. Each repeat consisted of one culture vessel. The number of germinated seeds and mean leaf and root numbers and lengths were calculated at the end of the incubation period of 30 d. Data were given as mean±standard deviation (SD). Means were compared using Duncan's multiple range test at a significance level of $P< 0.05$. IBM SPSS Statistics 22 software was used for statistical analysis. The data were normalized to 0–1 interval and hierarchical clustering analysis was performed according to the Euclidean distance and unweighted pair group method with arithmetic mean (UPGMA). BioVinci data visualization software, version 1.1.5, was used to create the clustering heatmap.

3. Results and Discussion

The general appearances of the cultures at the end of the incubation period are given in Figure 1. The percentage of germinated seeds greatly varied among the media tested (Fig. 2). SH medium had the highest (83±2.74%) germination percentage, whereas the seeds cultured on LS medium had the lowest (46±4.18%) germination rate. The results showed that the SH medium gave 80%, 30%, and 22% higher germination rates than LS, MS, and DG media, respectively. Statistically, the same germination rates were found from MS and DG media. Seed germination in many species is affected by soil's chemical and physical properties and by several environmental conditions in nature (Borawska-Jarmułowicz et al., 2017; Benvenuti & Mazzoncini, 2018). However, environmental conditions

such as light intensity, photoperiod, temperature, and relative humidity remain stable in tissue culture studies allowing researchers to test the effects of culture medium composition, simulating soil's chemical components. Therefore, culture medium components such as macro- and microelements and supplements such as plant growth regulators are decisive in seed germination success in such studies (Acemi & Özen, 2019). The current study is solely based on testing the effects of medium compositions that means the differences in all developmental parameters are culture medium-specific. Therefore, the highest germination rate obtained from SH medium might be attributed to its rich KNO_3, PO_4, and vitamin content since the other media contain these components at lower levels. Especially, considering that the difference between LS and MS media is only vitamin-sourced and the better germination performance in MS than LS medium, it can be concluded that the vitamin composition of culture medium plays a vital role in the seed germination of *P. pratensis*. On the other hand, faster seed germination in *P. pratensis* after priming the seeds with KNO_3 solution has been reported by Pill and Korengel (1997). Ervin et al., (2017) stated that ammonium tends to be oxidized in time and leads to the decreased availability of P for plants following soil acidification that is an unfavorable condition for bluegrass. The same researchers also concluded that without using P- or K-containing fertilizers, only sulfates of ammonium and Fe reduce the growth and spread of *P. annua* (Ervin et al., 2017). Therefore, the better seed germination performance of SH and DG than MS and LS media might be explained by their lesser NH_4 but higher P contents. Furthermore, ammonium phosphate may increase nutrient availability with a lesser salt effect. In this context, $NH_4H_2PO_4$ has been previously shown to induce nutrient availability in *Hypericum* × *moserianum* (Pizzeghello et al., 2019).

Mean leaf numbers developed from per seed are shown in Figure 3. The highest number of leaves (2.12±0.18) was found in the DG medium, whereas LS medium gave the lowest (1.13±0.04) result. MS and LS media gave statistically the same results, while the same statistical result was found between SH and DG media. Therefore, the DG and SH media were found better for leaf development than the other media tested. A similar trend with mean leaf numbers was also found in the mean leaf lengths (Fig. 4). SH and DG media gave statistically the same results, while the same statistical relationship was found between MS and LS media. The most elongated leaves (4.28±0.28 cm) were measured from the plants developed on SH medium, while LS medium gave the lowest (2.32±0.26 cm) mean leaf lengths. Therefore, better leaf elongation performance in *P. pratensis* was found in the SH and DG media than the other media tested. In addition to the above-discussed differences among macroelement compositions of the media tested, the LS medium's drawback in supplying leaf growth for *P. pratensis* may be due to the lack of vitamins such as pyridoxine and nicotinic acid in its formulation. The exogenously provided non-phosphorylated B_6 vitamers have been shown to reduce singlet oxygen accumulation in plants (Vanderschuren et al., 2013). Also, Colinas et al. (2016) showed that balancing B_6 vitamers is vital for the metabolism and plant development in *Arabidopsis*. Vitamin B has many beneficial roles in plants, such as activation of plant disease resistance (Ahn et al., 2005), alleviating the effects of several environmental stresses, and regulating post-embryonic root development (Chen & Xiong, 2005) since they are cofactors required by numerous enzymes. For instance, nicotinic acid has been found to alleviate the effects of salt stress in *Allium cepa* (Ali, 2002) and *Ricinus communis* (Hussein et al., 2014). Although LS medium includes thiamine (vitamin B_1) as vitamin B, it seems that the lack of nicotinic acid (vitamin B_3) and pyridoxine (vitamin B_6) is the main disadvantage of LS medium in comparison with other media tested in this study.

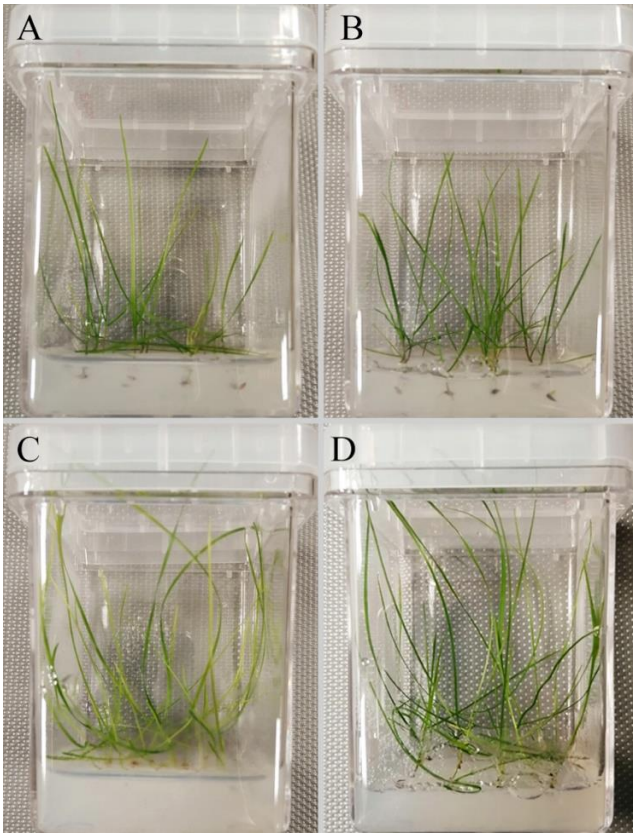

Figure 1. The appearance of the cultures at the end of the incubation period. Seeds germinated in LS (A), MS (B), DG (C), and SH media (D).

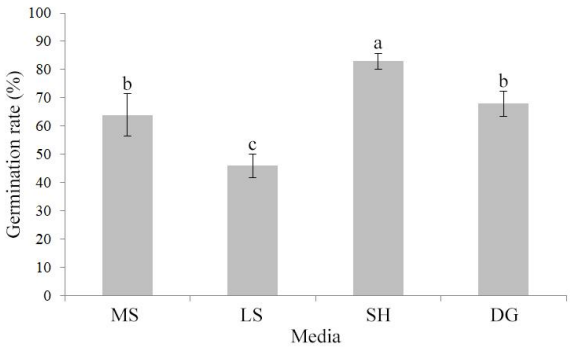

Figure 2. Effect of culture medium on *in vitro* germination of *P. pratensis* seeds. Data represent mean±SD. The bars with the same superscript letters are not significantly different by Duncan's multiple range test ($P < 0.05$).

The root development performance of *P. pratensis* in the media tested is shown in Fig. 5. The highest number of roots (3.09±0.26) was found in the SH medium, whereas the LS medium gave the lowest (1.84±0.1) mean root

number. MS and DG media gave statistically the same results. The root elongation was found better in the SH medium than the other media tested (Fig. 6). However, MS and LS media gave statistically the same results. The longest roots (1.43±0.19 cm) were measured from the plants developed on SH medium, while DG medium gave the lowest (0.81±0.08 cm) mean root lengths. These results suggested that SH medium better induces the rhizogenesis in *P. pratensis* than the other media tested. The turfgrass roots can release phytosiderophores under Fe- or Zn-deficient conditions that serve as natural chelating agents to extract micronutrients such as iron manganese, copper, and zinc from the soil (Ueno et al., 2007). However, a significant portion of the differentiation among the media's effects might come mostly from the macroelement and vitamin compositions since they cover relatively more space in a medium's formulation than microelements. Nevertheless, it should be noted that some microelements such as molybdenum (Mo) and zinc (Zn) are required for the biosynthesis of abscisic acid and indole-3 butyric acid that modulate the growth of primary and lateral roots (Kaiser et al., 2005). Furthermore, the role of another microelement, cobalt, in lateral root formation has been demonstrated in *Oryza sativa* (Hsu et al., 2013). Therefore, even microelements are required in low concentration, the disadvantage of DG medium in root elongation might stem from its minimal Mo and Co content. Another significant difference among the media tested is their *myo*-inositol contents. SH medium has the highest amount of *myo*-inositol that is ten times higher than those of the other media tested. *Myo*-inositol plays a significant role in many biosynthetic pathways of stress-molecule production and cell wall formation in plants (Loewus & Murthy, 2000). Also, it takes part in phosphate storage, cell communication, and transportation of plant hormones (Luo et al., 2011). In a tissue culture study, *myo*-inositol has been found to enhance the shoot growth and root development in the *Malus domestica* and *Pyrus communis* explants. The authors stated that higher levels of *myo*-inositol could be required to improve the explants' morphogenetic ability (Toma et al., 2012).

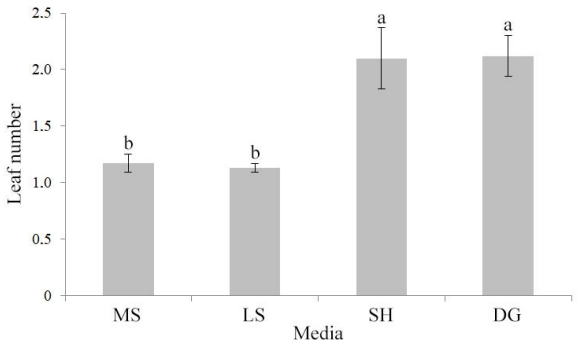

Figure 3. Effect of culture medium on *in vitro* leaf production in *P. pratensis*. Data represent mean±SD. The bars with the same superscript letters are not significantly different by Duncan's multiple range test ($P < 0.05$).

The developmental data-based hierarchical clustering heatmap shows the relationship among the media tested (Fig. 7). All the media tested were grouped into two main clusters. SH and DG media were found in the same cluster, while MS and LS media were placed in another cluster suggesting that the media's effects in the same cluster were more similar than the other media. The normalized data having the red color better developmental results in *P. pratensis*, while the data represented by blue color shows the lower developmental performance.

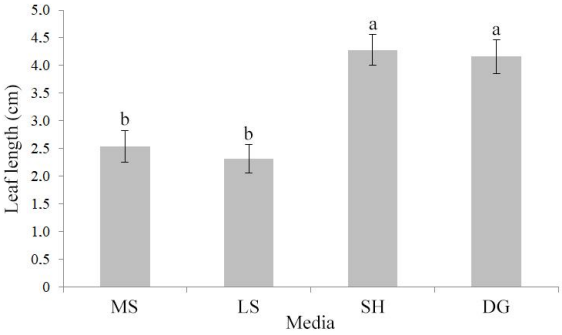

Figure 4. Effect of culture medium on *in vitro* leaf elongation in *P. pratensis*. Data represent mean±SD. The bars with the same superscript letters are not significantly different by Duncan's multiple range test ($P < 0.05$).

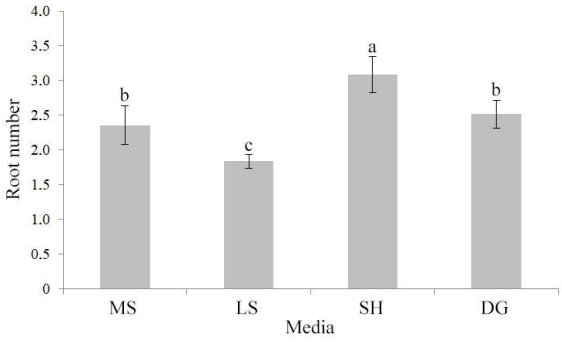

Figure 5. Effect of culture medium on *in vitro* root production in *P. pratensis*. Data represent mean±SD. The bars with the same superscript letters are not significantly different by Duncan's multiple range test ($P < 0.05$).

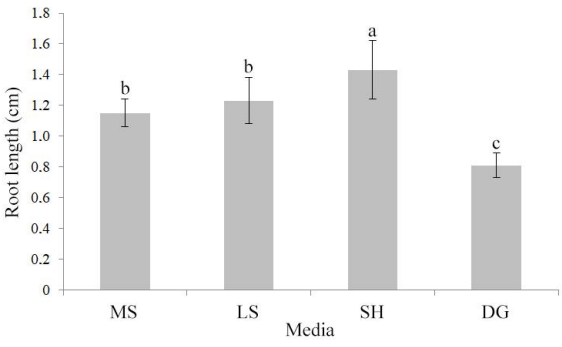

Figure 6. Effect of culture medium on *in vitro* root elongation in *P. pratensis*. Data represent mean±SD. The bars with the same superscript letters are not significantly different by Duncan's multiple range test ($P < 0.05$).

4. Conclusion

In the present study, influences of different culture media on *in vitro* seed germination and plant development in *P. pratensis* L. were evaluated based on the developmental parameters. In conclusion, SH media should be preferred

over the other medium to achieve a higher seed germination rate and better plant development in *P. pratensis*. The findings of this study would contribute to the improvement studies on the forage and grass species. Also, the effects of growth promoters should be tested to increase the efficiency of the medium.

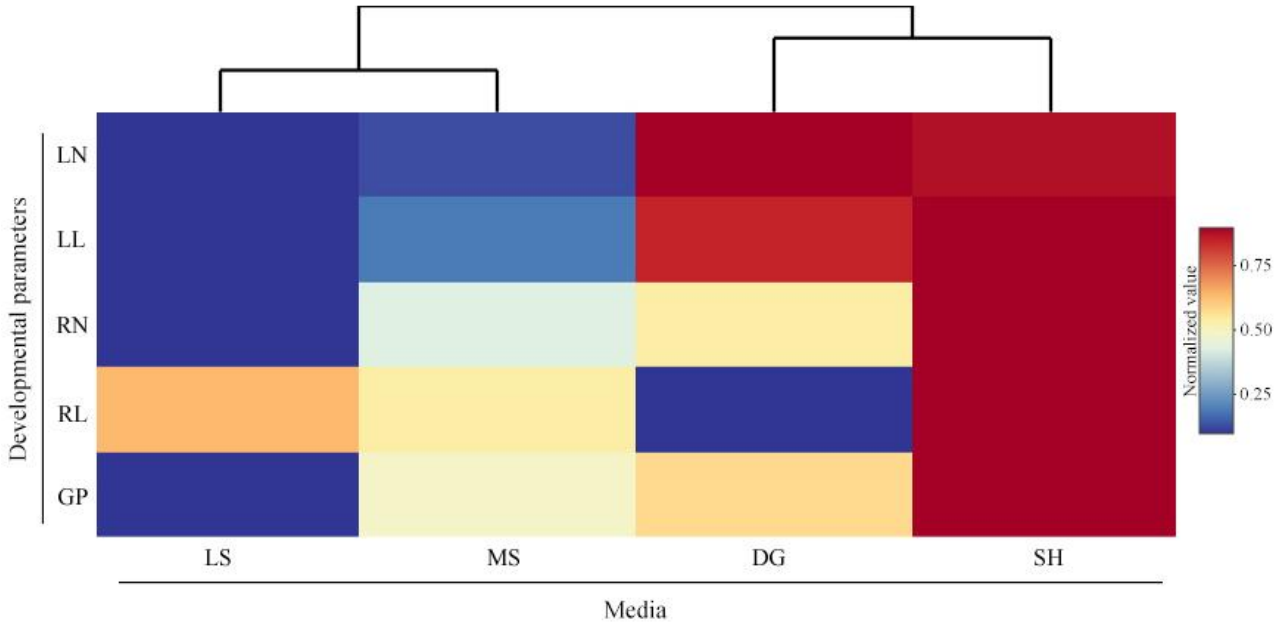

Figure 7. Hierarchical clustering heatmap-based comparison of the developmental data. Leaf number (LN), Leaf length (LL), Root number (RN), Root length (RL), Germination percentage (GP)

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Conflict of interest: The author declares that there is no conflict of interest.

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Research Article/Araştırma Makalesi

Lead and Cadmium Induced Oxidative Stress in the Epididymis and Spleen of Rats: Effects of Sesamol

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Abstract: Lead and cadmium are known as environmental pollutants extensively found in food and water that induce hazards to animals' and people's health. Sesamol is a dietary antioxidant that is found in some plants. Epididymis is known to play an important role in the maturation and storage of the sperm. The spleen is an important organ involved in the immune response. The present study aims to analyze the oxidative stress in spleen and epididymis. Therefore, Lead (LN) (90 mg/kg bw per day, 1/25 LD_{50}), Cadmium ($CdCl_2$) (3 mg/kg bw per day, 1/25 LD_{50}), and sesamol (50 mg/kg bw per day) were given to rats by gavage for 28 days. Antioxidant enzyme activities in epididymis and spleen tissues [superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione S transferase], malondialdehyde (MDA) were investigated at the end of 28 days comparatively with the control group. It is revealed that there is a significant decline in the antioxidant enzymes and increase in MDA levels in the spleen and epididymis tissues of the lead and cadmium treated rats compared to the control group. A small increase in these parameters was also observed in the sesamol treated groups.

Keywords: Antioxidants, heavy metals, lipid peroxidation, organ toxicity.

Sıçanların Epididimis ve Dalak Dokularında Kurşun ve Kadmiyuma Bağlı Oksidatif Strese Karşı Sesamolün Etkileri

Öz: Kurşun ve kadmiyum, hayvan ve insan sağlığına zarar veren gıda ve su kirleticilerinde yaygın olarak bulunan bilinen çevre kirleticileridir. Sesamol, bazı bitkilerde bulunan bir diyet antioksidanıdır. Epididimisin spermin olgunlaşması ve depolanmasında önemli bir rol oynadığı bilinmektedir. Dalak, bağışıklık tepkisinde rol oynayan önemli bir organdır. Bu çalışma, dalak ve epididimdeki oksidatif stresi analiz etmeyi amaçlamaktadır. Bu amaçla sıçanlara kurşun (günde 90 mg/kg canlı ağırlık, 1/25 LD_{50}), kadmiyum (günde 3 mg/kg canlı ağırlık, 1/25 LD_{50}) ve sesamol (günde 50 mg/kg canlı ağırlık) verildi. 28 gün boyunca gavaj, 28 gün sonunda epididimis ve dalak dokularındaki antioksidan enzim aktiviteleri [süperoksit dismutaz (SOD), katalaz (CAT), glutasyon peroksidaz (GPx) ve glutatyon S transferaz], malondialdehit (MDA) kontrol grubu ile karşılaştırmalı olarak araştırıldı. Kontrol grubuna göre kurşun ve kadmiyum uygulanan sıçanların dalak ve epididimis dokularında antioksidan enzimlerinde istatistiksel olarak anlamlı bir azalma ve MDA düzeylerinde artış olmuştur. Bu parametrelerde sesamol ile tedavi edilen gruplarda daha az artış gözlenmiştir.

Anahtar kelimeler: Antioksidanlar, ağır metal, lipit peroksidasyonu, organ toksisitesi.

1. Introduction

Lead and cadmium are extremely toxic and widespread heavy metals used in the environment. Materials containing cadmium are plastic, glass, and metal alloys (Marchlewicz et al., 2004; Merra et al., 2014; Baş & Kalender, 2016). It has been reported that lead caused some toxicity on reproductive systems (Marchlewicz et al., 2004). It has been known that cadmium causes oxidative damage in many organisms through mechanisms such as inhibition of major anti-oxidative enzymes like catalase (CAT) and superoxide dismutase (SOD) and enzymes that include glutathione (Djuric et al., 2015). Heavy metals have toxic effects on renal cells, hepatocytes, and also on mammalian brain cells. The relationship between metals and toxicity of the kidney, liver, and brain has been demonstrated in previous studies (Apaydın et al., 2016; Uzunhisarcikli et al., 2016; Baş et al., 2015a).

Imbalance between the reactive oxygen species (ROS) and antioxidant defenses is caused by oxidative stress (Zhou et al., 2011). Many investigations have evaluated the antioxidant activity of various materials using different techniques. Antioxidant compounds are widely used because of their protective roles in the body and against oxidative stress-mediated pathological processes (Gulcin, 2020).

Sesamol (3,4-methylenedioxyphenol), known as a potent antioxidant in sesame seed oil, is also an anti-inflammatory agent (Parihar et al., 2006). Sesamol has protective roles on organ injuries (Chu et al., 2010). Sesamol decreases lipid peroxidation and potently reduces oxidative stress (Hsu et al., 2008).

The immune system is very sensitive to toxic substances. Spleen is an organ that takes a role in the immune responses and antibody-production. Lymphocytes in the spleen are activated when presented with foreign antigens (Fang et al., 2010; Merra et al., 2014).

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On the other hand, epididymis plays an important role in sperm maturation and storage. Moreover, many substances are synthesized in the epididymis cells (Zhou et al., 2011). Therefore, in this study, the effects of cadmium and lead on these tissues have been studied. At the same time, it was examined whether sesamol has a role on these effects.

2. Material and Methods

2.1. Animals and chemicals

90 days of age Male Wistar rats (200-250 g body weight) were purchased from the Laboratory Animals Raising and Experimental Researches Center of Gazi University. All experimental procedures that treated animals were performed in accordance with protocols and guidelines of the Animal Experiments Local Ethics Committee (Protocol no: G.Ü.ET-17.086), Gazi University, Turkey. All rats were housed in plastic cases and fed ad libitum food and water. Cadmium chloride, lead nitrate, and sesamol were obtained from Sigma (St. Louis, MO) and dissolved in distilled water.

2.2. Animal treatments

Rats were divided into 8 groups with 6 rats in each group, randomly. Animals were treated with lead nitrate (product code: 1002741224), cadmium chloride (product code: 1002563650), and sesamol (product code: 102085395) orally via gavage. After the experiments, rats were sacrificed under euthanasia by a combination of ketamine and xylazin, then spleen and epididymis tissues were removed for spectrofotometrical analysis. Lead nitrate (90 mg/kg bw; 1/25 LD_{50}) (Plastunov & Zub, 2008; Sharma et al., 2010), cadmium chloride (3 mg/kg bw; 1/25 LD_{50}) (El-Demerdash et al., 2004), and sesamol (50 mg/kg bw) (Hemalatha et al., 2013) were given to rats via gavage for 28 days.

2.3. Biochemical analysis

The tissues were dissected and washed in sodium phosphate buffer (pH 7.2). After washing, samples were taken and stored at -80°C until the analysis. The tissues were homogenized using a teflon homogenizer (Heidolph Silent Crusher M). MDA content and antioxidant enzyme activity were determined by measuring the absorbance of the samples in a spectrophotometer (Shimadzu UV 1700, Kyoto, Japan). All processes were carried out at 4°C. Lipid peroxidation was determined by malondialdehyde production and measured in spleen and epididymis homogenates according to the method of Ohkawa et al. (1979) based on TBARS formation and it was expressed as MDA content. Superoxide dismutase level in tissues was estimated by the method of Marklund & Marklund (1974) and its activity was expressed as units/mg protein. Catalase was assayed according to the method of Aebi (1984) and expressed as mmol/min/mg protein of hydrogen peroxide unit to mmol/min/mg protein. Glutathione-S-tranferase enzymes activity was assayed by the method of Habig et al. (1974) and expressed as µmol/min/mg protein of CDNB-GSH conjugate formed. Glutathione peroxidase was assayed by the method of Paglia & Valentine (1987) and its activity was expressed as nmol/min/mg protein.

2.4. Statistical analyses

All statistical calculations were done by SPSS 20.0 version (SPSS Inc., Chicago, IL). The values are expressed as the mean ± standard deviation (SD). Differences between groups were done by one-way analysis of variance (ANOVA). $P<0.05$ values were stated statistically significant.

3. Results

No mortality was observed in LN and $CdCl_2$ treated groups. Also, there were no statistically significant differences between the control and sesamol-treated groups in MDA levels and activities of SOD, GST, CAT, and GPx in epididymis and spleen of rats (Fig. 1-4).

Matched against with the control group, there were statistically significant enhancing in MDA levels and decreasing in activities of antioxidant enzymes in LN and/or $CdCl_2$ treated groups of epididymis of rats. Parameters that were analyzed in this study were more altered in LN+$CdCl_2$ treated rats. Application of sesamol with LN or $CdCl_2$ reversed these changes partially against LN or $CdCl_2$ used groups but protective effect of sesamol was seen only on GST activity against LN + $CdCl_2$ group. Sesamol did not show a protective effect on other parameters against the group in which lead and mercury were used together (Fig. 1 and 3).

Figures 2 and 4 exhibit that the anti-oxidative activities of enzyme (SOD, GST CAT, and GPx) and MDA levels showed significant difference among groups of spleen of rats. LN and $CdCl_2$ treated groups compared to the control group was observed statistically significant decrease on enzyme activities and increase on MDA levels. These changes were higher when LN plus $CdCl_2$ co-treated. A significant elevation was observed in SOD, GST CAT, and GPx activities and lower in MDA level at the end of the experimental period in sesamol treated group compared to LN or $CdCl_2$ treated groups. However, the protective effect of sesamol against LN + $CdCl_2$ group was seen on only CAT, GPx, and MDA levels.

4. Discussion

Heavy metals are commonly used generally in world and affect the function and damage the structure of many organs by causing oxidative damages (Garcia-Nino & Pedraza-Chaverri, 2014; Apaydin et al., 2015; Baş et al., 2015b). Lead and cadmium treatments are consistent with oxidative damage in various tissues (Merra et al., 2014; Djuric et al., 2015). In previous studies, it has been shown that cadmium administration causes an increase in testicular $O_2^{\bullet -}$ concentrations by different doses (Djuric et al., 2015). Cadmium interferes with protein metal-binding functional groups altering essential bio-metals homeostasis as well as the activities of corresponding metallic-enzymes, metallothioneins (MTs) overproduction, and competing with essential bioelements for protein binding sites in different organs (Djuric et al., 2015).

When metals and antioxidants are applied together, it is observed that the toxic effect of metals is reduced (Apaydın et al., 2016; Garcia-Nino & Pedraza-Chaverri, 2014; Kalender et al., 2015). In previous studies, researchers reported that the reason for the decrease in the antioxidant enzyme activity is due to the metals binding to ions in these enzymes (Djuric et al., 2015).

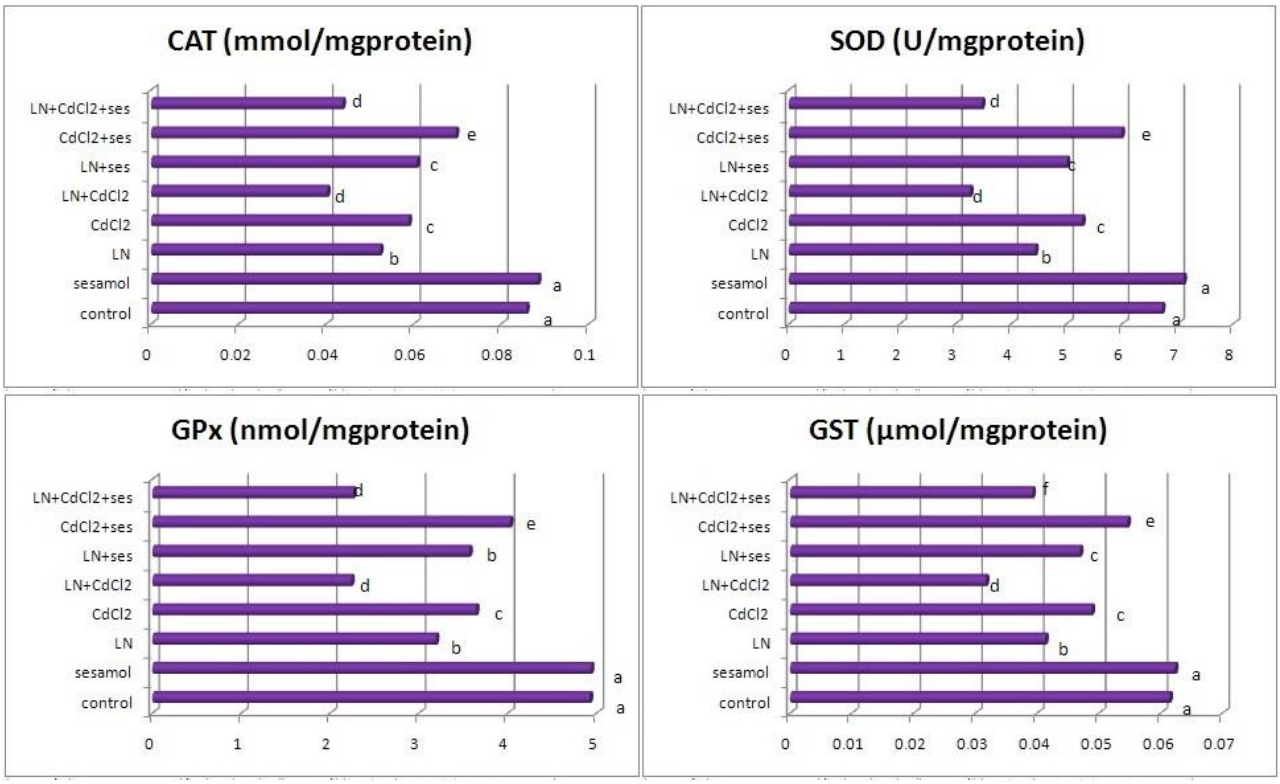

Figure 1. Preventive effects of sesamol (ses) against lead nitrate (LN) and cadmium chloride ($CdCl_2$) on antioxidant enzyme activities in epididymis of rats. Each bar represents mean ± SD (Significance at $P < 0.05$). Columns superscripts with different letters are significantly different.

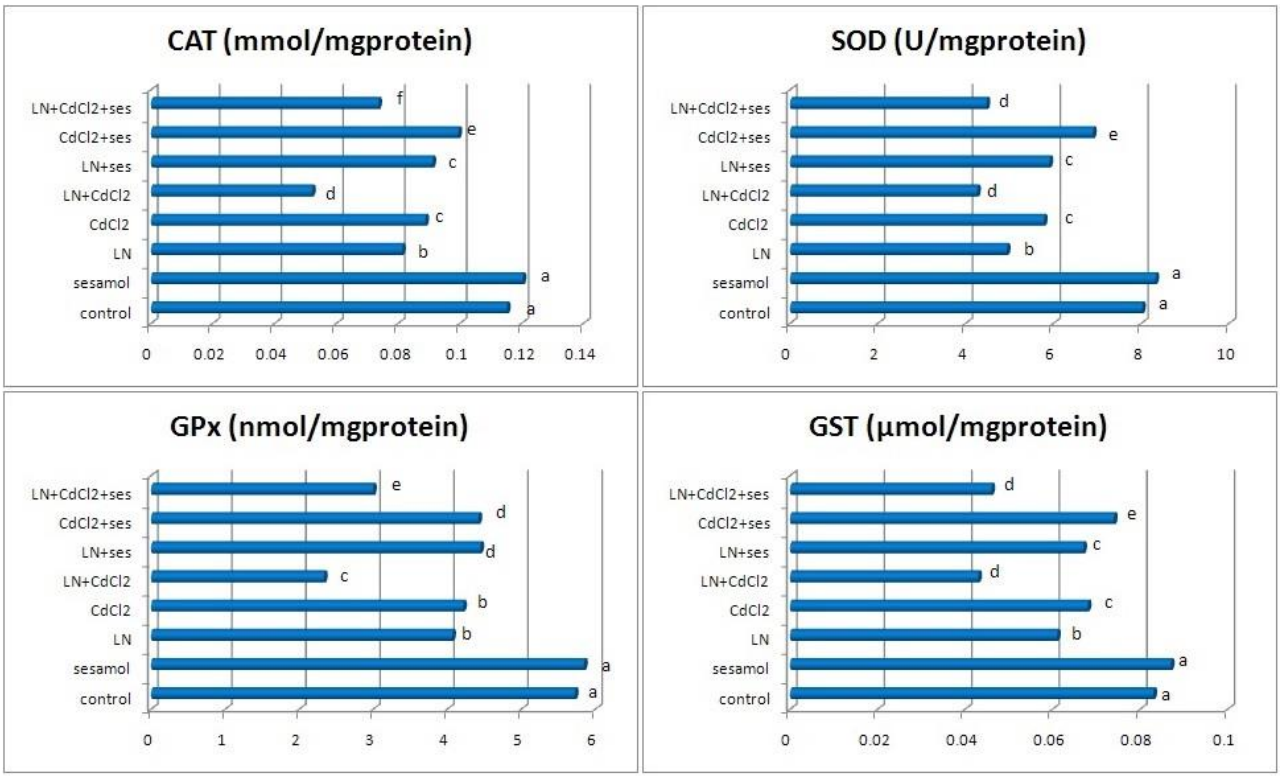

Figure 2. Preventive effects of sesamol (ses) against lead nitrate (LN) and cadmium chloride ($CdCl_2$) on antioxidant enzyme activities in spleen of rats. Each bar represents mean ± SD (Significance at $P < 0.05$). Columns superscripts with different letters are significantly different.

Lipid peroxidation is known to damage the integrity of cellular membranes, leading to the leakage of cytoplasmic enzymes and it also a marker of cellular oxidative damage (Tunali & Yanardag, 2006; Neogy et al., 2008). Antioxidant enzymes are primarily defense systems that protect biological macromolecules Antioxidant enzymes are primarily defense system which prevents biological macromolecules (Neogy et al., 2008). Cells include many antioxidant enzymes such as catalase, superoxidase dismutase, glutathione-S-transferase, and glutathione peroxidase (Neogy et al., 2008).

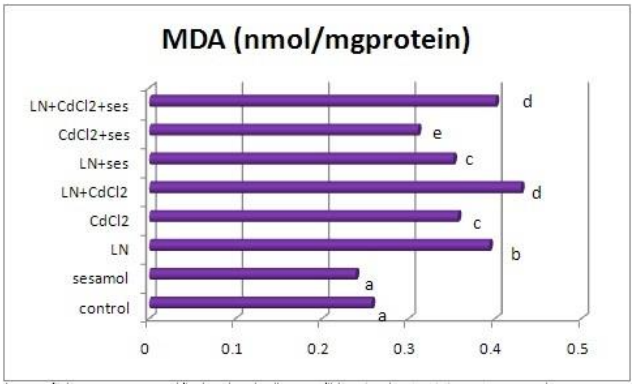

Figure 3. Preventive effects of sesamol (ses) against lead nitrate (LN) and cadmium chloride ($CdCl_2$) on MDA levels in epididymis of rats. Each bar represents mean ± SD (Significance at $P < 0.05$). Columns superscripts with different letters are significantly different.

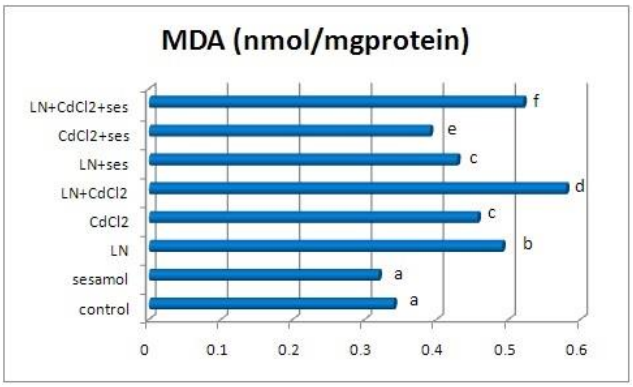

Figure 4. Preventive effects of sesamol (ses) against lead nitrate (LN) and cadmium chloride ($CdCl_2$) on MDA levels in spleen of rats. Each bar represents mean ± SD (Significance at $P < 0.05$). Columns superscripts with different letters are significantly different.

It is known that supplementation of an external source of antioxidants may offer some protection for cell damages. The term antioxidant refers to a wide spectrum of compounds which neutralizes free radical injuries (Garcia-Nino & Pedraza-Chaverri, 2014).

In the present study, administration of lead and cadmium increased lipid peroxidation in the epididymis and spleen. Similarly, environmental contaminants caused negative effects via increasing lipid peroxidative damages and caused differences in antioxidant levels in the epididymal sperm the male reproductive system (Latchoumycandane et al., 2003), and spleen tissues (Merra et al., 2014). In addition, splenotoxicity is important for many subchronic and chronic experiments (Tunali & Yanardag, 2006; Ma et al., 2008). It has also been shown in many studies that the biomarker enzymes measured in the study can be induced in heavy metal exposure (Agarwal et al., 2010).

Epididymis and spleen are affected by subacute cadmium and lead intoxication. It may be due to oxidative stress and lipid peroxidation causing serious harm to these tissues. When heavy metals are used together with sesamol, we showed that ameliorative effect.

Ethics committee approval: This study was performed in accordance with ethical standards of animal experiments. Legal research ethics committee approval permissions for the study were obtained from the Gazi University, Animal Experiments Local Ethics Committee (No: G.Ü.ET-17.086).

Conflict of interest: The authors declares that there is no conflict of interest.

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Research Article / Araştırma Makalesi

Evaluation of the Effects of Flaxseed Feeding in Mice Exposed to Oxidative Stress with Various Biomarkers

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Abstract: The aim of the study was to determine the antioxidant effects of the consumption of flaxseed as a dietary supplement in mice exposed to N-methyl N-nitrosourea (MNU) as an oxidative stress agent. For this aim, 60 female mice (*Mus musculus, BALB/c*), 8 weeks old, weighing an average of 20 (±2 g) grams, were divided into six experimental groups and fed in various forms. A single dose (50 mg/kg) of MNU was administered by intraperitoneal injection. Groups were determined as the control group (standard pellet), Group 1 (1st week 50 mg/kg ip MNU + standard pellet/12 weeks), Group 2 (1st week 50 mg/kg ip MNU + 10% flaxseed pellet/12 weeks), Group 3 (first 6-week standard pellet + 6 weeks 50 mg/kg ip MNU + Last 6 weeks 10% flaxseed pellet), Group 4 (10% flaxseed pellet/12 weeks), and Group 5 (10% flaxseed pellet/12 week + 50 mg/kg ip MNU at 6th weeks). Flaxseed was added to the standard mice diet at a rate of 10% and administered orally (*ad libitum*). At the end of the 12th week, cervical dislocation was applied to the mice and their liver tissues were taken to evaluate the selected biochemical markers (AST, ALT, LDH, GST, GR, GPX, CAT, CaE, and EROD). There was a statistically significant decrease in LDH activity in all groups compared to the control ($p<0.05$). The increase in CaE activity in groups 1, 2, and 4 was found statistically significant ($p<0.05$). Also, the alterations in ALT activity in groups 3, 4, and 5 were found statistically significant ($p<0.05$). In conclusion, the results from the biomarkers suggest that the giving of flaxseed alone did not cause a negative effect in mice, while MNU was toxic and flaxseed did not reduce the MNU effect.

Keywords: Biomarker, Flaxseed, N-methyl N-nitrousurea, oxidative stress.

Oksidatif Strese Maruz Kalmış Farelerde Keten Tohumu ile Beslemenin Etkilerinin Çeşitli Biyobelirteçler ile Değerlendirilmesi

Öz: Çalışmanın amacı, keten tohumu katkılanmış diyet tüketiminin N-Metil N- Nitrosoure (MNU) ile oksidatif stres oluşturulmuş farelerde antioksidatif etkilerinin belirlenmesidir. Farelere tek doz (50 mg/kg) MNU intraperitonal enjeksiyon ile uygulanmıştır. Çalışmada ağırlıkları ortalama 20 (±2 gr) gram, 8 haftalık 60 adet dişi fare (*Mus musculus*, *BALB/c*) altı gruba ayrılarak çeşitli formlarda beslenmiştir. Gruplar; Kontrol (standart pellet), Grup 1 (1. hafta 50 mg/kg i.p. MNU + standart pellet /12 hafta), Grup 2 (1. hafta 50 mg/kg i.p. MNU + %10 keten tohumu içeren pellet/12 hafta), Grup 3 (İlk 6 hafta standart pellet + 6. haftada 50 mg/kg i.p. MNU + son 6 hafta %10 keten tohumu içeren pellet), Grup 4 (%10 keten tohumu içeren pellet/12 hafta), Grup 5 (%10 oranında keten bitkisi tohumu pellet/12 hafta + 6. haftada 50 mg/kg i.p MNU) olarak belirlenmiştir. Keten tohumu, standart fare diyetine %10 oranında katılarak, farelere oral yolla (*ad libitum*) verilmiştir. On iki haftalık uygulama sonunda servikal dislokasyon uygulanmış ve karaciğer dokuları seçilmiş biyokimyasal belirteçleri (AST, ALT, LDH, GST, GR, GP_X, CAT, CaE ve EROD) değerlendirmek üzere alınmıştır. Tüm gruplarda LDH aktivitesinde kontrole göre istatistiksel olarak anlamlı bir azalma belirlenmiştir ($p<0.05$). CaE aktivitesi 1., 2. ve 4. gruplarda önemli düzeyde artış göstermiştir. Ayrıca, 3., 4. ve 5. grupların ALT aktivitesindeki değişimlerin kontrol grubuna göre istatistiksel olarak anlamlı olduğu belirlenmiştir ($p<0.05$). Sonuç olarak tek başına verilen keten tohumunun farelerde olumsuz bir etkiye sebep olmadığı, biyobelirteçlerden elde edilen sonuçların MNU etkisini yansıttığı ve keten tohumunun söz konusu etkiyi azaltıcı etkisinin olmadığı düşünülmektedir.

Anahtar kelimeler: Biyobelirteç, Keten tohumu, N-metil N-nitrosure, oksidatif stress.

1. Giriş

Son yıllardaki bilimsel çalışmalar diyet ve hastalıklar arasındaki ilişkiyi açık bir şekilde ortaya koymuş olup, epidemiyolojik çalışmalar diyetin kronik hastalıkların önlenmesi ve hastalıklardan korunmadaki rolüne işaret etmektedir. Beslenme alışkanlıklarının daha fazla meyve, sebze ve tahıl tüketecek şekilde değiştirilmesi kronik hastalıkların önlenmesinde etkin ve pratik bir yaklaşımdır (Haschke et al., 2001). Fonksiyonel gıdalar, besleyici etkilerinin yanı sıra bir ya da daha fazla etkili bileşene bağlı olarak sağlığı koruyucu, düzeltici ve/veya hastalık riskini azaltıcı etkiye sahip olup, bu etkileri bilimsel ve klinik olarak ispatlanmış gıdalar olarak tanımlanmaktadır (TBMM, 2004). Bilimsel çalışmalar besin bileşenlerinin, kardiyovasküler hastalıklar, kanser ve osteoporoz gibi hastalıkların önlenmesine katkıda bulunduğu ve sağlık üzerinde olumlu etkilerinin olduğuna ilişkin sonuçlar vermektedir (Coşkun, 2005).

Keten tohumu özellikle bileşiminde yer alan yüksek orandaki omega-3 yağ asitleri, α-linolenik asit (ALA) ve lignan olarak bilinen fenolik bileşenlerden dolayı, fonksiyonel gıda olarak tüketimi giderek artan bir gıdadır. Ayrıca protein, lif, magnezyum, fosfat ve kalsiyum bakımından da zengindir (Bernacchia et al., 2014).

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Bileşiminde %35-45 yağ, %28 diyet lif, %20 protein, %4 kül, 790-1030 mg/100 g toplam fenolik asit ve 13 mg/g lignan bulunmaktadır (Singh et al., 2011; Katare et al., 2012; Heimbach, 2009). Toplam yağ asidi içeriğinde yüksek oranda (%49-60) ω-3 yağ asidi, orta düzeyde (%12-17) linoleik asit ve ω-6 yağ asidi bulunmaktadır (Blasbaig et al., 2011). *In vitro* deneyler ve hayvan modellerinin, ω-3 çoklu doymamış yağ asidi bakımından zengin diyetlerin kolon veya göğüs kanserleri gibi çeşitli kanserlere karşı koruyucu olabileceği (Chamberland & Moon, 2015; Sorice et al., 2016), ω-6 yağ asitleri ile tedavinin ise kanser hücresi çoğalmasını artırabileceği ileri sürülmektedir (Iyengar et al., 2013). Keten tohumu, bitkisel lignanların en zengin kaynağı olarak bilinmektedir. Bazı çalışmalarda, yüksek oranda sekoizolarikirezinol diglukosit (SDG) tüketiminin meme kanserine yakalanma olasılığını düşürdüğü gösterilmiş, bunun için de lif ve memeli lignanlarının öncü maddeleri olan SDG açısından zengin gıdaların sıkça tüketiminin önemli olduğu belirtilmiştir (Hall et al., 2006; Konuklugil & Bahadır, 2004). Keten tohumunun hücre proliferasyonunu azalttığı, meme kanseri hastalarında, özellikle menopoz sonrası kadınlarda tümör büyümesini azaltma potansiyeline sahip olduğu ve bu tür kanser riskini azalttığı rapor edilmektedir (Bernacchia et al., 2014; Saggar et al., 2009; Thompson et al., 2005). Keten tohumunun yapısındaki tokoferoller ve flavonoidler lipid peroksidasyonunu geciktirmektedir. Ayrıca, antioksidan ve lignanların hücre çoğalmasını engelleyerek antikanserojenik ve kardiyovasküler hastalıklarda koruyucu olduğu ve oksidatif stresi azaltıcı etki gösterdiği bildirilmektedir (İşleroğlu et al., 2005). Yapılan bir çalışmada keten tohumunun toplam ve LDL kolesterol seviyelerini düşürdüğü, yemek sonrası glikoz emilimini ve bazı iltihap belirtilerini azalttığı bilinmektedir. Ayrıca, ω-3 yağ asitlerinin plazmadaki seviyelerini yükselttiği belirtilmiştir (Rodriguez Leyva & Pierce, 2010).

Beslenme, oksidatif stresin yaşam düzenleyicilerinden biridir. Besin tüketimi ile ilişkili oksidatif stres, hücre ortamını değiştirerek, çeşitli organların önemli sinyal yolaklarında moleküler değişikliklere neden olabilmektedir. Araştırmalar, hücre metabolizmasındaki değişikliklerin çeşitli kanser türlerinin ilerlemesinde önemli bir rol oynayabileceğini ortaya koymuştur. Organizmada reaktif oksijen türleri oksidatif stresi artırarak, antioksidan enzim sistemini olumsuz etkilemektedir (Pandey et al., 2003).

N-Metil-N-nitrosourea (MNU), DNA yapısını bozarak kanser ve dejeneratif hastalıklara neden olabilir (Tsubura et al., 2011; APA, 2020). Bu çalışmada, intraperitonal enjeksiyon (i.p) ile MNU'ya maruz bırakılan farelerin keten tohum katkılanmış olan diyetle beslenmelerinin karaciğer dokusunda çeşitli biyokimyasal parametreler üzerine etkileri değerlendirilmesi amaçlanmıştır.

2. Materyal ve Metot

2.1. Keten tohumu

Çalışmada keten bitkisinin (*Linum usitatissimum* L.) sarı renkli tohuma sahip ve Türkiye'de tescilli olan Sarı 85 çeşidi kullanıldı.

2.2. Deney hayvanları

Bu çalışmada İnönü Üniversitesi Deney Hayvanları Araştırma Merkezinden temin edilen 20 (±2 gr) gram ağırlığında 60 adet dişi fare (*Mus musculus*, *BALB/c*) (8 haftalık) kullanıldı. Hayvanlar, 22°C'de 12:12 saatlik bir ışık/karanlık döngüsü ile iyi havalandırılan bir odada 12 hafta boyunca ayrı kafeslerde barındırıldı. Deneyler, Ulusal Sağlık Enstitüsü Hayvan Araştırmaları Rehberi'ne uygun olarak gerçekleştirildi ve Malatya, İnönü Üniversitesi Deney Hayvanları Etik Kurulu tarafından onaylanmış olan test protokolü uygulandı (Protokol No: 2006/25).

2.3. Uygulama

Bu çalışmada 60 adet dişi fare (*Mus musculus*, *BALB/c*), rastgele her biri 10 fare içeren 6 farklı gruba ayrıldı. Tablo 1'de uygulama grupları gösterilmiştir. Gruplar; Kontrol grubu (standart pellet), Grup 1 (1. hafta 50 mg/kg i.p. MNU + standart pellet /12 hafta), Grup 2 (1 hafta 50 mg/kg i.p. MNU + %10 keten tohumu içeren pellet/12 hafta), Grup 3 (İlk 6 hafta standart pellet + 6. haftada 50 mg/kg i.p. MNU + 6 hafta %10 keten tohumu içeren pellet), Grup 4 (% 10 keten tohumu içeren pellet/12 hafta) ve Grup 5 (%10 oranında keten tohumu içeren pellet/12 hafta + 6. haftada 50 mg/kg i.p MNU) olarak belirlendi. MNU uygulaması hayvanlara tek doz olmak üzere, uygulamanın farklı dönemlerinde (1. veya 6. haftada) intraperitonal yolla gerçekleştirildi. Keten tohumu farelerin besinlerine (standart pellet) %10 oranında ilave edilerek verildi. Bunun için standart fare diyeti laboratuvar tipi bir değirmen ile kabaca parçacıklara öğütüldü ve bu diyete %10 öğütülmüş keten tohumu ilave edildi. Karışımın yeniden pellet haline gelmesi için %10 mısırözü yağı katıldı ve yem karışımı 1 cm^3 hacimli silindirik pelletler halinde preslenerek hazırlandı. Hazırlanan diyet hayvanların yemliklerine günlük 15gr/gün olacak şekilde konularak *ad libitum* tüketimleri sağlandı. Kontrol ve MNU grubu hayvanları sadece yağ karıştırılmış ve preslenmiş diyet ile beslendi. Deneye alınan tüm hayvanlar 12. haftanın sonunda servikal dislokasyon yolu ile sakrifiye edildi ve karaciğer dokuları alındı. Bu dokular biyokimyasal analizlere kadar -80ºC'de muhafaza edildi.

Tablo 1. Uygulama grupları.

Table 1. Application groups

	MNU uygulanan		**Normal diyetle beslenen**		**Keten tohumu ilavesi ile beslenen**	
Gruplar	Başlangıçta	6. haftada	İlk 6 hafta	Son 6 hafta	İlk 6 hafta	Son 6 hafta
Kontrol			✓	✓		
Grup 1	✓		✓	✓		
Grup 2	✓				✓	✓
Grup 3		✓	✓			✓
Grup 4					✓	✓
Grup 5		✓			✓	✓

2.4. Biyokimyasal analizler

Dokular potasyum fosfat tamponunda (pH 7.4) homojenize edildikten sonra elde edilen homojenatın 1/10, 4°C'de 16.000 xg devirde 20 dakika süre ile santrifüj edildi. Santrifüj sonrası elde edilen supernatantlar temiz ependorf tüplerine alınarak sitozolik enzim aktivitelerinin tayininde kullanıldı. Homojenatın geriye kalan kısmı literatürde belirtilen bir seri homojenizasyon ve santrifügasyon işlemlerinden sonra mikrozomal EROD aktivitesi tayininde kullanıldı (Whyte et al., 2000).

Karaciğer dokusunda sitozolik Aspartat Aminotransferaz (AST, µmol/dakika/mg protein) (Biolabo: 80025), Alanin Aminotransferaz (ALT, µmol/dakika/mg protein) (Biolabo: 80027) ve Laktat Dehidrogenaz (LDH, µmol/dakika/mg protein) (Biolabo: 92111) aktiviteleri; Glutatyon S-Transferaz (GST, µmol mol/dakika/mg protein) (Habig et al., 1974), Glutatyon Redüktaz (GR, nmol/dakika/mg protein) (Cribb et al., 1989), Glutatyon Peroksidaz (GP_X, µmol/dakika/mg protein) (Stephensen et al., 2000), Karboksilesteraz (CaE, µmol/dakika/mg protein) (Nousiainen & Torronen, 1984; Santhoshkumar & Shivanantappa, 1999), Katalaz (CAT, µmol/dakika/mg protein) (Luck, 1965) ve mikrozomal 7-Etoksirezorufin-*O*-Deetilaz (EROD, pmol/dakika/mg protein) (Whyte et al., 2000) enzim aktiviteleri literatürde belirtilen yöntemlere göre tespit edildi. Sitozolik, mikrozomal enzim aktivitesi ve toplam protein miktarı ölçüm işlemleri santrifüj işleminden hemen sonra, mikroplaka okuyucu sistemleri (Versamax Mikroplate Reader ve Gemini XS Fluoresans Microplate Reader; Molecular Devices Corp., USA) kullanılarak yapıldı. Örneklerde spesifik enzim aktivitesinin hesaplanması için tüm örneklerde toplam protein miktarı BSA (Bovine Serum Albumin) standart eğrisine bağlı olarak tespit edildi (Bradford, 1976).

2.5. İstatistik analiz

Her bir deneyden en az 3 tekrarlı olarak elde edilen bulgular bir paket program kullanılarak istatistiksel olarak değerlendirildi (SPSS 19.0). Kontrol ve uygulama grupları arasındaki farkların istatistiksel önem düzeyleri "One way ANOVA" testi ve "DUNCAN" post-hoc testiyle belirlendi. Gruplar arası farklılığın önemli olup olmadığı $p<0.05$ düzeyinde önemlilik derecesine göre saptandı. Non-parametrik değerlendirmelerde Mann Whitney-U testi kullanıldı.

3. Bulgular

Tablo 2'de AST, ALT, LDH, GST, GR, GP_X, CaE, CAT enzim aktivitesi ile mikrozomal EROD enzim aktivite değerleri gösterilmiştir. Karaciğer AST, GST, GR, GPx ve CAT aktiviteleri ile EROD aktivitesinde kontrol ve uygulama grupları arasındaki farklılıkların önemli olmadığı tespit edilmiştir ($p>0.05$). Diğer yandan, ALT enzim aktivite değerlerinde 3. grupta kontrole göre artış gözlenirken ($p<0.05$), 4. ve 5. gruplarda aktivitede düşüş belirlenmiştir ($p<0.05$). Karaciğer LDH değerlerinin, tüm gruplarda kontrole göre bir azalış gösterdiği, en fazla düşüşün 0.72±0.032 µmol/dakika/mg protein değeri ile sadece keten tohumu diyetiyle beslenen 4. grup fare dokularında olduğu saptanmıştır ($p<0.05$). Karaciğer CaE aktivitesi değerlerinin 5. grup dışında kalan tüm uygulama gruplarında kontrole göre arttığı, 1., 2. ve 4. gruplardaki aktivite artışının kontrole göre istatistiksel olarak önemli olduğu saptanmıştır ($p<0.05$).

Tablo 2. Karaciğer dokusunda AST, ALT, LDH, GST, GR, GPX, CaE, CAT aktivitesi ile hepatik postmitokondriyal EROD aktivite değerleri.

Table 2. Hepatic postmitochondrial EROD activity values with AST, ALT, LDH, GST, GR, GPX, CaE, CAT activity in liver tissue.

Grup	*n*	AST	*n*	ALT	*n*	LDH	*n*	GST	*n*	GR	*n*	GPX	*n*	CaE	*n*	CAT	*n*	EROD
K	8	1.29 ± 0.11	7	0.52 ± 0.14	8	1.11 ± 0.06	8	0.67 ± 0.07	8	65.9 ± 11.8	7	0.79 ± 0.07	8	5.04 ± 0.46	7	5600 ± 494	7	97.1 ± 22.9
1	8	1.51 ± 0.12	8	0.49 ± 0.03	8	0.91 ± 0.05 *	8	0.68 ± 0.03	8	58.3 ± 7.3	8	0.86 ± 0.08	8	6.00 ± 0.42 *	8	5952 ± 588	8	98.9 ± 13.5
2	9	1.41 ± 0.09	9	0.48 ± 0.02	9	1.05 ± 0.03 *	9	0.58 ± 0.03	9	63.5 ± 4.9	9	1.01 ± 0.07	8	5.53 ± 0.34 *	8	6340 ± 490	9	83.3 ± 6.8
3	8	1.46 ± 0.08	8	0.55 ± 0.02*	8	0.75 ± 0.03 *	8	0.59 ± 0.02	8	50.2 ± 6.4	8	0.87 ± 0.05	8	5.09 ± 0.30	8	6103 ± 628	8	118 ± 17.0
4	5	1.58 ± 0.33	5	0.42 ± 0.03*	5	0.72 ± 0.03 *	5	0.56 ± 0.06	5	36.8 ± 4.7	5	0.78 ± 0.03	5	6.37 ± 0.48 *	5	5091 ± 207	5	129 ± 13.0
5	5	1.23 ± 0.14	5	0.40 ± 0.01*	5	0.81 ± 0.05 *	5	0.57 ± 0.02	5	45.6 ± 4.7	5	0.85 ± 0.05	5	5.03 ± 0.26	5	4753 ± 470	5	88.9 ± 13.8

GR ve EROD dışındaki enzimlerin aktiviteleri µmol/dakika/mg protein±standart hata cinsinden ifade edildi. GR aktivitesi nmol/dakika/mg protein±standart hata, EROD aktivitesi ise pmol/dakika/mg protein±standart hata cinsinden ifade edildi.

* Kontrolden farkın önemli olduğunu göstermektedir ($p<0.05$)

Mikrozomal EROD aktivitesinin gruplar arasında istatistiksel olarak önemli düzeyde bir farklılık göstermediği saptanmış olmakla birlikte, EROD aktivitesi 12 hafta boyunca keten tohumu ilave edilmiş diyetle beslenen hayvanlarda (4. grup) ve MNU uygulamasına maruz kalıp altı hafta boyunca keten tohumu ilaveli diyet alan farelerde (3. grup) göreceli bir artış sergilemiştir ($p>0.05$).

4. Tartışma

Bu çalışmada, i.p yolla MNU'ya maruz kalan dişi farelerin keten tohumu katkılanmış (%10) diyet ile beslenmelerinin, oksidatif stres üzerine etkilerinin değerlendirilmesi amaçlanmıştır. Bunun için farklı uygulama gruplarında belirli sürelerle MNU ve/veya keten tohumu etkisine maruz bırakılan hayvanlarda karaciğer doku örneklerinde çeşitli seçilmiş metabolik enzimlerin, detoksifikasyon enzimlerinin ve antioksidan enzimlerin düzeyleri (AST, ALT, LDH, GST, GR, GPx, CaE, CAT ve EROD) belirlenmiştir. Çalışmalar sonucunda uygulama yapılan gruplardaki fareler ile kontrol grubu fareleri karşılaştırılarak oksidatif stres etmenine karşı keten tohumunun bir koruyucu etkisinin olup olmadığı değerlendirilmiştir.

Oksidatif stres, antioksidan metabolizmaya bağlı olarak yüksek seviyelerde reaktif oksijen türlerinin ve serbest radikallerin oluştuğu fizyolojik bir durumdur (Saha et al., 2017). Oksidatif stresin DNA molekülüne zarar verebileceği, sinyal yollarını değiştirebileceği ve meme, akciğer, karaciğer, kolon, prostat, yumurtalık ve beyin kanserleri dahil olmak üzere çeşitli kanserlerin ilerlemesine yol açabileceği bilinmektedir (Lee et al., 2017; Zhang et al., 2017; Wang et al., 2016; Saed et al., 2017).

Serbest radikallerin zararlı etkileri, fonksiyonel gıdalar gibi bazı maddeler tarafından azaltılabilir veya ortadan kaldırılabilir. Keten tohumu, ω-3 yağ asidi, ALA ve birçok fitokimyasal madde açısından zengin olduğu için fonksiyonel bir gıda olarak kabul görmektedir (Katare et al., 2012). Epidemiyolojik araştırmalar, özellikle ω-3 çoklu doymamış yağ asitleri açısından zengin bir diyet ile beslenilmesinin insan meme kanserinin gelişimine karşı koruma sağladığını iddia etmektedir (Tsubura et al., 2011). Keten tohumu içeriğindeki moleküllerin antioksidan kapasitesinin yüksek olması nedeniyle, oksidatif stres kaynaklı hastalıklara karşı önleyici/koruyucu olabileceği iddia edilmektedir (Udenigwe & Aluko, 2012; Bhatia et al., 2007).

MNU, DNA ile etkileşime girebilen alkilleyici bir ajandır. MNU uygulaması sonucunda mutasyonların meydana gelmesi hedef organlarda kanser riskini artırabilir veya aşırı DNA hasarı onarılmadığında hassas doku veya organlarda hücre ölümüne neden olabilir. Literatürde MNU uygulayarak hayvan dokularında kanser oluşturulan çalışmalar mevcuttur (Tsubura et al., 2011). Hücre içinde oksijenin metabolize edildiği her yerde antioksidanlar, oksijen ara metabolitlerini azaltmak için hızlı ve özgül olarak çalışırlar. Antioksidan savunmada, öncelikle etkili olanlar enzimatik antioksidanlardır (Xu & Qian, 2011). Bu nedenle doku hasarını saptamada biyobelirteç olarak kullanılan enzim aktivite değerleri önem taşımaktadır. Organizmalarda, radikal oksijen türlerinin oluşumu ile hücresel bileşenlerin oksidatif hasarı sonucu gerçekleşen oksidatif strese engel olmak için, antioksidan enzimlerin aktivite (GST, GR, GPx, CAT) düzeylerinin artması ile antioksidan savunmanın arttırılması amaçlanır (Pandey et al., 2003). Oksidatif stresin değerlendirilmesinde kullanılan parametrelerden CAT, GPx ve GR oksidatif stresin önüne geçilmesinde diğer antioksidan enzimlere göre daha fazla rol almaktadır. Ancak çalışmamızda, GST, CAT, GPx ve GR aktiviteleri için kontrol ile uygulama grupları karşılaştırıldığında çeşitli değişimler olsa da bu değişimlerin istatistiksel olarak önemli olmadığı saptanmıştır. Ksenobiyotik metabolizmasında II. basamak biyotransformasyon olaylarında yer alan GST'nin, ksenobiotiklere maruz kalmanın bir biyobelirteci olarak kullanılabileceği çeşitli çalışmalarda gösterilmiş ve çeşitli organik bileşiklere maruz kalma sonrası hepatik GST aktivitesinin böbrek, bağırsak gibi diğer organlara kıyasla daha fazla indüklendiği bildirilmiştir. (Ferrari et al., 2007; Skouras et al., 2003). GST aktivitesinin artışı, kirleticilerin yarattığı strese organizmanın gösterdiği adaptasyon olarak değerlendirilmektedir (Ozmen et al., 2008). Çalışmamızda karaciğer GST aktivitesinin MNU uygulanan 2. 3. ve 5 gruplarda istatistiksel olarak anlamlı bulunmasa da bir düşüş gösterdiği tespit edilmiştir. MNU uygulaması ve beraberinde keten tohumu tüketen tüm bu gruplarda, MNU'nun oluşturduğu toksik etkiyi ortadan kaldırmada keten tohumunun olumlu katkılar sağlayabileceği fikri oluşmaktadır. Benzer şekilde, Rickard et al. (1999) yaptığı bir çalışmada, keten tohumu ile beslenmenin, tümör gelişimi üzerinde etkili olmadığını, fakat MNU indüksiyonu ile oluşturulan meme tümöründeki ilerlemeyi durdurduğunu rapor etmiştir. Başka bir çalışmada CCl_4 enjeksiyonu yapılan albino sıçanlarda, keten tohumu ilaveli diyetle beslenmenin karaciğer üzerinde iyileştirici etkileri gösterilmiştir (Rajesha et al., 2006). Benzer şekilde karaciğer hasarının tamirinde keten tohumunun koruyucu rolünün belirlenmesi ile ilgili olarak sıçanlar üzerinde yapılan çalışmalarda da keten tohumunun iyileştirici etkisi olduğu belirtilmektedir (Bishri, 2013; Bernacchia et al., 2014).

Farelerde MNU maruziyetinin toksik etkilerini değerlendirmek için CaE aktivitesi belirlenmiştir. CaE aktivitesi 1., 2. ve 4. grupta önemli düzeyde artış göstermiştir ($p<0.05$). En fazla artışın ise sadece keten tohumu tüketen grupta (Grup 4) olduğu belirlenmiştir. Bu artışın, keten tohumu içeriğindeki biyokimyasal moleküllerden kaynaklanabileceği düşünülmektedir. Önceki çalışmalar, CaE'nin hem Faz-I detoksifikasyon enzimi olduğunu hem de bazı toksik ajanlar tarafından inhibe edildiğini göstermektedir (Wheelock et al., 2005, Ozkaya et al., 2018). Ayrıca, CaE'nin ksenobiyotiklere karşı koruyucu rolünden dolayı, özellikle 1. ve 2. gruplardaki CaE aktivesindeki artışa MNU maruziyetinin neden olduğu düşünülmektedir.

ALT ve AST hepatosit hasarı sonucu kana salınan intrasellüler enzimlerdir. Bu enzimin kanda artışı karaciğer hasarı göstergesidir (Ersoy, 2012). ALT aktivitesinde, 3. gruptaki farelerin karaciğer dokusunda kontrole göre önemli bir artış belirlenmesine rağmen 4. ve 5. gruplarda kontrole göre anlamlı düşüş belirlenmiştir. Rekha & Hamid (2013) tarafından gerçekleştirilen bir çalışmada, pestisit uygulamasının albino rat karaciğerlerinde detoksifikasyonuna sebep olarak ALT değerlerini arttırdığı tespit edilmiştir. Lamfon (2011) tarafından gerçekleştirilen bir diğer çalışmada ise, Metalaxyl fungusit uygulamasının fare karaciğer hücrelerinde hasara neden olmak suretiyle serum AST ve ALT düzeylerini arttırdığı, zencefil uygulamasının ise karaciğer hücrelerinde toksisiteyi azaltarak AST ve ALT düzeylerinde tekrar bir düşüşe neden olduğu rapor edilmiştir. LDH, hücre hasarı için iyi bilinen bir biyokimyasal belirteçtir ve genellikle kimyasal veya toksik maddelerin neden olduğu hepatotoksisiteyi değerlendirmek için kullanılır (Sokmen et al., 2012). Oksidatif stres koşulu altında çeşitli dokularda LDH enzim aktivitesinin karaciğer dokusu hasarlarına bağlı olarak artabileceği iddia edilmektedir (Kumar et al., 2003). Ancak çalışmamızda, LDH aktivitesinin tüm gruplarda kontrole göre azaldığı ve bunun istatistiksel olarak anlamlı olduğu bulunmuştur ($p<0.05$). Gruplar kendi içinde değerlendirildiğinde sadece keten tohumu ile beslenen 4. gruptaki LDH enzim aktivite değeri en düşük olarak tespit edilmiştir. 4. Gruptaki düşük LDH aktivitesinin, keten tohumunun koruyucu etkisinden dolayı olduğu düşünülmektedir.

Son yıllarda organizmada sağlık durumunun ve toksik ajanlara yanıtın belirlenmesinde özellikle polisiklik aromatik hidrokarbon türü çevresel kirleticilere karşı oluşan yanıtta EROD anahtar belirteç enzim olarak kabul edilmektedir. Hepatik EROD aktivitesinin ksenobiyotikler, sıcaklık, hormonlar, beslenme ve sağlık durumu gibi çok sayıda faktörlerden etkilendiği de bildirilmiştir (Whyte et al., 2000). Çeşitli stres durumlarına (kirleticiler, kimyasallar vb.) maruz kalma sonrası karaciğer tahribatı nedeniyle bu enzimlerin karaciğerdeki aktivitesinin arttığı bilinmektedir (Rao, 2006). Çalışmamızda, MNU ve keten tohumu uygulanan grupların EROD aktivitesi düzeylerinde bazı değişimler

olmasına rağmen, bu değişimlerin istatistiksel olarak önemli olmadığı belirlenmiştir. Ayrıca, hem mikrozomal EROD hem de sitozolik CAT, GPx, GR, GST ve AST enzim aktivitelerinde gruplar arasında anlamlı farklılıkların olmamasının, çalışmada uygulanan doz ve süreyle ilgili olabileceği düşünülmektedir.

Sonuç olarak belirtilen süre ve dozda MNU uygulaması sonucunda farelerin karaciğer dokularındaki bazı enzim aktivitelerinde tespit edilen farklılıklar, antioksidan savunma sistemindeki bozulmaların bir göstergesi olabilir. Ancak, genel olarak MNU uygulanan farelerde keten tohumunun uygulanan doz ve süreye bağlı olarak iyileştirici/koruyucu etkisinin olmadığı ileri sürülebilir. Elde edilen veriler doğrultusunda, MNU ve keten tohumunun etkilerinin daha iyi anlaşılabilmesi için yeni çalışmalar ile desteklenmesi gerekmektedir.

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Research Article/Araştırma Makalesi

Notes on the Distribution of the Genus *Oreochromis* in the East Mediterranean Region of Turkey

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Abstract: Tilapias are freshwater species which are the common name of three genera of cichlids, *Oreochromis*, *Sarotherodon,* and *Coptodon*. Many of the species belonged to these genera have been used for aquaculture products. Although their main native distribution areas are tropical and subtropical regions, they have spread to other areas due to their high invasive characteristics. This study was aimed to investigate the distribution of genus *Oreochromis* in the East Mediterranean Region of Turkey. The study was conducted at 18 stations located in Adana and Mersin from 2014 to 2017. The introduction of the species in these systems was mainly recorded weed control, transfers of fisherman, and escape from aquaculture facilities. However, the distribution of genus *Oreochromis* in Turkey and their effects on the aquatic ecosystems are unknown. The results showed that the species of genus *Oreochromis* were easily adapted the different conditions such as various flow rates, salinity, and temperature.

Keywords: Tilapia, *Oreochromis*, freshwater systems, native, invasive.

Oreochromis Cinsinin Türkiye'nin Doğu Akdeniz Bölgesi'ndeki Dağılımına İlişkin Notlar

Öz: Tilapialar, üç cins çiklit olan *Oreochromis*, *Sarotherodon* ve *Coptodon*'un ortak adı olan tatlı su türleridir. Bu cinslere ait türlerin çoğu, su ürünleri yetiştiriciliğinde kullanılır. Ana doğal dağılım alanları tropikal ve subtropikal bölgeler olmasına rağmen, yüksek istilacı özelliklerinden dolayı diğer bölgelere yayılmışlardır. Bu çalışma Türkiye'nin Doğu Akdeniz Bölgesi'nde *Oreochromis* cinsinin dağılımını araştırmayı amaçlamıştır. Çalışma, 2014-2017 yılları arasında Adana ve Mersin'de bulunan 18 istasyonda gerçekleştirildi. Bu sistemlere türlerin girişi, ağırlıklı olarak yabancı ot kontrolü, balıkçı transferleri ve su ürünleri tesislerinden kaçış olarak kaydedildi. Fakat *Oreochromis* cinsinin Türkiye'deki dağılımı ve sucul ekosistemler üzerindeki etkisi halen bilinmemektedir. Sonuçlar, *Oreochromis* türlerinin çeşitli akış hızlarına, tuzluğa ve sıcaklığa sahip farklı koşullara adapte olduğunu göstermektedir.

Anahtar kelimeler: Tilapia, *Oreochromis*, tatlı su sistemleri, doğal, istilacı.

1. Introduction

The family Cichlidae, within the order Perciformes, is one of the most abundant families of fish. They are tropical freshwater species and their native habitats are mainly located tropical and subtropical regions of America and Africa, as well as Madagascar, India, and Sri Lanka (Salzburger & Meyer, 2004; Maan & Sefc, 2013). However, they can also be found as non-native species such as Florida in America (Schofield et al., 2014), in North, South and Central America (Cassemiro et al., 2018), in Thailand (Nico et al., 2007), in Germany (Lukas et al., 2017), in Portugal (Carecho et al., 2018), and in Russia (Zworykin & Pashkov, 2010). The family is represented by 250 genera, including Tilapia (Froese & Pauly, 2019).

Tilapia is the most well-known member of this family as a common name of the three genera that are *Oreochromis*, *Sarotherodon*, and *Coptodon* (Mohamed & Al-wan, 2020), each includes many species such as Nile tilapia *Oreochromis niloticus*, Magadi tilapia *Sarotherodon alcalicus grahami,* and Guinean tilapia *Coptodon guineensis*. Among tilapias, the genus *Oreochromis* is represented with 89 species. They inhabit in mainly freshwater as well as estuaries as non-native species in almost 100 countries and as native species in Africa. They possess a big slender body shape with small eyes and a terminal mouth (Froese & Pauly, 2019).

According to the Food and Agriculture Organization of the United Nations (FAO, 2018), this genus has an important possession of aquaculture. The farming of tilapias and other cichlids is currently increasing, having reached 1.6 million tonnes in 2016. For instance, Nile tilapia *Oreochromis niloticus* was the most produced aquaculture followed by carps, at 8% of total production in 2016. In addition, the other species of *Oreochromis* spp. was also produced at 2% of the total in 2016. The most produced culture of *Oreochromis* spp. is currently established on hybrids between *O. niloticus, O. aureus,* and *O. mossambicus* species (D'Amato et al., 2007). They are not only used as food sources but also used for their skin as the leather of making clothing, shoes, belts, and other accessories due to being a large fish. They have also been used as a disease control vector such as for malaria and Zika (FAO, 2018). Besides their use in aquaculture, leather textile, and vector, they are popular in the aquarium trade due to their various

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coloration and behavior habits (Welcomme, 1988).

In Turkey, the aquaculture of tilapia production has changed over the years. According to the Turkish Statistical Institute (2020), tilapia was produced 32 tons in 2014, 12 tons in 2015, 58 tons in 2016, 8 tons in 2017, and 12 tons in 2018. However, some of the species escaped and released into the other freshwater systems (Dikel & Celik, 1998; Celik & Gökce, 2003; Gökce et al., 2003). *O. aureus* and *O. niloticus* were introduced and now are found as non-native species in open systems including reservoirs, rivers, and closed systems. Their introduction in the freshwater systems resulted in different aims (Innal, 2012). It was reported that *O. niloticus* found in Asi River (Gürlek, 2004), Köyceğiz Lake (Yılmaz, 2009), Damsa Dam Lake (Mert & Çiçek, 2010), Sakarya River basin (Emiroğlu, 2011), Pınarbaşı Creek (Burdur) (Innal & Sungur, 2009), and *O. aureus* found in Seyhan Dam Lake (Gökce et al., 2003). However, the knowledge of the distribution and effects of *Oreochromis* spp. in the natural freshwaters of Turkey is scarce. Therefore, in this study, the existence of this species in Adana and Mersin region of Turkey and their possible introduction to these systems have been reported.

2. Material and Methods

The study was carried out from November 2014 to June 2017 in the Eastern Mediterranean Region (Adana and Mersin) in accordance with the internationally accepted principles for laboratory animal use and care that followed the Local Ethics Committee of Experimental Animals (Decision Number: 93773921-18, Date: 20 February 2013). A total of 18 sites (Ceyhan River, Bahçe Channel, Kulak Creek, Çakırören Creek, Karagöçer Creek, Köprügözü Channel, Terliksiz DSI pump channel, Seyhan River, Baharlı Creek, Berdan River, Atalar Channel, Keloğlu Channel, Kapızlı Creek, Göksu River, Paradeniz Lagoon and Channel, Akgöl Channel, Kurtuluş Village Channel, Arkum Channel), representing a variety of habitats (including river and creeks, irrigation canals, lagoonal canals), were repeatedly surveyed. Sampling sites with its locality and habitat description are given in Table 1. Sampling localities are given in the map below (Fig. 1).

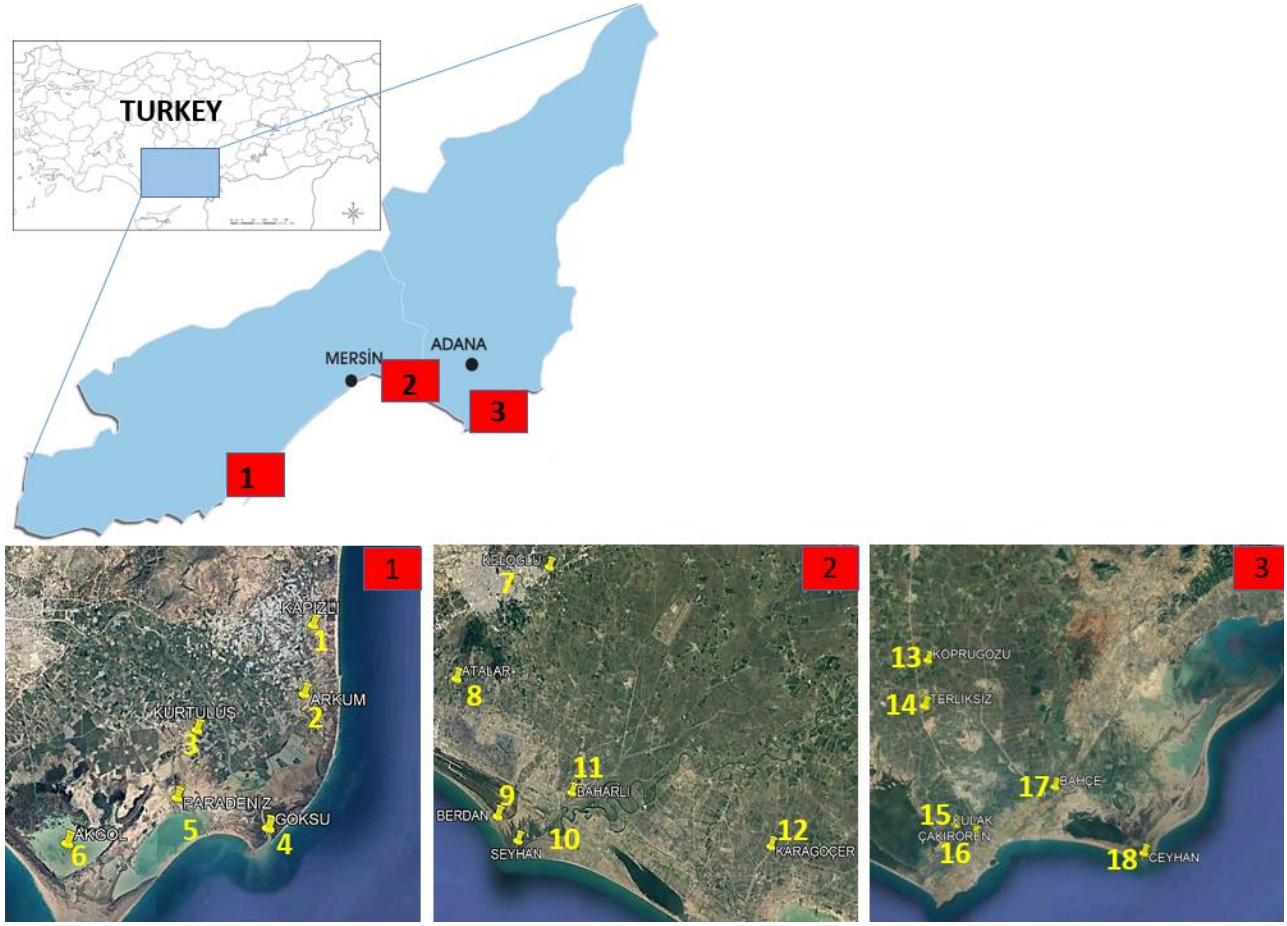

Figure 1. Map of Turkey showing sampling localities. Region 1. (1- Kapızlı Creek, 2- Arkum Channel, 3- Kurtuluş Village Channel, 4- Göksu River, 5- Paradeniz Lagoon and Channel, 6- Akgöl Channel); Region 2. (7- Keloğlu Channel, 8- Atalar Channel, 9- Berdan River, 10- Seyhan River, 11- Baharlı Creek, 12- Karagöçer Creek); Region 3. (13- Köprügözü Channel, 14- Terliksiz DSI pump channel, 15- Kulak Creek, 16- Çakırören Creek, 17- Bahçe Channel, 18- Ceyhan River)

Oreochromis species of the systems were sampled using gill nets of various mesh sizes (10, 17, 23, and 30 mm bar lengths), cast nets, and fish traps. The fish were kept in 4% formaldehyde until they were brought to the Biology Laboratory of Burdur Mehmet Akif Ersoy University. Before the determination of the species, the fish in formaldehyde were kept in water for a day to remove the formaldehyde. Then, the fish were kept in 70% ethanol to be analyzed. Fish species were identified according to Kottelat and Freyhof (2007).

3. Results

Species of *Oreochromis* established in the sampling localities and their purpose for introduction are given in Table 2.

Water channels in Göksu Wetland and Çukurova region are subject to many introductions and transfers of *Oreochromis* species for weed control and aquaculture purpose. *Oreochromis* species were detected in 18 systems in Mersin and Adana region. In the studied systems, non-indigenous *Oreochromis* species have been identified along with native fish species *Acanthobrama orontis* (Berg, 1949), *A. marmid* (Heckel, 1843), *Luciobarbus pectoralis* (Heckel, 1843), *Garra culiciphaga* (Pellegrin, 1927), *Cyprinus carpio* (Linnaeus, 1758), *Chondrostoma ceyhanensis* (Küçük et al., 2017) and, members of Mugilids.

Table 1. Sampling sites with its locality and habitat description

No	Locality	Region	Flow velocity	Latitude	Longitude
1	Kapızlı Creek	Silifke-Mersin	Steady	36°22'27.06"N	34°03'54.72"E
2	Arkum Channel	Silifke-Mersin	Steady	36°20'55.02"N	34°03'41.42"E
3	Kurtuluş Village Channel	Silifke-Mersin	Steady	36°20'05.77"N	34°00'45.18"E
4	Göksu River	Silifke-Mersin	Fast	36°17'57.80"N	34° 2'53.12"E
5	Paradeniz Lagoon and Channel	Silifke-Mersin	Steady	36°18'35.10"N	34°00'13.55"E
				36°18'27.51"N	34° 0'39.00"E
6	Akgöl Channel	Silifke-Mersin	Steady	36°19'50.65"N	33°56'14.74"E
7	Keloğlu Channel	Tarsus-Mersin	Steady	36°55'33.16"N	34°56'35.15"E
8	Atalar Channel	Tarsus-Mersin	Steady	36°50'50.15"N	34°51'13.66"E
9	Berdan River	Tarsus-Mersin	Moderate	36°47'47.40"N	34°56'18.43"E
10	Seyhan River	Tarsus-Mersin	Fast	36°43'50.61"N	34°54'45.74"E
11	Baharlı Creek	Tarsus-Mersin	Steady	36°45'54.22"N	34°57'53.13"E
12	Karagöçer Creek	Karataş-Adana	Slow	36°43'35.23"N	35°09'25.18"E
13	Köprügözü Channel	Karataş-Adana	Slow	36°44'37.23"N	35°20'41.79"E
14	Terliksiz DSI pump channel	Karataş-Adana	Slow	36°40'00.28"N	35°19'56.71"E
15	Kulak Creek	Karataş-Adana	Steady	36°35'34.79"N	35°22'52.84"E
16	Çakırören Creek	Karataş-Adana	Slow	36°36'08.90"N	35°21'44.36"E
17	Bahçe Channel	Karataş-Adana	Steady	36°37'41.81"N	35°28'13.09"E
18	Ceyhan River	Karataş-Adana	Fast	36°34'10.83"N	35°33'36.15"E

Table 2. Species of *Oreochromis* established in the sampling localities and their purpose for introduction

No	Locality	Species	Purpose of introduction
1	Kapızlı Creek	*Oreochromis* sp.	Weed control
2	Arkum Channel	*Oreochromis* sp.	Unknown
3	Kurtuluş Village Channel	*Oreochromis niloticus*	Unknown
4	Göksu River	*Oreochromis niloticus*	Unknown
5	Paradeniz Lagoon and Channel	*Oreochromis niloticus*	Unknown
6	Akgöl Channel	*Oreochromis* sp.	Unknown
7	Keloğlu Channel	*Oreochromis* sp.	Weed control
8	Atalar Channel	*Oreochromis* sp.	Weed control
9	Berdan River	*Oreochromis niloticus*	Weed control and transfers of fisherman
10	Seyhan River	*Oreochromis niloticus*	Escape from Aquaculture facilities
11	Baharlı Creek	*Oreochromis niloticus*	Escape from Aquaculture facilities
12	Karagöçer Creek	*Oreochromis* spp.	Weed control
13	Köprügözü Channel	*Oreochromis* sp.	Weed control
14	Terliksiz DSI pump channel	*Oreochromis* sp.	Weed control
15	Kulak Creek	*Oreochromis niloticus*	Weed control and transfers of fisherman
16	Çakırören Creek	*Oreochromis* sp.	Weed control
17	Bahçe Channel	*Oreochromis niloticus*	Weed control and transfers of fisherman
18	Ceyhan River	*Oreochromis niloticus*	Escape from Aquaculture facilities

4. Discussion

Freshwater ecosystems possess rich biodiversity, an active role in water cycling, and an important source of food and water for human populations (Havel et al., 2015). They are invaded by non-native species via international shipping, aquaculture, ornamental fish training, biological control of diseases, new fisheries techniques, and inter-basin transfers (Tarkan et al., 2015). Although the biological invasions by non-native species may have positive effects on the native ecosystems such as aquaculture, ornamental and recreational purposes (Ewel et al., 1999), they cause adverse effects on native biota including extinctions of endemic and native species and even human health as carrying parasites (Ferrari & Hoffman, 1992).

The first introduction of non-native tilapias is inferred to occur in Java (Indonesia) in the 1930s because of the aquarium releasing of Mozambique tilapia, *O. mossambicus* (Courtenay & Williams, 1992). Fish began to be used as aquaculture products - in terms of being in the category of white meat such as chicken meat and being cheap compared to beef and pork in the 1970s. similar chicken meat and cheap economic prices comparing with beef and pork in the 1970s. As they are known as 'aquatic chicken', tilapias are very convenient to produce and trade in the aquaculture sector. It was shown in 1993 that *O. niloticus* grew 60% faster than the other tilapias (Canonico et al., 2005). Besides, they have important characteristics to be selected as valuable aquaculture products, including the relationship between extreme environment conditions and sex-differences (Hekimoğlu et al., 2019; Dussenne et al., 2020), as a biomarker for disease control (Huang et al., 2018; Chen et al., 2019; Yin et al., 2018; Wu et al., 2019), and for evaluating the effects of environmental pollutants (Yıldırım et al., 2006; Benli & Ozkul, 2010; Beryl et al., 2019; Ibrahim, 2020). Hence, the Genetic Improvement of Farmed Tilapia (GIFT) program was established and GIFT has invested many types of research and projects to improve the farming performance of tilapias, especially in Asia (Dey et al., 2000).

Turkey is the country with the richest biodiversity in Europe and the Middle East. Its geographic location provides high endemism and genetic diversity (Demirayak, 2002) and also increases the introduction of non-native species from Europe, Asia, and the Middle East (Tarkan et al., 2015). The number of non-native fish species recorded in

Turkey has increased in recent years. Thirty non-native fish species were recorded in 2019, including *Oreochromis aureus* (Steindachner 1864) and *O. niloticus* (Linnaeus 1758) (Innal & Sungur, 2019). *Oreochromis* spp. was first introduced in Turkey in the 1970s by transplanting into Lake Burdur but they were all dead due to temperature differences (Gürlek, 2004). Then, several tilapia species brought from Syria and introduced into Seyhan Dam Lake by General Directorate of State Hydraulic Works in 1976. Afterward, owing to scientific researches their counts were increased and transferred to several Fisheries Institutes and research facilities. In the meantime, they were introduced intentionally/ accidentally in the freshwater systems (Altun et al., 2006). They can easily adapt to the newly introduced environment, reproduce rapidly, and become invasive where they affect native species, especially endemic species. However, they distribute easily and rapidly through lentic and lotic ecosystems, human impact is the first reason to introduce the non-native species in the natural ecosystems (Tarkan, 2013). According to the General Directorate of State Hydraulic Works (2020), studies are continued to introduce with suitable fish species within the scope of aquaculture activities in reservoirs and dam lakes including commercial hunting, fish farming in net cages, aquaculture, amateur hunting, and development of other aquaculture models. Besides, some species are used for the biological control of diseases. It is stated that the first vaccination of *Gambusia holbrooki* in the freshwaters of Turkey as a precaution for the biological control of malaria against vector mosquitoes between 1920 and1929 (Walton et al., 2012). Even though *Oreochromis mossambicus* were used as a bioindicator organism in different types of studies including disease infection (Yılmaz et al., 2013; Gültepe et al., 2014; Yilmaz et al., 2014) in the universities of Turkey by making its culture, the species has not been recorded in open freshwater systems in Turkish.

Although the genus of *Oreochromis* distribution of Turkey has been studied, the effects of the species have not been known in these systems. However, there is some information about their effects on the other aquatic systems. Bittencourt et al. (2014) reported that the fish composition of the Amazonas River (Brazil) changed the invasion of *O. niloticus*. Although the other native cichlid species were in the river, in a short time *O. niloticus* was higher biomass than the others. Because of filter-feeding omnivorous species, Nile tilapia caused the changing of plankton biomass during and after an algal bloom in tropical lakes in the Rio Grande do Norte (Brazil) (Vasconcelos et al., 2018). The changes in the population of Nile tilapia and red-spotted sunfish (*Lepomis miniatus*) that live in the same environmental conditions were shown with the experimental design of the estuaries of the Gulf of Mexico. The red-spotted sunfish population decreased when there was a predator in the tank with both species. Thus, it was indicated that Nile tilapia was more competitive than the other species (Martin et al., 2010). According to Khan et al. (2011), the native fish species of Pakistan have been under threat due to the introduction of non-native species including *O. niloticus, O. aureus,* and *O. mossambicus*. Comparıng the feeding behavior of Nile tilapia with/without introducing another species (Nile perch, *Lates niloticus*) in Lake Nabugabo (Uganda) showed that herbivorous feeding was features of Nile tilapia without Nile perch (Bwanika et al., 2006).

The present study showed that freshwater systems in Adana and Mersin are the convenient habitat for *Oreochromis* spp. Because they are easily adapted to the different environmental conditions, including salinity and temperature (Ford et al., 2019), they inhabit these systems where the morphological, chemical, and biological structures of 18 systems differ from each other, especially in Ceyhan, Seyhan, and Goksu River. They were detected in the systems where some endemic species were also detected. Their effects on these endemic species have not been known yet. During the field studies, it was observed that *Oreochromis* spp. found dense populations in Seyhan River and Baharlı Creek. The introduction of the species in these stations was determined as weed control, transfers of fisherman, and escape from the aquaculture facilities. Therefore, we can specify the factors affecting the distribution of this genus as anthropogenic factors. Another anthropogenic factor was identified that this genus is used as a food source. People who lived in the villages around the stations caught *Oreochromis* spp. from the stations, especially from creeks. Due to the pollution of these systems, health problems may occur in the future for those who feed on these fish and in the aquatic ecosystem in which they are located.

5. Conclusion

As a consequence of this research, it is revelaed that *Oreochromis* spp. inhabits within the freshwaters in Adana and Mersin due to the resembling environmental conditions of their native habitats. Their feeding characteristics and high reproduction rate cause their predominant population there. Besides, there are many native fish species in the stations and the effects of *Oreochromis* spp. are still not clear. Further research should be done to figure out the effects of *Oreochromis* spp. on native and endemic fish species and sufficient control methods should be developed for this invading species.

Ethics committee approval: This study was performed in accordance with ethical standards of animal experiments. Legal research ethics committee approval permissions for the study were obtained from the Mehmet Akif Ersoy University, Animal Experiments Local Ethics Committee (No: 93773921-18).

Conflict of interest: The authors declares that there is no conflict of interest.

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Research Article/Araştırma Makalesi

Effects of Altitude and Temperature on Erythrocyte Morphology of *Emys orbicularis* (Linnaeus, 1758) and *Mauremys rivulata* (Valenciennes, 1833)

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Abstract: The decrease in erythrocyte size of animals live at high altitudes yields an evolutionary advantage to survive by providing adaptation to colder temperatures and low partial oxygen pressures. We examined the effect of geographical changes on the erythrocyte morphology of two terrapins, *Emys orbicularis* and *Mauremys rivulata*, and analyzed whether such erythrocyte characteristics as size and volume changed at high altitudes and different temperatures. We found out that the erythrocyte characteristics varied both within and between the populations. They varied depending on altitude for *E. orbicularis* and on temperature for *M. rivulata*. However, the erythrocyte characteristics were not correlated with the environmental parameters, except between sunshine duration and erythrocyte length, size, and nucleus volume for *E. orbicularis*.

Keywords: Hematology, blood cell, European pond turtle, Caspian turtle.

Yükseklik ve Sıcaklığın *Emys orbicularis* (Linnaeus, 1758) ve *Mauremys rivulata* (Valenciennes, 1833)'nın Eritrosit Morfolojisi Üzerine Etkisi

Öz: Yüksek rakımlarda yaşayan hayvanlarda eritrosit boyutlarındaki azalma, düşük sıcaklıklara ve düşük kısmi oksijen basıncına adaptasyon sağlayarak hayatta kalmalarına evrimsel bir avantaj kazandırmaktadır. Coğrafik değişikliklerin eritrosit morfolojisi üzerindeki etkilerini iki tatlısu kaplumbağası türü olan *Emys orbicularis* ve *Mauremys rivulata*'da inceledik ve boyut ve hacim gibi eritrosit özelliklerinin yüksek rakım ve farklı sıcaklıklarda değişip değişmediğini analiz ettik. Eritrosit özelliklerinin hem populasyon içerisinde hem de populasyonlar arasında farklılık gösterdiğini belirledik. *E. orbicularis* için yüksekliğe bağlı olarak, *M. rivulata* içinse sıcaklığa bağlı olarak değişiklik göstermektedir. Bununla birlikte, *E. orbicularis* için güneşlenme süresi ile eritrosit uzunluğu, büyüklüğü ve nukleus hacmi arasındaki korelasyon dışında, eritrosit özellikleri ile çevresel parametreler arasında korelasyon görülmemektedir.

Anahtar kelimeler: Hematoloji, kan hücresi, Benekli Kaplumbağa, Çizgili Kaplumbağa.

1. Introduction

High-altitude habitats force animals to adapt to low oxygen levels (Lu et al., 2015). These animals must maintain the balance between O_2 supply and O_2 demand (Ramirez et al., 2007; Storz et al., 2010). When O_2 is lower than needed, all vertebrates face death owing to its essential role for brain functions (Lutz & Kabler, 1997). Lower vertebrates are prone to survive at low O_2 levels due to the efficient anaerobic periods in short terms; however, turtles are even more resilient since their anaerobic periods can be much longer than those of the other animals (Jackson, 2002). High-altitude environments lead to physiological difficulties for animals due to the harsh conditions particularly colder temperatures and low partial oxygen pressures (P_{O2}) as compared to low-altitude environments (Storz & Moriyama, 2008; Su et al., 2018). High-altitude hypoxia leads to an increase in the number of erythrocytes (Su et al., 2018). Vertebrates have a capacity to endure high altitudes and manage to survive despite the decreases in O_2 tension that potentially restrict the aerobic life (Samaja et al., 2003; Weber, 2007). Although some investigations stated slight or no correlation between blood parameters and altitudinal distribution in reptiles (e.g. Dessauer, 1970; Ruiz et al., 1993), others showed higher hematologic values in upland-distributed species (Vinegar & Hillyard, 1972). The variation in erythrocyte sizes with regard to altitudinal differences may be explained by the effect of surface on gas exchange; for example, a small blood cell permits more gas exchange than a larger one. As in anurans, the ones that live at higher altitudes have smaller erythrocytes (Baraquet et al., 2013). Temperature is another important factor that affects the blood parameters and the number of the red blood cells. Hemoglobin contents increase in cold environments whereas they decrease at warmer temperatures (Washburn & Huston, 1968; Moye et al., 1969).

Haematology of Anatolian terrapins were studied previously (e.g., Yılmaz & Tosunoğlu, 2010; Tosunoğlu et al., 2011). Some of these studies focused on the erythrocyte morphology (e.g., Metin et al., 2006; Arıkan & Çiçek, 2010; Çiçek et al., 2015), but none of them discussed the effects of environmental factors. Herein, we aimed to understand the relationship between geographical and environmental factors, such as altitude, temperature, precipitation, and sunshine duration differences, and the erythrocyte morphology of *E. orbicularis* and *M. rivulata*.

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2. Material and Methods

We conducted field surveys at seven localities in southern Anatolia (Table 1). All the populations of *E. orbicularis* varied by altitude [0-1193 m above sea level (asl)] as well as mean temperature, annual precipitation, and sunshine duration (between 1940 and 2019; the Turkish State Meteorological Service). The three populations of *M. rivulata* were similar in terms of altitude (0 and 11 m asl); therefore, we compared them with regard to other environmental variables.

Table 1. Environmental characteristics of study sites and the number of specimens sampled for each location.

Loc. no	Loc. name	Coordinate	Altitude	Annual Mean Temperature (°C) (1940-2019)	Annual Mean of Daily Sunshine Duration (h) (1940-2019)	Total Monthly Precipitation Average (mm) (1940-2019)	Species	Number F	M	J	Total
1	Muğla/ Dalaman	36°47'29"N 28°48'49"E	0 m	15.5	87	1214.8	*E. orbicularis*	3	3	-	6
							M. rivulata	3	4	-	7
2	Hatay/ Tekebaşı	36°03'18"N 36°02'03"E	0 m	18.3	86	1168.2	*E. orbicularis*	4	2	1	7
							M. rivulata	4	3	-	7
3	Mersin / Silifke	36°36'39"N 33°57'21"E	11m	19.1	89.5	615.8	*E. orbicularis*	1	-	-	1
							M. rivulata	4	1	-	5
4	Isparta/ Barla	38°00'32"N 30°47'6"E	917 m	12.2	85	570.2		2	6	-	8
5	Burdur/ Gölhisar	37°08'14"N 29°30'26"E	935 m	13.2	89	428		4	4	-	8
6	Konya/ Karapınar	37°42'54"N 33°35'36"E	1093m	11.6	88.7	327.7	*E. orbicularis*	6	4	-	10
7	Konya/ Suğla	37°20'25"N 32°02'22"E	1193m	12	94.4	340.7		1	-	-	1

The current study was conducted in the context of the project that was confirmed by Animal Ethics Committee of Ege University (Approval number: 2010-013). We collected blood samples from the caudal vein by using heparinized glass capillaries. Five to ten blood smears were prepared immediately for each individual and all individuals were released after the blood samples had been collected. The prepared blood smears were stained with Wright's stain. For each blood smear, 40 erythrocytes were chosen randomly and measured by using an eyepiece ocular micrometer (Olympus CX31). Erythrocyte length (EL), erythrocyte width (EW), nuclear length (NL), and nuclear width (NW) were measured. The volumes of erythrocytes (EV) and their nuclei (NV) were calculated according to the following formulae: $EV = (EL \times EW^2) \times (\pi/6)$ [μm^3] and $NV = (NL \times NW^2) \times (\pi/6)$ [μm^3]. In addition, the nucleocytoplasmic ratios (NR) were calculated according to the following formula: NR = NV / (EV – NV) (Uca, Arıkan, & Çiçek, 2017). The blood smears of 41 *E. orbicularis* and 19 *M. rivulata* specimens were examined.

All statistical analyses were performed by PAST vers.3 (Hammer et al., 2001). Normality of the measurements was tested by using the Kolmogorov-Smirnov D test. If the data set was distributed normally (Kolmogorov–Smirnov D test, $P \geq 0.05$), then parametric tests were used for comparison. The correlations between erythrocyte characteristics and environmental parameters were compared via the Spearman correlation test.

3. Results

The erythrocytes of both *E. orbicularis* and *M. rivulata* are ellipsoidal and have an ellipsoidal nucleus located at the center of the cell (Fig. 1 and 2). The cytoplasms are yellowish pink and the nuclei are dark purple due to Wright's stain.

The comparison of *E. orbicularis* populations showed that the mean EL, EW, and EV were minimum at Loc3 which was the hottest locality with the annual mean temperature of 19.1°C. The mean NL, NW, NS, and NV were minimum while the mean EW, EV, and ES were maximum at Loc4 which had the minimum sunshine duration.

Loc6 and Loc7 have similar environmental characteristics such as temperature, precipitation, and altitude because of their proximity. They are both situated in the same province, Konya. They have the minimum annual mean temperature and the total monthly precipitation average while having the maximum altitude among the study sites. All the mean nuclear measurements were maximum at these two localities (Table 2). However, there is only one environmental parameter, sunshine duration, correlated to EL, ES, and NV. While ES and NV were positively affected from sunshine duration, EL was affected negatively. Apart from this, there is no statistically significant correlation detected between erythrocyte morphology and the other environmental parameters (Table 4).

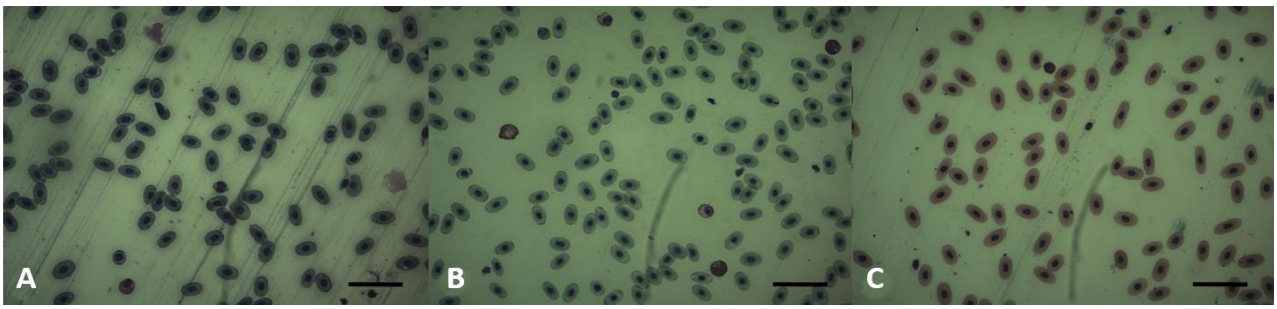

Figure 1. Photomicrographs of *Emys orbicularis* erythrocytes from A. Loc7 (1193 m asl); B. Loc1 (0 m asl) and C. Loc2 (0 m asl). Horizontal bar: 20 µm.

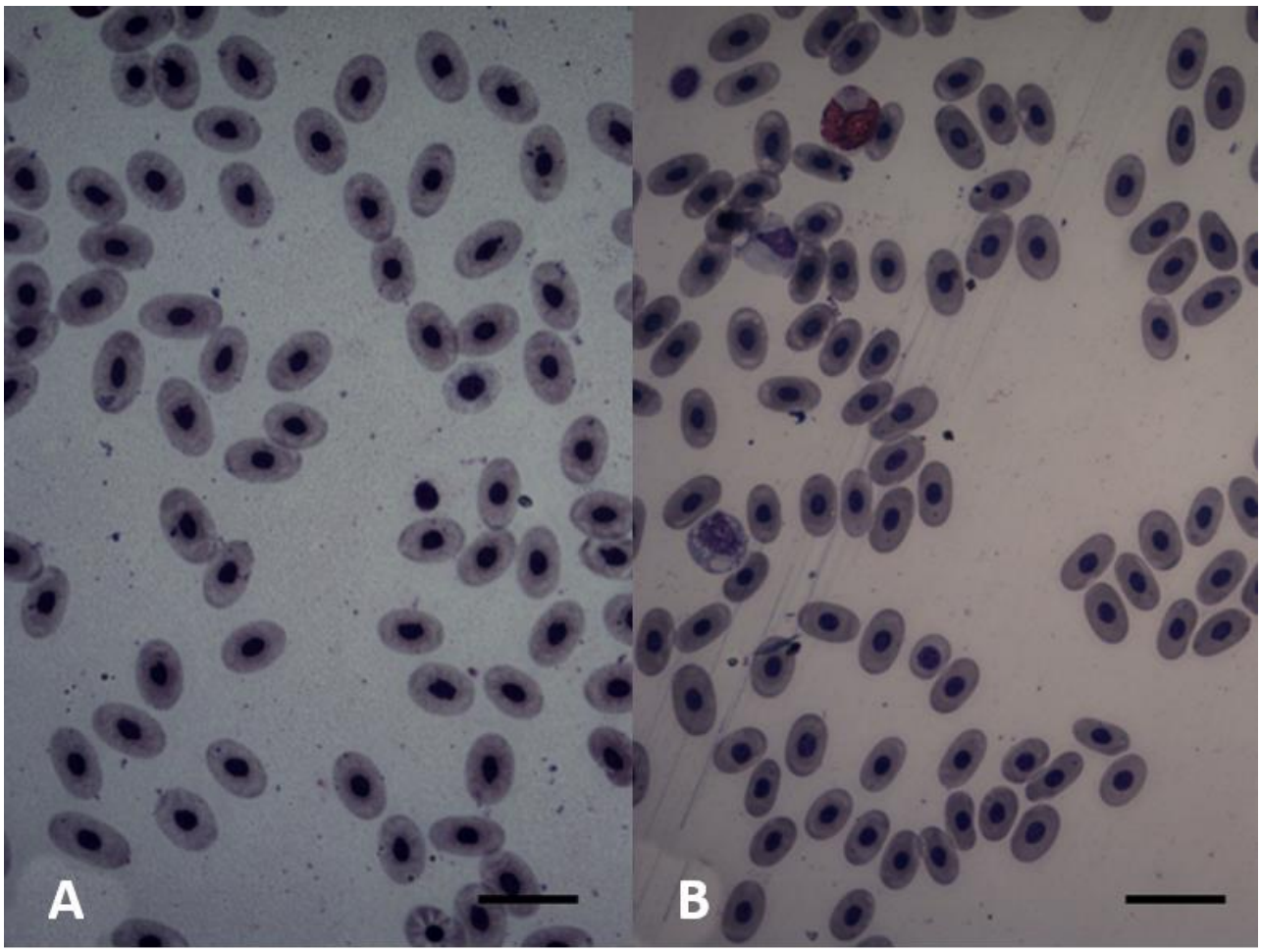

Figure 2. Photomicrographs of *Mauremys rivulata* from A. Loc1 and B. Loc3. Horizontal bar: 20 µm.

Because of all the populations of *M. rivulata* studied were at sea level, we did not compare them in terms of altitude. All measurements except NR were maximum at Loc1 which was the westernmost locality with the minimum annual mean temperature among the study sites. Moreover, all the values except EW were minimum at Loc3 which have the maximum annual mean temperature but the minimum sunshine duration and annual mean precipitation. Although Loc2 was the easternmost locality, measurements from this population have average results except EW and NR (see Table 3). Furthermore, there is no statistically significant correlation detected between erythrocyte characteristics and the environmental parameters for *M. rivulata*.

4. Discussion

Environmental conditions like season, geographical position, mean temperature, and precipitation influence many physiological processes such as age, sex, reproduction, and blood parameters of vertebrates (Moye et al., 1969; Jacobson, 2007). Erythrocytes are the transporters of oxygen and carbon dioxide.

Table 2. The mean erythrocyte measurements of *Emys orbicularis*.

Measurement* Locality	EL (µm)	EW (µm)	EL/EW	NL (µm)	NW (µm)	NL/NW (µm)	NS (µm²)	ES (µm²)	NS/ES (µm²)	NR (µm³)	EV (µm³)	NV (µm³)
Loc1	19.56 ±1.84	11.15 ±1.04	1.76 ±0.19	5.59 ±0.76	4.29 ±0.76	1.32 ±0.15	19.20 ±5.77	174.71 ±26.11	0.11 ±0.03	0.047 ±0.022	1291.25 ±300.68	57.72 ±28.71
Loc2	20.37 ±1.73	11.25 ±1.29	1.83 ±0.25	5.72 ±0.77	4.23 ±0.68	1.38 ±0.29	19.05 ±4.09	180.34 ±27.6	0.10 ±0.02	0.044 ±0.018	1373.56 ±352.99	55.26 ±19.48
Loc3	18.09 ±1.26	10.12 ±1.05	1.80 ±0.24	5.50 ±0.73	4.28 ±0.62	1.30 ±0.22	18.58 ±4.14	143.9 ±19.29	0.13 ±0.03	0.0105 ±0.003	928.02 ±227.81	54.42 ±19.19
Loc4	19.91 ±1.57	11.87 ±1.28	1.69 ±0.19	5.46 ±0.77	3.94 ±0.47	1.39 ±0.24	16.94 ±3.23	186.09 ±27.82	0.09 ±0.17	0.0326 ±0.0112	1493.71 ±369.68	45.30 ±13.77
Loc5	19.08 ±1.58	11.80 ±1.35	1.63 ±0.20	5.53 ±0.74	4.32 ±0.65	1.30 ±0.25	18.80 ±3.92	177.35 ±28.79	0.10 ±0.02	0.0425 ±0.0168	1418.32 ±379.38	55.48 ±19.13
Loc6	19.81 ±1.86	11.08 ±1.40	1.80 ±0.22	5.92 ±0.71	4.25 ±0.62	1.41 ±0.22	19.90 ±4.38	172.96 ±30.29	0.11 ±0.02	0.0489 ±0.0186	1302.81 ±426.42	58.00 ±21.18
Loc7	18.81 ±1.32	11.68 ±1.28	1.62 ±0.18	6.00 ±0.70	4.53 ±0.61	1.35 ±0.25	21.34 ±3.84	173 ±26.02	0.12 ±0.02	0.0525 ±0.0175	1367.46 ±359.24	65.62 ±18.65
Total	19.67 ±1.77	11.42 ±1.34	1.74 ±0.22	5.65 ±0.77	4.21 ±0.65	1.36 ±0.24	18.81 ±4.39	176.94 ±28.95	0.10 ±0.02	0.0423 ±0.0191	1370.02 ±380.36	54.45 ±21.04

Table 3. The mean erythrocyte measurements of *Mauremys rivulata*.

Measurement* Locality	EL (µm)	EW (µm)	EL/EW (µm)	NL (µm)	NW (µm)	NL/NW (µm)	NS (µm²)	ES (µm²)	NS/ES (µm²)	NR (µm³)	EV (µm³)	NV (µm³)
Loc1	20.47	11.58	1.78	6.49	4.65	1.41	23.80	186.44	0.12	0.0563	1456.85	75.44
	±1.49	±1.17	±0.19	±0.92	±0.62	±0.27	±4.96	±25.77	±0.02	±0.0185	±342.87	±23.53
Loc2	18.62	10.69	1.76	5.85	4.47	1.33	20.66	156.29	0.13	0.0627	1125.48	63.28
	±1.52	±1.09	±0.23	±0.82	±0.67	±0.25	±4.65	±20.16	±0.03	±0.0259	±246.90	±21.81
Loc3	17.50	10.93	1.61	5.74	4.23	1.37	19.20	150.30	0.12	0.0555	1105.36	55.76
	±1.27	±0.99	±0.18	±0.75	±0.63	±0.22	±4.36	±18.09	±0.03	±0.0248	±222.94	±20.75
Total	18.83	11.02	1.72	6.01	4.45	1.37	21.15	163.40	0.13	0.0587	1217.02	64.65
	±1.84	±1.15	±0.22	±0.89	±0.67	±0.25	±5.00	±26.18	±0.03	±0.0238	±313.27	±23.29

Table 4. Correlation between erythrocyte morphology and environmental parameters according to spearman rho test (Bold values indicate statistically significance).

		EL (µm)	EW (µm)	EL/EW (µm)	EV (µm³)	ES (µm²)	NL (µm)	NW (µm)	NI/NW (µm)	NV (µm³)	NS (µm²)	NS/ES (µm²)	NR (µm³)
Altitude	**cc**	-0.342	0.234	-0.618	0.414	0.414	0.218	0.414	-0.288	0.183	0.450	0.198	0.541
	p	0.452	0.613	0.139	0.355	0.355	0.638	0.355	0.531	0.694	0.310	0.670	0.210
Annual Mean	**cc**	-0.143	-0.321	0.468	-0.464	-0.107	-0.631	-0.500	0.000	0.145	-0.643	-0.321	-0.571
Temperature (°C)	**p**	0.760	0.482	0.289	0.294	0.819	0.129	0.253	1.000	0.756	0.119	0.482	0.180
Annual Mean of Daily	**cc**	**-0.893**	-0.321	-0.396	0.393	**0.821**	-0.523	0.429	**-0.750**	**0.800**	0.250	-0.464	0.536
Sunshine Duration (h)	**p**	**0.007**	0.482	0.379	0.383	**0.023**	0.229	0.337	**0.052**	**0.031**	0.589	0.294	0.215
Total Monthly	**cc**	0.179	-0.143	0.396	-0.393	-0.214	-0.360	-0.357	0.286	-0.091	-0.393	-0.214	-0.464
Precipitation Average (mm)	**p**	0.702	0.760	0.379	0.383	0.645	0.427	0.432	0.535	0.846	0.383	0.645	0.294

*EL: Erythrocyte Length; EW: Erythrocyte Width; NL: Nucleus Length; NW: Nucleus Width; NS: Nucleus Size; ES: Erythrocyte Size; NR: Nucleocytoplasmic Ratio; EV: Erythrocyte Volume; NV: Nucleus Volume.

** cc: correlation coefficient; p: significance level

The size and shape of erythrocytes influence the efficiency and rate of gas exchange (Hartman & Lessler, 1964). Small cells have a larger surface/volume ratio than large cells have; thus, they have a greater rate of oxygen exchange than larger ones do (Stacy et al., 2011; Javanbakht et al., 2013).

Because erythrocytes play an essential role in vertebrate physiology, it is important to understand their morphological variations to be able to identify changes depending on the environmental factors. Changes in temperature and rainfall affect food availability which influences the metabolism of ectotherms (e.g., Litzgus, & Hopkins, 2003; Setlalekgomo et al., 2012).

The increase in the number of red blood cells depending on altitude is an adaptation to be able to provide more gas exchange in a relatively short time. Therefore, the size and volume of the erythrocytes decrease at higher altitudes (Ramirez et al., 2007) whereas the number increases. Lu et al. (2015) reported that *Phrynocephalus erythrurus*, a reptile that lives at high altitudes, has more erythrocytes with less cell volume than those living at low altitudes. Accordingly, similar results were found for the Tibetan chicken (Su et al., 2018). Temperature is another factor that affects cell morphology. It has been known that the erythrocyte volumes and sizes increase at colder temperatures (e.g., Washburn & Huston, 1968; Moye et al., 1969). In parallel with these results, we calculated the minimum erythrocyte sizes and volumes from Loc3.

We found out that the mean values of erythrocyte sizes and volumes of *E. orbicularis* varied among the study sites; however, these variations did not correlate with environmental changes. There was a difference of 1193 m between the lowest and highest localities; yet, we discovered that the minimum and maximum values of the measurements were at Loc3 and Loc4, respectively. Altitude difference between these localities is 906 m, nearly the same between Loc1 and Loc7, but the annual mean temperature difference is nearly twice than those between Loc1 and Loc7 (3.5°C between Loc1-Loc7, 7.1°C between Loc3-Loc4). Thus, we concluded that the temperature also affects the erythrocyte sizes but we do not have correlation on erythrocyte morphology for our sample.

Making a comparison of erythrocyte sizes with the literature is not straightforward because few studies indicate in which altitude the samples were taken and the others do not provide any information about environmental parameters. For this reason, it is not possible to discuss all environmental factors with the published literature. Maximum values for EL and ES were reported 22.5 µm, 249.4 µm² respectively for *Emys trinacris* (Arizza et al., 2014). Maximum ES for Anatolian populations of *E. orbicularis* is 225.1 µm² reported from captive specimens (Metin et al., 2006).

Our results indicated that sunshine duration positively correlate with ES and NV whereas negatively correlate with EL for *E. orbicularis*. This means the turtles have longer sunshine duration, have much bigger spherical erythrocytes, and have larger nucleus volume. Basking is a common behavior in ectotherms and many freshwater turtles bask either at the surface of the water or on the substrates (Bulté & Blouin-Demers, 2010). Sunshine duration becomes crucial for regulation of metabolic rate to this respect. Bulté & Blouin-Demers (2010) suggested that basking behavior allows turtles to increase their metabolic rate by up to 30.1%.

Erythrocytes of *M. rivulata* were large both in size and in volume at Loc1, while they were small at warmer Loc3. Because of the altitudes were the same among the localities of *M. rivulata*, the differences between the Loc1 and Loc3 were temperature which could be an effective environmental variable on erythrocyte morphology and precipitation. Loc1 had been a cooler habitat with more rainfall for the turtles in which we measured larger cells and nuclei.

In conclusion, our results support the literature knowledge that the temperature and the altitude affect the erythrocyte sizes. The turtles living in the higher temperature or/and elevation are tend to have smaller erythrocyte sizes. However, these variations on erythrocyte sizes do not correlate with environmental parameters in all studied populations. Further studies with more populations that have various intermediate environmental characteristics are needed for a better point of view.

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Conflict of interest: The authors declares that there is no conflict of interest.

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Research Article / Araştırma Makalesi

The Ornithological Diversity of the Province of Kilis

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Abstract: In this study, it is aimed to determine the bird diversity of Kilis province that is located in the southeast Anatolia, Turkey. For this purpose, field survey was conducted between February 2017 and June 2018. As a result, 129 species, belonging to 43 families (16 ordos) that live in settlements, agricultural fields, wetlands, forests, and steppes were recorded. They were categorized as 76 residents, 43 summer migrants, 8 winter visitors, and 2 transit migrants. According to the Red Data Book of Turkey, these species were listed as 2 "Vulnerable", 3 "Near threatened", and 124 "Least concern".

Keywords: Avifauna, *Clanga clanga*, *Streptopelia turtur*, redlist.

Kilis İli'nin Ornitolojik Çeşitliliği

Öz: Bu çalışmada Türkiye'de Güneydoğu Anadolu'da yer alan Kilis ilinin kuş çeşitliliğinin belirlenmesi amaçlanmıştır. Bu amaçla Şubat 2017 ve Haziran 2018 tarihleri arasında saha araştırması yapılmıştır. Bu çalışma sonucunda, yerleşimlerde, tarım alanlarında, sulak alanlarda, ormanlar ve bozkırlarda yaşayan (16 ordo) 43 familyaya ait 129 tür kaydedilmiştir. Türler, 76 yerli, 43 yaz göçmeni, 8 kış ziyaretçisi ve 2 transit göçmen olarak kategorize edilmiştir. Türkiye Kırmızı listesine göre, bu türlerin 2'si "hassas", 3'ü "neredeyse tehdit altında", 124'ü "asgari endişe" olarak listelenmektedir.

Anahtar kelimeler: Avifauna, *Clanga clanga*, *Streptopelia turtur*, kırmızı liste.

1. Introduction

Located at the intersection of three continents, Turkey is a bridge and in crossroads in terms of biodiversity (Karakaş, 1999; Ambarlı et al., 2016; Küçükosmanoğlu et al., 2019). Moreover, three (the Caucasus, the Mediterranean, and Irano-Anatolian) of the world's 34 hot spots that are rich in biodiversity, which must be protected immediately, are located in Turkey (Küçükosmanoğlu et al., 2019). Turkey displays the continent features in terms of bird species diversity. Two of the four bird migration routes in the Palearctic region cross over Anatolia. Among the most important reasons behind Turkey's avifaunistic richness are its location on major migration routes, geographical location, abundance of wetlands, and habitat diversity (Erciyas Yavuz, 2014; Erciyas Yavuz et al., 2015; Karaardıç & Erdoğan, 2019).

Ornithofaunistic research in Turkey with several exceptions, are devoted to exhibit local ornithofauna. Introducing a complete ornithofauna is possible by handling such local studies together (Kiziroğlu, 2015). Although there is no comprehensive study on the determination of the bird species in Kilis province, there is an extensive study on the determination of bird species in the province of Gaziantep which is the single neighbor of Kilis (Toprak et al., 2008). Also, bird observations made in this province on Kuşbank and Trakuş websites also have great contributions in determining the bird diversity of the province (Anonim, 2020a).

Kilis province, with its intact forest area located on the northwestern provincial border, with the agricultural lands located in the east and with 6 rivers and dams and ponds on these rivers, has important resting, feeding, and breeding areas for both migratory species and resident species. There is no important bird area (IBA) for the bird species in Kilis province. However, there is the Elbeyli Key Biodiversity Area (KBA) (Eken et al., 2006). This area is the important resting and feeding area for the bird species in the east region of Kilis.

The aim of this study is to determine the bird diversity of Kilis. Our study will contribute to the studies done to list the bird species that are Turkey's biological richness.

2. Material and Methods

Kilis is situated in the C6 square in the southern part of Turkey and is bordered by Syria to the south and Gaziantep to the north, east, and west, with coordinates $36^{0}37'$-$37^{0}2'$ N, $36^{0}42'$-$37^{0}34'$ E and its total area is 1.642 km^2. Kilis province is zoo-geographically located in the western Palearctic. Also, the area falls in the Mediterranean and Irano-Turanian floristic regions and possesses a semiarid Mediterranean climate (Solak et al., 2014). Average annual temperature of experimental area was 17.1°C according to the long-term meteorological data (1959-2019) (Anonim, 2020b). The forest and shrub vegetation of study area is composed of *Pinus brutia*, *Pinus pinea*, *Arbutus andrachne*, *Pistacia lentiscus*, *Erica arborea*, *Styrax officinalis*, *Cistus creticus*, and some members of Juniperus, Cupressus, Quercus, Fraxinus, Populus, Acacia, Olea and Acer (Solak et al., 2014).

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The study was conducted between the dates of February 2017-June 2018, in a total of 13 field surveys on 41 days. 659 field spots between 371 and 972 meters were visited in Polateli, Elbeyli, and Musabeyli regions. Within the scope of the project, field survey was carried out on all 21 parcel on 1/25.000 scale map of Kilis province. The field survey was carried out for 3 days in winter, 9 days in spring, 3 days in summer, and 4 days in autumn in 2017 and 6 days in winter, 13 days in spring, and 3 days in summer in 2018. Field studies were conducted in wetlands, forests, steppes, settlements, and agricultural areas. The sites were selected by evaluation of the satellite images or on-site assessment.

In the survey and the assessment of bird population, 12x50 binoculars, telescope (with 15-60 magnification), DSLR camera with 100-400 mm and 50-500 mm lenses, video camera, and GPS were used as the basic equipment. "Collins Bird Guide, Red Data Book and The Pocket Guide Birds of Türkiye" were used in identification and status of species in Kilis (Svensson et al., 2009; Kiziroğlu, 2015; IUCN, 2021). Generally, counting and dot counting methods were used over the line transect when determining the species. Both direct and indirect observations such as sounds, wing sounds, feathers, singing of the birds were used in the field survey.

Field survey was carried out to monitor both breeding and after breeding population in the study area. Investigations were conducted using transect method. Also, spot observation method (waiting for 45-60 minute durations on spots that have full sight of the area) was used to survey water birds and shore birds. Observations were conducted over 5 hours after the dawn and 3 hours before the nightfall since birds are very active and meteorological events like heat and moisture prevent determining the bird species from long distances. Species name, number of individuals, breeding status, habitat type, threats, date, hour and geographical coordinates were recorded. The geographical coordinates of the observed species were identified using the geographical positioning system (GPS) device Garmin Etrex 10. Coordinates were recorded as latitude and longitude in decimal degrees and referenced to the World Geodetic system established in 1984 (WGs84). These locations and their coordinates have been recorded in Noah's Ark Biodiversity Database (Anonim, 2020c).

3. Results and Discussion

As a result of this study, 129 species belonging to 43 families were determined. The list regarding the seasonal status of the birds determined during our field study and the classification of the birds determined in terms of order and family level, breeding status and Redlist status are as follows (Table 1).

Table 1. According Lepage (2020) the list of bird species determined.

Scientific Name	English Name	BERN	CITES	IUCN	Status	Breeding Status
Alectoris chukar	Chukar Partridge	III	OL	LC	R	B
Francolinus francolinus	Black Francolin	III	OL	LC	R	B
Coturnix coturnix	Common Quail	III	OL	LC	R	B
Spatula querquedula	Garganey	III	OL	LC	S	B
Spatula clypeata	Northern Shoveler	III	OL	LC	R	B
Anas platyrhynchos	Mallard	III	OL	LC	R	B
Apus apus	Common Swift	III	OL	LC	S	B
Apus pallidus	Pallid Swift	II	OL	LC	S	B
Apus affinis	Little Swift	III	OL	LC	S	B
Cuculus canorus	Common Cuckoo	III	OL	LC	S	B
Columba livia	Rock Dove	III	OL	LC	R	B
Columba palumbus	Common Wood Pigeon	OL	OL	LC	R	B
Streptopelia turtur	European Turtle Dove	III	OL	VU	S	B
Streptopelia decaocto	Eurasian Collared Dove	III	OL	LC	R	B
Spilopelia senegalensis	Laughing Dove	III	OL	LC	R	B
Gallinula chloropus	Common Moorhen	III	OL	LC	R	B
Fulica atra	Eurasian Coot	III	OL	LC	R	B
Tachybaptus ruficollis	Little Grebe	II	OL	LC	R	B
Podiceps cristatus	Great Crested Grebe	III	OL	LC	R	B
Himantopus himantopus	Black-winged Stilt	II	OL	LC	R	B
Vanellus vanellus	Northern Lapwing	III	OL	NT	R	B
Charadrius dubius	Little Ringed Plover	II	OL	LC	S	B
Actitis hypoleucos	Common Sandpiper	II	OL	LC	S	B
Chroicocephalus genei	Slender-billed Gull	II	OL	LC	R	B
Chroicocephalus ridibundus	Black-headed Gull	III	OL	LC	R	B
Ichthyaetus ichthyaetus	Pallas's Gull	III	OL	LC	W	NB
Larus michahellis	Yellow-legged Gull	III	OL	LC	R	B

Scientific Name	English Name	BERN	CITES	IUCN	Status	Breeding Status
Larus armenicus	Armenian Gull	III	OL	NT	R	U
Ciconia nigra	Black Stork	II	II	LC	S	U
Ciconia ciconia	White Stork	II	OL	LC	S	B
Phalacrocorax carbo	Great Cormorant	III	OL	LC	R	B
Ixobrychus minutus	Little Bittern	II	OL	LC	S	B
Nycticorax nycticorax	Black-crowned Night Heron	II	OL	LC	S	B
Ardeola ralloides	Squacco Heron	II	OL	LC	S	B
Bubulcus ibis	Western Cattle Egret	II	OL	LC	R	B
Ardea cinerea	Grey Heron	III	OL	LC	R	B
Ardea purpurea	Purple Heron	II	OL	LC	S	B
Ardea alba	Great Egret	II	OL	LC	R	B
Egretta garzetta	Little Egret	II	OL	LC	R	B
Pandion haliaetus	Western Osprey	III	II	LC	R	U
Clanga clanga	Greater Spotted Eagle	III	II	VU	W	U
Hieraaetus pennatus	Booted Eagle	III	II	LC	R	U
Accipiter nisus	Eurasian Sparrowhawk	III	II	LC	R	B
Circus aeruginosus	Western Marsh Harrier	III	II	LC	R	B
Circus cyaneus	Hen Harrier	III	II	LC	R	U
Milvus migrans	Black Kite	III	II	LC	R	B
Buteo rufinus	Long-legged Buzzard	III	II	LC	R	B
Buteo buteo	Common Buzzard	III	II	LC	R	B
Tyto alba	Western Barn Owl	II	II	LC	S	B
Athene noctua	Little Owl	II	II	LC	R	B
Asio otus	Long-eared Owl	II	II	LC	S	B
Upupa epops	Eurasian Hoopoe	II	OL	LC	S	B
Coracias garrulus	European Roller	II	OL	LC	S	B
Merops apiaster	European Bee-eater	II	OL	LC	S	B
Dendrocopos syriacus	Syrian Woodpecker	II	OL	LC	R	B
Falco tinnunculus	Common Kestrel	II	II	LC	R	B
Lanius collurio	Red-backed Shrike	II	OL	LC	S	B
Lanius senator	Woodchat Shrike	II	OL	LC	S	B
Garrulus glandarius	Eurasian Jay	OL	OL	LC	R	B
Pica pica	Eurasian Magpie	OL	OL	LC	R	B
Coloeus monedula	Western Jackdaw	OL	OL	LC	R	B
Corvus frugilegus	Rook	OL	OL	LC	R	B
Corvus cornix	Hooded Crow	OL	OL	LC	R	B
Corvus corax	Northern Raven	III	OL	LC	R	U
Poecile lugubris	Sombre Tit	II	OL	LC	R	B
Cyanistes caeruleus	Eurasian Blue Tit	II	OL	LC	R	B
Parus major	Great Tit	II	OL	LC	R	B
Alauda arvensis	Eurasian Skylark	III	OL	LC	R	B
Galerida cristata	Crested Lark	III	OL	LC	R	B
Calandrella brachydactyla	Greater Short-toed Lark	II	OL	LC	S	B
Melanocorypha bimaculata	Bimaculated Lark	II	OL	LC	S	B
Melanocorypha calandra	Calandra Lark	II	OL	LC	R	B
Pycnonotus xanthopygos	White-spectacled Bulbul	III	OL	LC	R	B
Hirundo rustica	Barn Swallow	II	OL	LC	S	B
Delichon urbicum	Common House Martin	II	OL	LC	S	B
Cecropis daurica	Red-rumped Swallow	II	OL	LC	S	B
Cettia cetti	Cetti's Warbler	II	OL	LC	R	B

Scientific Name	English Name	BERN	CITES	IUCN	Status	Breeding Status
Aegithalos caudatus	Long-tailed Tit	III	OL	LC	R	B
Phylloscopus trochilus	Willow Warbler	II	OL	LC	T	U
Phylloscopus collybita	Common Chiffchaff	II	OL	LC	R	B
Acrocephalus arundinaceus	Great Reed Warbler	II	OL	LC	S	B
Acrocephalus melanopogon	Moustached Warbler	II	OL	LC	R	U
Acrocephalus scirpaceus	Eurasian Reed Warbler	II	OL	LC	S	U
Iduna pallida	Eastern Olivaceous Warbler	II	OL	LC	S	B
Argya altirostris	Iraq Babbler	III	OL	LC	R	U
Sylvia curruca	Lesser Whitethroat	II	OL	LC	S	B
Sylvia melanocephala	Sardinian Warbler	II	OL	LC	R	B
Sylvia mystacea	Menetries's Warbler	II	OL	LC	S	B
Sitta neumayer	Western Rock Nuthatch	II	OL	LC	R	B
Sitta tephronota	Eastern Rock Nuthatch	II	OL	LC	R	B
Sturnus vulgaris	Common Starling	OL	OL	LC	R	B
Turdus merula	Common Blackbird	III	OL	LC	R	B
Turdus philomelos	Song Thrush	III	OL	LC	W	NB
Cercotrichas galactotes	Rufous-tailed Scrub Robin	II	OL	LC	S	B
Erithacus rubecula	European Robin	II	OL	LC	R	B
Luscinia megarhynchos	Common Nightingale	II	OL	LC	S	B
Irania gutturalis	White-throated Robin	II	OL	LC	S	B
Phoenicurus ochruros	Black Redstart	II	OL	LC	W	NB
Phoenicurus phoenicurus	Common Redstart	II	OL	LC	T	U
Saxicola rubicola	European Stonechat	II	OL	LC	S	U
Saxicola torquatus	Common Stonechat	II	OL	LC	R	U
Saxicola maurus	Siberian Stonechat	II	OL	LC	W	NB
Oenanthe oenanthe	Northern Wheatear	II	OL	LC	S	B
Oenanthe isabellina	Isabelline Wheatear	II	OL	LC	S	B
Oenanthe pleschanka	Pied Wheatear	II	OL	LC	S	B
Oenanthe finschii	Finsch's Wheatear	II	OL	LC	R	B
Oenanthe hispanica	Black-eared Wheatear	II	OL	LC	S	B
Passer domesticus	House Sparrow	OL	OL	LC	R	B
Passer hispaniolensis	Spanish Sparrow	III	OL	LC	R	B
Passer moabiticus	Dead Sea Sparrow	III	OL	LC	R	B
Passer montanus	Eurasian Tree Sparrow	III	OL	LC	R	B
Petronia petronia	Rock Sparrow	II	OL	LC	R	B
Gymnoris xanthocollis	Yellow-throated Sparrow	III	OL	LC	S	B
Motacilla flava	Western Yellow Wagtail	II	OL	LC	S	B
Motacilla cinerea	Grey Wagtail	II	OL	LC	R	B
Motacilla alba	White Wagtail	II	OL	LC	R	B
Anthus pratensis	Meadow Pipit	II	OL	NT	W	NB
Fringilla coelebs	Common Chaffinch	III	OL	LC	R	B
Chloris chloris	European Greenfinch	II	OL	LC	R	B
Rhodospiza obsoleta	Desert Finch	III	OL	LC	R	B
Linaria cannabina	Common Linnet	III	OL	LC	R	B
Carduelis carduelis	European Goldfinch	II	OL	LC	R	B
Serinus serinus	European Serin	II	OL	LC	R	B
Spinus spinus	Eurasian Siskin	II	OL	LC	W	NB
Emberiza calandra	Corn Bunting	III	OL	LC	R	B
Emberiza citrinella	Yellowhammer	II	OL	LC	W	NB
Emberiza cia	Rock Bunting	II	OL	LC	W	NB

Scientific Name	English Name	BERN	CITES	IUCN	Status	Breeding Status
Emberiza caesia	Cretzschmar's Bunting	II	OL	LC	S	B
Emberiza melanocephala	Black-headed Bunting	II	OL	LC	S	B

B: Breeding; NB: Non-Breeding; U: Unknown

According to Kiziroğlu (2015); Resident (R), Summer migrant (S), Winter visitor (W), Transit migrant (T), Vagrant (V)

According to IUCN (2021), Red List categories; LC: Least concern, NT: Near threatened, VU: Vulnerable, EN: Endangered, CR: Critically endangered

According to BirdLife (2015), BERN categories; II: Annex II, III: Annex III, OL: Out of List

According to BirdLife (2015), CITES categories; II: Annex II, III: Annex III, OL: Out of List

In the light of the data obtained as a result of field studies "Yellow-legged Gull (*Larus michahellis*)", Menetries's Warbler (*Sylvia mystacea*)", "The Iraq Babbler (*Argya altirostris*)", "European Stonechat (*Saxicola rubicola*)", "Siberian Stonechat (*Saxicola maurus*)", "Yellowhammer (*Emberiza citrinella*)", "Cretzschmar's Bunting (*Emberiza caesia*)", "Yellow-throated Sparrow (*Gymnoris xanthocollis*)" are listed as new records for the Kilis province.

Finally, as a result of this study, some species that spread in a limited area (Dead Sea Sparrow "*Passer moabiticus*", The Iraq Babbler "*Argya altirostris*", Menetries's Warbler "*Sylvia mystacea*" and Yellow-throated Sparrow "*Gymnoris xanthocollis*") were identified.

4. Conclusion

This study is important as it is the first, regular and long-term research study on the ornithofauna of the area. Among the identified species, it was observed that important species that are in danger of extinction use the area for feeding, breeding, and resting during migration.

According to IUCN Redlist Categories, 2 Vulnerable (Greater Spotted Eagle "*Clanga clanga*" and European Turtle-dove "*Streptopelia turtur*") and 3 Near Threatened (Northern Lapwing "*Vanellus vanellus*", Armenian Gull "*Larus armenicus*" and Meadow Pipit "*Anthus pratensis*") were identified (BirdLife International, 2017a, 2017b, 2018, 2019a, 2019b). "European Turtle-dove (*Streptopelia turtur*)" is a summer migrant and "Northern Lapwing (*Vanellus vanellus*)" is a resident. In our bird list of the results, European Turtle-dove and Northern Lapwing are given as Breeding birds. In Çamurlu mound, two pairs of "European Turtle-dove" were observed that reproduce with nests in June 2018. In Save dam, a pair of "Northern Lapwing" was observed that reproduces with nest in May 2017. As a result, Çamurlu mound and Save dam are important areas for these species and must be protected.

Also, some species (Yellow-legged Gull "*Larus michahellis*", Menetries's Warbler "*Sylvia mystacea*", The Iraq Babbler "*Argya altirostris*", European Stonechat "*Saxicola rubicola*", Siberian Stonechat "*Saxicola maurus*", Yellowhammer "*Emberiza citrinella*", Cretzschmar's Bunting "*Emberiza caesia*", Yellow-throated Sparrow "*Gymnoris xanthocollis*") are listed as new records for the Kilis province as it is determined that these species were not observed in Kilis province in the literature review. Finally, in this province the existence of these species (Dead Sea Sparrow "*Passer moabiticus*", The Iraq Babbler "*Argya altirostris*", Menetries's Warbler "*Sylvia mystacea*" and Yellow-throated Sparrow "*Gymnoris xanthocollis*"), which have spread in a limited area in Turkey, is revealed in our results. Although spread in a limited area, these species, except the Iraq Babbler, are breeding in Kilis.

92 species and 1 subspecies belonging to 38 families were identified in the study to determine the bird diversity between 2003 and2004 in Gaziantep province, which is approximately 45 km northeast of the Kilis province (Toprak et al., 2008). 69 species detected in the study conducted by Toprak et al. (2008) were also detected in our study. However, 24 species detected in the study conducted by Toprak et al. (2008) were not detected in our study. In the study conducted by Toprak et al. (2008), 61 species detected in our study were not detected. These 61 species include both the new records species for the Kilis province and the species which have spread in a limited area in Turkey. 361 species belonging to 68 families were identified in Hatay province, which is approximately 103 km southwest of the Kilis province (Atahan et al., 2008; Ünal, 2016; Lepage, 2021). Hatay has such a large variety of birds, due to its location on an important bird migration route. However, The Iraq Babbler "*Argya altirostris*", Yellow-throated Sparrow "*Gymnoris xanthocollis*" and Dead Sea Sparrow "*Passer moabiticus*" species detected as new records for Kilis province could not be detected in the province of Hatay. This results indicates that these ornithological studies should be repeated regularly in order to follow the distribution status of the species.

Threats such as excessive use of pesticides due to agricultural activities, destruction of nests, excessive use of water, habitat destruction, poaching, and burning of reeds were determined for all bird species during the field studies conducted within the scope of the Biodiversity inventory of Kilis province. The amounts of punishment and counts of inspection should be increased in order to prevent the catching of the species and poaching. In order to prevent excessive use of pesticides and destruction of nests, inspections should be increased and awareness raising meetings should be held for local people. The activities that cause habitat destruction should not be allowed to be carried out in or near the areas where the birds are densely populated; however, if it must be done, it should be done by considering the annual life cycles of the birds such as breeding and hatching. Excessive and unconscious water use should be prevented through local public awareness meetings, administrative fines, and legal regulations. Lastly, hunting the "European Turtle-dove (*Streptopelia turtur*)", which is protected on a world scale, should be banned by the Central Hunting Commission Decisions as soon as possible.

Although, in the literature review, it was determined that this province's bird species list consists of 271 species belonging to 57 families (Kiziroğlu, 2015; Anonim, 2020c), results of this study determined 129 species belonging to

43 families. It is stated in the literature study that the main reason why some bird species cannot be observed in the field studies is the water shortage due to the excessive use in 2017 and 2018. Due to the decrease of water in rivers, dams and ponds, bird species that need their feeding and breeding depending on the water or water edge prefer suitable regions outside the province. For example; the ducks and shore birds determined during the every Mid-Winter Water Bird (KOSK) counts in the Küplüce, Seve and Balıklı ponds was not determined during the 2018 KOSK counts due to the excessive water decrease. To summarize, when the literature data is compared with the field surveys, the ornithofauna of the Kilis consists of 280 bird species belonging to 61 families. If these results are considered as a whole, 280 bird species, which were determined in Kilis province, correspond to more than half of the Turkey's bird list.

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Research Article / Araştırma Makalesi

Chromosome Analysis of *Micaria formicaria* (Araneae: Gnaphosidae) from Turkey

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Abstract: In this study, *Micaria formicaria* (Sundevall, 1831) karyotype properties of the species were analyzed for the first time by using standard giemsa staining method. Gonads derived from the testicles were put through hypotonic application and, fixation and staining processes then, chromosome preparations were prepared. In this study, the number of diploid chromosomes belonging to the species was determined as 2n=22. All chromosomes including sex chromosomes are telocentric type; the sexual chromosome system was determined as X_1X_20. In the meiosis I phases, 10 autosomal bivalent and two heteropicnotic sexual chromosomes with at least one chiasm point were observed. In meiosis II phases, sexual chromosomes showed an isopicnotic feature.

Keywords: Spider, karyotypes, cytogenetic, meiosis.

Türkiye'den *Micaria formicaria* (Araneae: Gnaphosidae) Türünün Kromozom Analizi

Öz: Bu çalışmada *Micaria formicaria* (Sundevall, 1831) türünün karyotip özellikleri, standart giemsa boyama yöntemi kullanılarak ilk kez analiz edilmiştir. Testislerden elde edilen gonadlar hipotonik uygulama, fiksasyon ve boyama işlemlerinden geçirilerek kromozom preparatları hazırlanmıştır. Çalışmada türe ait diploid kromozom sayısı 2n=22 olarak belirlenmiştir. Eşey kromozomları da dâhil tüm kromozomlar telosentrik tipte olup eşey kromozom sistemi X_1X_20 olarak tespit edilmiştir. Mayoz I evrelerinde en az bir kiyazma noktası bulunan 10 otozomal bivalent ve heteropiknotik özellikte iki eşey kromozomu görülmüştür. Mayoz II evrelerinde ise eşey kromozomları izopiknotik özellik göstermiştir.

Anahtar kelimeler: Örümcek, karyotip, sitogenetik, mayoz.

1. Giriş

Örümcekler, şimdiye kadar tanımı yapılmış 49200 tür ile hayvanlar âlemindeki en büyük takımlardan biri olarak kabul edilmektedir (World Spider Catalog, 2021). Artan çalışmalarla birlikte her gün yeni türler keşfedilmekte ve tanımlanan tür sayısının 170.000'e kadar ulaşması beklenmektedir (Sebastian & Mathew, 2009).

Gnaphosidae familyası, 162 cins ve 2566 tür ile örümcek takımı içerisinde bulunan yedinci büyük aile olarak bilinmektedir (World Spider Catalog, 2021). Gnaphosidae familyası üzerine yapılan sitogenetik çalışmaların tanımlanan tür sayısı ile karşılaştırıldığında yeterli olmadığı görülmektedir. Günümüzde karyotip özellikleri bilinen sadece 54 gnafozid türü bulunmaktadır (Araujo et al., 2020). Çalışılan bu türlerde diploid kromozom sayısı 2n=22 ile 30 arasında değişmektedir. Kromozom morfolojisi akrosentrik tipte olup eşey kromozom sistemi $♂X_1X_2$ ve $♀X_1X_1X_2X_2$ olarak belirlenmiştir (Araujo et al., 2020). Kaydedilen bu özellikler çalışılan türlerin büyük çoğunluğunda görülmektedir. Ancak *Drassodes lutescens* (L. Koch, 1839) (Kumbıçak et al., 2014) *Urozelotes rusticus* (L. Koch,1872) (Srivastava & Shukla, 1986) ve tanımı yapılmamış iki *Drassodes* (Srivastava & Shukla, 1986) türünde diploid kromozom sayısı 2n=21 ve X0 eşey kromozom sistemi gibi özelliklere nadiren de olsa rastlanmaktadır.

Gnaphosidae familyası ülkemizde 32 cins ve 147 tür ile temsil edilmektedir (Danışman, Kunt, & Öztürk, 2019). Gnafozidler, Lycosidae ve Salticidae ile ülkemizde sitogenetik olarak en çok çalışılan örümcek ailelerinden biridir. Gnafozid örümcekler üzerine yapılan sitogenetik analizlere ülkemizde yapılan çalışmalarla büyük katkı sağlanmaktadır. Karyotip özellikleri belirlenen 54 türün %44'ünün (24 tür) veri girişi ülkemizdeki çalışmalar sonucu yapılmıştır (Araujo et al., 2020). Ayrıca ülkemizde *Zelotes aeneus* (Simon, 1878) türünde kaydedilen 2n=20 diploid kromozom sayısı ve *Drassodes lutescens* türünde kaydedilen 2n=21 ve X0 eşey kromozom sistemi Gnaphosidae familyası türleri arasındaki farklı karyotip özelliklerinin anlaşılması açısından önem arz etmektedir (Taşdemir, Varol, & Akpınar, 2012; Kumbıçak et al., 2014).

Bu çalışmayla Gnaphosidae familyasına ait *Micaria formicaria* (Sundevall, 1831) türünün sitogenetik özelliklerinin analiz edilmesi ile elde edilen verilerin uluslararası sitogenetik veri bankasına girişi yapılarak Gnaphosidae familyası sitogenetik verilerine katkı sağlanması amaçlanmaktadır.

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2. Materyal ve Metot

Micaria formicaria türüne ait örneklerin toplandığı arazi çalışması örümceklerin üreme davranışlarının arttığı Mart – Mayıs (2018) aylarında gerçekleşmiştir. Örümcekler doğrudan elle toprak yüzeyinden ve taş altlarından canlı olarak yakalanmıştır. Arazi çalışması; Adana-Pozantı, Mersin-Gülek, Kahramanmaraş-Göksun olmak üzere 3 farklı alanda gerçekleştirilmiştir (Tablo 1). Toplanan örnekler ayrı plastik tüpler içerisinde canlı olarak laboratuvara getirilmiştir. Örneklerin tür teşhisleri pedipalp yapısına ve vücut desenlerinin özelliklerine göre bazı literatürler kullanılarak (Miller, 1971; Heimer & Nentwig, 1991; Roberts, 1995; Almquist, 2006) Dr. Zübeyde KUMBIÇAK tarafından yapılmıştır. Çalışmada kullanılan örnekler Nevşehir Hacı Bektaş Veli Üniversitesi, Fen Edebiyat Fakültesi, Genetik Araştırma Laboratuvarında muhafaza edilmektedir.

Tablo 1. *Micaria formicaria* örneklerinin toplandığı lokalitelerin koordinatları.

Table 1. The localities coordinates of *Micaria formicaria* specimens were collected.

Örnek sayısı	Toplama Tarihi	Lokalite Bilgileri
3♂	26 Mart 2018	Adana, Pozantı 37°25'35''N, 34°51'42''E
5♂	1 Nisan 2018	Mersin, Gülek 37°15'47''N, 34°45'15''E
2♂	12 Mayıs 2018	Kahramanmaraş, Göksun 38°00'32''N, 36°28'57''E

Bu çalışma, Bedo (1984) metodunda bazı değişiklikler yapılarak gerçekleştirilmiştir. Erkek örümcekler, pedisel bölgesinden ayrılarak öldürülmüş ve opistosoma kısmından gonadlar elde edilmiştir. Gonadlar, 2–3 ml hipotonik çözelti (0.075 M KCl) eklenmiş tüplerin içerisinde 40 dakika bekletilmiştir. Süre sonunda 2 defa olmak üzere 2000 rpm'de 5'er dakika süreyle santrifüj yapılmış ve her defasında süpernatant kısım uzaklaştırılmıştır. Fiksasyon aşamasına hazır duruma gelen gonadların bulunduğu tüpe fiksatif eklenerek karıştırılmış ve 2000 rpm'de 2 defa olmak üzere 10'ar dakika santrifüj yapılmıştır. Süpernatant kısım atıldıktan sonra elde edilen materyalin üzerine 1ml fiksatif eklenerek karıştırılmış ve karışımdan bir miktar alınarak lam üzerine bırakılmıştır. Preparatlar havada kurumaya bırakılmış daha sonra en az bir gün olmak üzere buzdolabında (+4ºC) bekletilmiştir. Elde edilen tüm kromozom preparatları faz kontrast mikrobunda incelenerek hücre bölünmesi içeren preparatlar tespit edilmiştir. Bu preparatlar daha sonra fosfat tampon içeren %5'lik Giemsa boyası (pH=6.8) ile 50 dk boyanmıştır. Boyama işlemi sonunda preparatlar sırasıyla musluk suyu ve distile su ile yıkanarak RT'de kurumaya bırakılmıştır. Preparatlar mikroskop incelemesi yapılıncaya kadar buzdolabında (+4ºC) muhafaza edilmiştir.

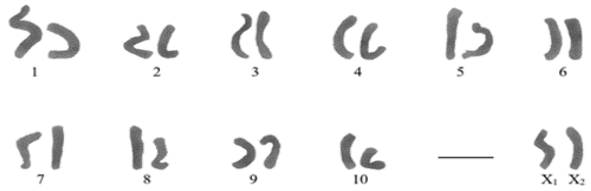
Hazırlanan preparatlar Olympus CX21 araştırma mikroskobunda 10X büyütmede incelenerek erkek ve dişi bireyler için mitotik metafaz ve mayoz evreleri tespit edilmiştir. Kromozomların ayrıntılı olarak incelenmesi ise 100X büyütmede gerçekleştirilmiştir.

Micaria formicaria türüne ait karyotip yapılması aşamasında en az 10 metafaz evresine ait fotoğraflar, Olympus BX53 araştırma mikroskobu ve DP26 kamera sistemi, CellSens programı (Olympus) ile çekilmiştir. Kromozomların uzunlukları CellSens programı ile ölçülmüş ve kromozomların sentromer konumları Levan et al., (1964)'ye göre belirlenmiştir. Kromozomların çiftler halinde sıralanması ise Adobe Photoshop CS6 programı ile gerçekleştirilmiştir.

3. Bulgular

3.1. Karyotip ve Eşey Kromozomu Sistemi

Micaria formicaria türünün erkek bireylerinin mitotik metafaz evresinde diploid kromozom sayısı 2n=22 olarak tespit edilmiştir (Şekil 1). Eşey kromozom sistemi X_1X_20 ve kromozom morfolojisi telosentrik tiptedir. Otozomal kromozom çiftlerinin relatif uzunlukları %11.98 ile %6.59 arasında değişmektedir. İkinci büyük kromozom çiftinden başlayarak en küçük çifte doğru kademli bir azalma görülmektedir. Eşey kromozomlarının relatif uzunlukları X_1 için %8.11 ve X_2 için %7.24 olarak belirlenmiştir (Tablo 2).



Şekil 1. *Micaria formicaria* ait karyogram, 10 çift otozomal kromozomlar ve X_1X_2 şeklindeki eşey kromozomları (Ölçüm=10 µm).

Figure 1. Karyogram of *Micaria formicaria*, 10 pairs of autosomal chromosomes and X_1X_2 shaped sex chromosomes (Measurement = 10 µm).

Tablo 2. *Micaria formicaria* türünün erkek bireyine ait karyotipte kromozom uzunlukları (p-kısa kol, q-uzun kol, p+q-toplam uzunluk, q/p-kol oranı), morfolojileri.

Table 2. Chromosome lengths (p-short arm, q-long arm, p + q-total length, q / p-arm ratio) and morphologies in karyotype of male individual of *Micaria formicaria* species.

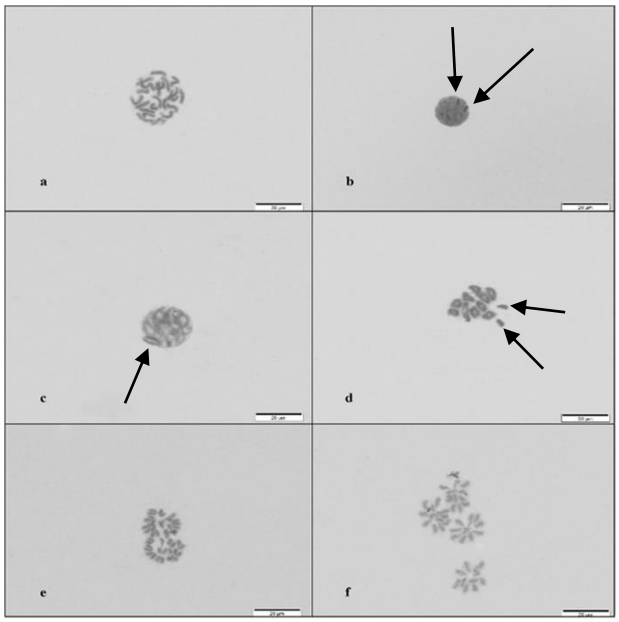
Kromozom No	p (µm)	q (µm)	q/p	Oransal boy (%)	Kromozom morfolojisi
1	0	19.19 ± 3.76	∞	11.98	Telosentrik
2	0	14.63 ± 2.42	∞	9.13	Telosentrik
3	0	14.20 ± 2.57	∞	8.86	Telosentrik
4	0	13.92 ± 2.41	∞	8.69	Telosentrik
5	0	13.48 ± 2.26	∞	8.41	Telosentrik
6	0	12.81 ± 1.83	∞	7.99	Telosentrik
7	0	12.48 ± 1.99	∞	7.79	Telosentrik
8	0	11.94 ± 1.65	∞	7.45	Telosentrik
9	0	11.56 ± 1.59	∞	7.21	Telosentrik
10	0	10.56 ± 1.19	∞	6.59	Telosentrik
X_1	0	13.00 ± 2.42	∞	8.11	Telosentrik
X_2	0	12.40 ± 2.42	∞	7.74	Telosentrik

3.2. Bazı Mitotik ve Mayotik Evrelerinin İncelenmesi

Mitotik metafaz evresinde yoğunlaşarak tam şeklini alan kromozomlar sayılabilir durumdadır. Bu evrede diploid kromozom sayısı 2n=22, kromozom morfolojisi ise eşey

kromozomları da dâhil telosentrik olarak tespit edilmiştir (Şekil 2a).

Mayotik Profaz I'in alt evresi leptotenden sonra eşey kromozomları vezikül hâlinde belirmeye başlayıp ilerleyen evrelerde kısalıp kalınlaşmaya bağlı olarak sayılabilir duruma gelmektedir. Bu sebeple; zigoten (Şekil 2b) ve pakiten (Şekil 2c) evrelerinde kısalıp kalınlaşmaya başlayan eşey kromozomları daha koyu boyanarak pozitif heteropiknotik özellik göstermektedir. Ayrıca pakiten evresinde eşey kromozomlarının terminal uçları ile birbirlerine bağlı oldukları görülmektedir (Şekil 2c). Diploten ve diyakinezde en az bir kiyazma noktası bulunan 10 otozomal bivalent ve 2 eşey kromozomu belirlenmiştir (Şekil 2d). Eşey kromozomları pozitif heteropiknotik özellik göstererek çekirdek yüzeyinde konumlanmaktadır. Anafaz I'de koyu boyanmayan eşey kromozomları izopiknotik özellikte olması sebebiyle otozomlardan ayırt edilememektedir. Bu evrede tüm kromozomlar telosentrik morfolojiden ötürü "V" şeklindedir ve n=11 (10 otozom+X_1X_2) ve n=10 (otozomlar) olmak üzere iki yeni çekirdek kaydedilmiştir (Şekil 2e). Anafaz II evresinde ikisi n=11 diğer ikisi n=10 olmak üzere dört yavru çekirdek "I" şeklinde görülmektedir (Şekil 2f).



Şekil 2. a. Mitotik metafaz, 2n♂: 22, b. Zigoten, c. Pakiten, d. Diploten, 10 otozomal bivalent ve iki eşey kromozomu, e. Anafaz I, f. Anafaz II.

Figure 2. a. Mitotic metaphase, 2n♂: 22, b. Zygotene, c. Pakiten, d. Diplotene, 10 autosomal bivalent and two sex chromosomes, e. Anaphase I, f. Anaphase II.

4. Tartışma ve Sonuç

Örümcekler üzerine yapılan sitogenetik çalışmalar yüzyıldan fazla süredir devam etmesine rağmen bu takımla ilgili veriler istenilen düzeyde değildir. Kromozomların elde edilmesindeki zorluklar ve kromozom yapılarının küçük olması gibi olumsuzluklar nedeniyle sadece 868 türün karyotip analizi yapılmıştır (Araujo et al., 2020).

Örümcekler hakkındaki sitogenetik verilerin büyük çoğunluğu Araneomorphae'nin alt gruplarından biri olan entelejin örümceklere dayanmaktadır (Šťáhlavský et al., 2020). Mevcut örümcek türlerinin yaklaşık %80'ini içeren entelejinler, düşük diploid sayı (2n=10-49) (Kořínková & Král, 2013), kiyazmatik mayoz (Kumbıçak, 2010) ve akrosentrik kromozom morfolojisinin baskın olduğu bir karyotiple nitelendirilmektedir (Araujo et al., 2020). Gnaphosidae familyası 2n=22-30 arasında değişen kromozom sayısı ve genellikle akrosentrik tipteki kromozom morfolojisi ile entelejin örümcekler için karakteristik olan karyolojik özellikleri göstermektedir. Gnaphosidae familyasının eşey kromozom sistemi ♂X_1X_2 ve ♀$X_1X_1X_2X_2$ şeklinde tespit edilmiş olup bu sistem X_1X_20 eşey kromozom sistemi olarak bilinmektedir (Araujo et al., 2020).

Gnaphosidae familyasından sitogenetik analizler sonucu elde edilen veriler homojenlik göstermektedir. Ancak *Drassodes lutescens* (L. Koch, 1839) (Kumbıçak et al., 2014), *Urozelotes rusticus* (L. Koch,1872) (Srivastava & Shukla, 1986) ve tanımı yapılmamış iki *Drassodes* (Srivastava & Shukla, 1986) türünde diploid kromozom sayısı 2n=21 ve X0 eşey kromozom sistemi gibi familya genelinden farklı özelliklerde kaydedilmiştir. Bu farklılığın nedenleri arasında; X_1X_20 eşey kromozom sistemindeki X kromozomlarından birinin aşamalı olarak ortadan kalkması veya X kromozomları arasında distal ya da proksimal füzyondan önce meydana gelen karşılıklı translokasyon gösterilmektedir (Maddison & Leduc-Robert, 2013).

Bu çalışmada Türkiye'den toplanan *M. formicaria* türünün karyotip özellikleri ilk kez analiz edilmiştir. Türün diploid kromozom sayısı 2n=22, eşey kromozom sistemi X_1X_20 ve kromozom morfolojisi telosentrik olarak bulunmuştur. Mayotik Profaz I'den itibaren vezikül halinde belirmeye başlayan eşey kromozomları pozitif heteropiknotik özellik göstermelerinden dolayı otozomlardan ayırt edilebilmektedir. Sonuç olarak elde edilen bu karyolojik veriler Gnaphosidae familyasına ait önceki verilerle uyumluluk göstermektedir.

Etik kurul onayı: Bu çalışma için etik kurul onayı alınmasına gerek yoktur.

Çıkar çatışması: Yazarlar, çıkar çatışması olmadığını beyan etmiştir.

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Research Article / Araştırma Makalesi

Counting Results of Great Bustard (*Otis tarda*, Linnaeus, 1758) between 2013-2020 in Eskişehir, Kütahya, and Afyonkarahisar Provinces

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Abstract: The Great Bustard (*Otis tarda*, Linneaus 1758) is a bird species categorized as "Least Concern" in Europe and "Vulnerable" in the world by the IUCN and listed in both CITES and the Bern Convention Annex II. According to the latest estimates, Turkey's population makes up only 1-2% of the global population, which is represented by 44.000-57.000 individuals. This population study was carried out in 7 different locations in Eskişehir, Kütahya, and Afyonkarahisar provinces between March 2013 and January 2020. In summary, the surveyed areas were recorded as having a total wintering population of 205 individuals, a total breeding population of 100 individuals, and a total summering population of 70 individuals. The surveyed areas represent an average of 17-29% of Turkey's population in wintering period, an average of 8-14% of Turkey's population in breeding period, and an average of 6-10% of Turkey's population in summering period. Urgent implementation of protective measures is required to prevent the population decline of the Great Bustard within the study areas. Detailed ethological studies on the species are recommended as a means of creating new measures to not only stop population decline but to promote population growth to healthy levels.

Keywords: Turkey, vulnerable, Aegean, Inner Anatolia.

Eskişehir, Kütahya ve Afyonkarahisar İllerinde 2013-2020 Yılları Arası Büyük Toy Kuşu (*Otis tarda*, Linnaeus, 1758) Sayım Sonuçları

Öz: Büyük Toykuşu (*Otis tarda*, Linneaus 1758), IUCN tarafından Avrupa'da 'Asgari Endişe Verici' ve Dünya çapında 'Hassas' olarak sınıflandırılan ve hem CITES hem de Bern sözleşmesinde EK-II'de (Kesin Koruma Altındaki Fauna Türleri) listelenen bir kuş türüdür. Son tahminlere göre Türkiye populasyonu, 44.000-57.000 birey ile temsil edilen dünya populasyonunun sadece %1-2'sini oluşturmaktadır. Bu populasyon çalışması, Mart 2013-Ocak 2020 tarihleri arasında, Eskişehir, Kütahya ve Afyonkarahisar illerinde 7 farklı lokasyonda gerçekleştirilmiştir. Özetle, incelenen alanların toplam 205 bireyden oluşan bir kışlama populasyonuna, toplam 100 bireyden oluşan bir üreme populasyonuna ve toplam 70 bireyden oluşan bir yazlama populasyona sahip olduğu kaydedildi. İncelenen alanlar kışlama döneminde Türkiye nüfusunun ortalama %17-29'unu, üreme döneminde Türkiye nüfusunun ortalama %8-14'ünü ve yazlama döneminde Türkiye nüfusunun ortalama %6-10'unu temsil etmektedir. Çalışma alanlarında Büyük Toykuşu populasyonunun azalmasını önlemek için acil koruyucu önlemlerin uygulanması gerekmektedir. Sadece nüfus düşüşünü durdurmak için değil, aynı zamanda nüfus artışını sağlıklı seviyelere çıkarmak için yeni tedbirler oluşturmanın bir yolu olarak türler üzerinde detaylı etolojik çalışmalar önerilmektedir.

Anahtar kelimeler: Türkiye; hassas; Ege; İç Anadolu.

1. Introduction

The Great Bustard (*Otis tarda*) is categorized as "Least concern" in Europe and "Vulnerable" in the world by the IUCN (Birdlife International, 2017). The species is listed in CITES Appendix I-II and the Bern Convention Annex II (Gao et al., 2008; Birdlife International, 2017). The Palearctic distribution range of the Great Bustard has decreased due to various threats during the last two centuries (Alonso et al., 2003b; Karakaş & Akarsu, 2009). Throughout the previous decades, many European populations of the Great Bustard have come close to extinction or have become seriously endangered, with the exception being the Iberian and Russian populations. The Iberian population is considered to be stable and the Russian population is increasing (Alonso et al., 2003a, 2003b; Karakaş & Akarsu, 2009; Birdlife International, 2017).

According to the latest estimates, Turkey's population makes up only 1-2% of the global population, which is represented by 44.000 to 57.000 individuals (Alonso & Palacin, 2010; Birdlife International, 2017). Various population size studies have been conducted on the Great Bustard in Turkey, with each study producing different results. The most up to date studies give the total population size as approximately 700-1200 individuals (Morales & Martin, 2002; Kılıç & Karakaş, 2005; Palacin & Alonso, 2008; Karakaş & Akarsu, 2009; Alonso & Palacin, 2010; Birdlife International, 2017). The species has two discrete subpopulations in Turkey. One of them is located in Eastern and Southeastern Anatolia and the other in the central and inner parts of Central Anatolia (Karakaş & Akarsu, 2009).

Recent Great Bustard population status studies have been conducted on a regional basis (Kılıç & Karakaş, 2005; Özbağdatlı & Tavares, 2006; Karakaş & Akarsu, 2009;

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Karataş, 2012; Azizoğlu, 2013; Karataş & Özelmas, 2013) and the breeding ecology of the Great Bustard in the Muş plain has been studied (Tanrıverdi, 2015). The population status of the Altıntaş Wildlife Development Area and the area between Körhasan – Aktaş – Ortaköy – Başkurt villages have previously been determined (Yarar & Magnin, 1997; Karataş & Özelmas, 2013). The area between Körhasan – Aktaş – Ortaköy – Başkurt villages (a part of the Aliken Key Biodiversity Area) is located in Eskişehir, and the Altıntaş Wildlife Development Area (WDA) is located in Kütahya. They are important bustard breeding areas in the national bustard action plan, since 40-60 individuals breed in the Inner-Western Anatolia region of Turkey (Eken et al., 2006; Özelmas & Karakaya, 2011). In addition, these two areas are a part of the 97 Key Bird Areas (Yarar & Magnin, 1997). Anecdotal evidence for the existence of this species in the province of Afyonkarahisar was given by the local people but had not been scientifically verified.

The presence of Great Bustard populations is also predicted to occur in areas other than the two main study areas. Therefore, our study's main purpose is to determine the population status of the Great Bustard which is distributed in different locations in Eskişehir, Kütahya, and Afyonkarahisar provinces in Turkey. Field surveys were conducted investigating the latest status of the species in areas where they have been proven to exist (Altıntaş Wildlife Development Area and Körhasan – Aktaş – Ortaköy – Başkurt). Also, the existence of the species was investigated in the areas which had unproven anecdotal evidence sourced from interviews conducted with the local residents (Başmakçı – Dazkırı – Evciler, Aydınlar – Yenice – Döğer, Kalkanlı – Kıravdan, Kaymazyayla – Zaferhamit – Yeniköy, Kaymaz – Bahçeçik – Gerenli). Lastly, we investigated the possible presence of the species in areas within the provinces of Eskişehir, Kütahya, and Afyonkarahisar.

2. Material and Methods

2.1. Study Area

The field survey was conducted in 7 different locations in Eskişehir, Kütahya, and Afyonkarahisar provinces. These areas are Körhasan – Aktaş – Ortaköy – Başkurt (Area 1), Kaymaz – Bahçecik – Gerenli (Area 2), Kaymazyayla – Zaferhamit – Yeniköy (Area 3), Kalkanlı – Kıravdan (Area 4), Altıntaş Wildlife Development Area (Area 5), Aydınlar – Yenice – Döğer (Area 6), and Başmakçı – Dazkırı – Evciler (Area 7).

Area 1 is located between Çifteler and Sivrihisar in Eskişehir province (Fig. 1). Area 2 is located in Sivrihisar, Eskişehir province (Fig. 2). Area 3 is located between Çifteler, Sivrihisar and Mahmudiye in Eskişehir province (Fig. 3). Area 4 is located in Odunpazarı, Eskişehir province (Fig. 4). Area 5 is located between Altıntaş and Aslanapa in Kütahya province (Fig. 5). Area 6 is located between Altıntaş and İhsaniye in Kütahya and Afyonkarahisar province (Fig. 6). Area 7 is located between Başmakçı, Dazkırı and Evciler in Afyonkarahisar province (Fig. 7). The coordinates of the areas are not given as the Great Bustard is a "Vulnerable" species and there is a lot of poaching pressure on the Great Bustard. Areas are respectively 16.210; 8.731; 13.438; 5.211; 15.040; 26.013; 26.771 hectares, and the elevation of the areas varies respectively between 830-930; 880-1010; 850-960; 925-994; 1000-1414; 1030-1170; 850-1080 meters. Not all the areas have a protection status, except for Area 5, which is classified as a Wildlife Development Area (Karataş & Özelmas, 2013).

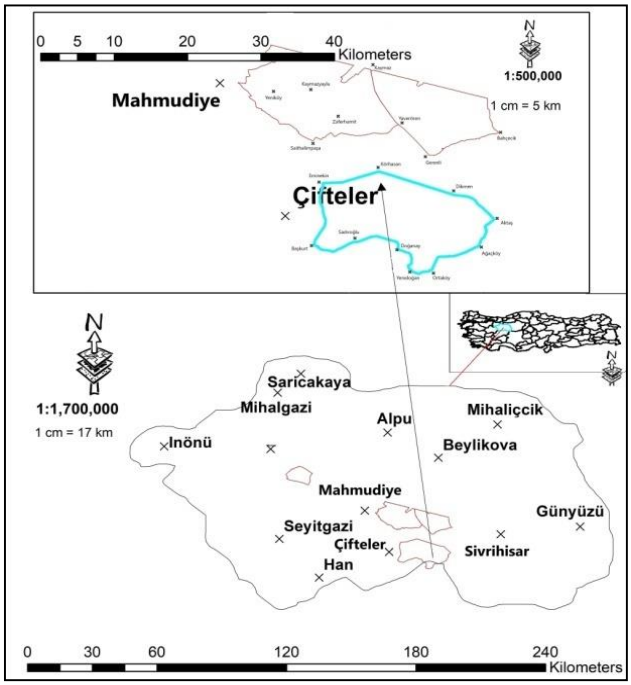

Figure 1. Map of Area 1.

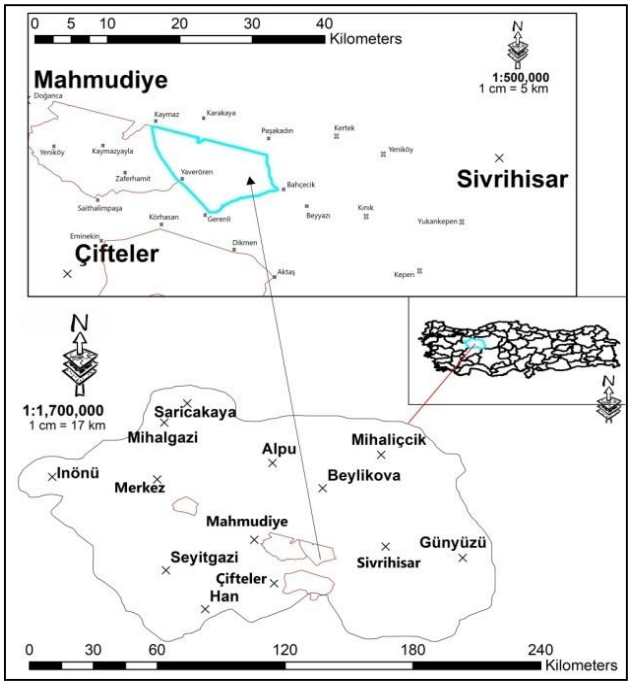

Figure 2. Map of Area 2.

In Area 1, there are cultivated areas, fallow lands, and uncultivated stony and marsh areas. Dry farming is carried out on the majority of the agricultural land in the area. However, irrigated farming practices have been increasing in recent years. In all areas, except Area 3, dry farming is carried out on the majority of the agricultural land and about half of the area is released fallow. However, irrigated farming is also performed partly. In Area 3, irrigated farming is carried out on most of the agricultural land in the area. However, dry farming is also performed partly.

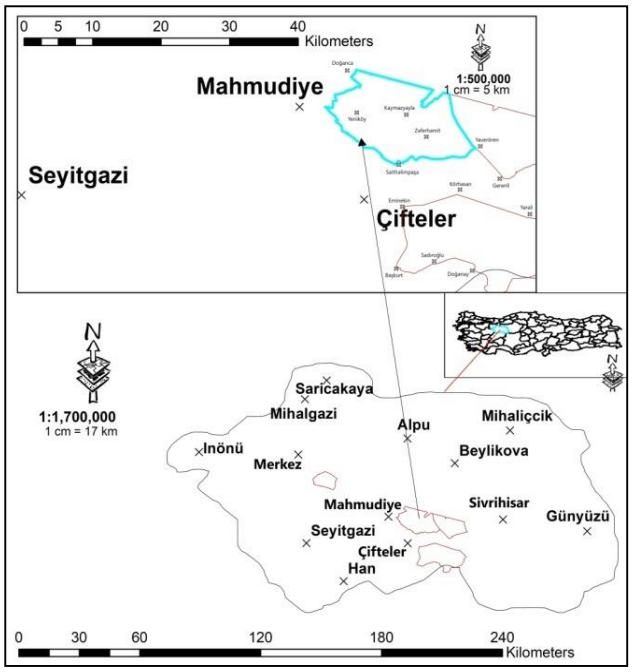

Figure 3. Map of Area 3.

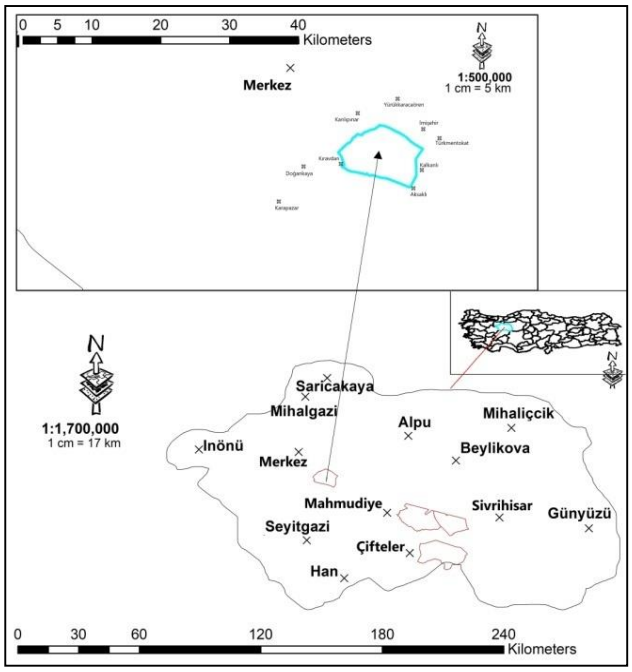

Figure 4. Map of Area 4.

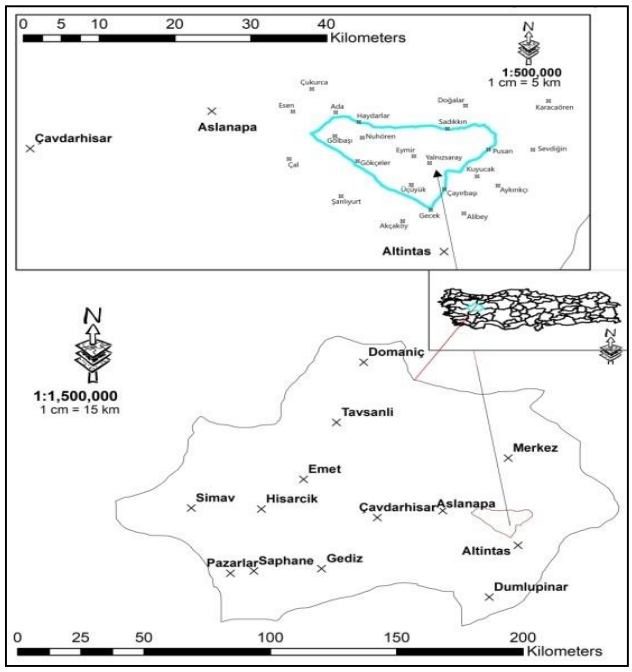

Figure 5. Map of Area 5.

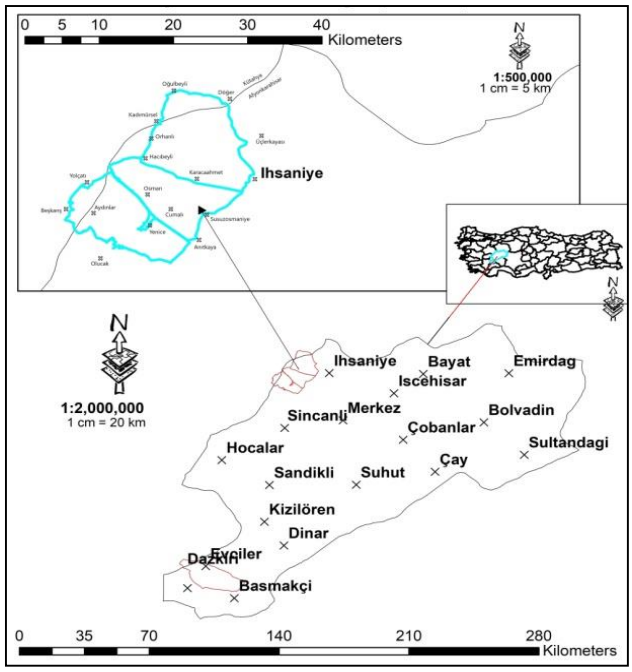

Figure 6. Map of Area 6.

2.2. Method

Field surveys were carried out for monitoring both the breeding and post breeding populations in the study area between March 2013 and January 2020, a total period of 7 years. Investigations were conducted using the transect and spot observation methods (Hellmich & Idaghdour, 2002). Observation of the species was carried out in two periods, the first period beginning at dawn and lasting for 5 hours and the second period beginning 3 hours before sunset and finishing once the sun has set. These observation periods were selected because of the absence of obscuring weather phenomena (heat shimmer, etc.) and due to these periods being the most active times for the Great Bustard (Martinez, 2008). Date, hour, coordinate, number of individuals, sex, and age were recorded during the observations. Also, information was obtained about the presence of the species in the area by interviewing the local people. To test the verifiability of the interviewees, a photograph of a Grey Heron (*Ardea cinerea*) was shown and was stated to be a Great Bustard (Hellmich & Idaghdour, 2002). Data obtained by interviews were omitted from the count results. However, areas with strong anecdotal evidence were investigated more thoroughly.

Finally, count results were categorized under two subheadings, "Breeding season" which was classified as being between March-July and "Post-Breeding season" which was classified as being between August-February. The Post-Breeding season was separated between August-November "Summering period" and between December-February "Wintering period".

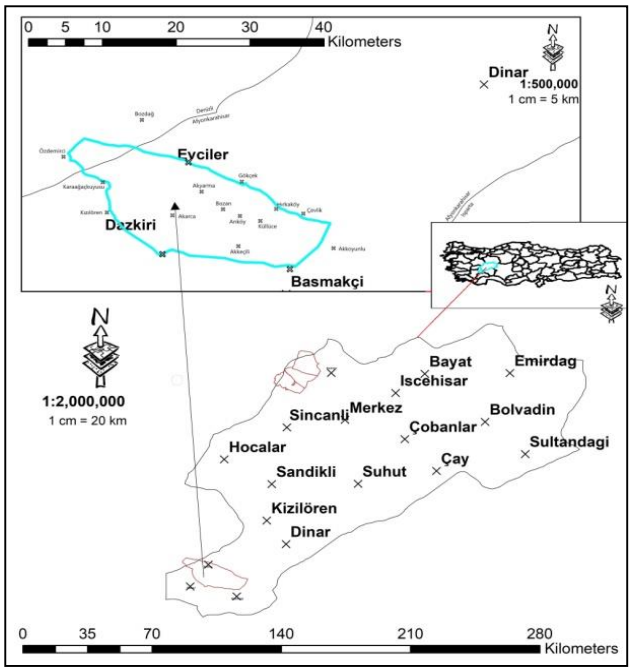

Figure 7. Map of Area 7.

3. Results

As a result of the field surveys, 1 locality was determined to be *Lek Area* in Eskişehir and 3 localities were determined to be *Lek Areas* in Afyonkarahisar where male individuals were observed exhibiting mating behaviors with a breeding plumage. 2 nests with 2 eggs were detected in Afyonkarahisar in May 2019 and 1 nest with 2 eggs in May 2019 and 1 nest with 3 eggs in May 2020 were recorded in Eskişehir.

As a result of the field observations carried out in Area 1 between March 2013 and January 2020, the greatest number of the recorded individuals in the breeding periods was 72, on 11.04.2015. The greatest number of the recorded individuals in the summering periods was 36, on 26.11.2018. The greatest number of the recorded individuals in the wintering periods was 33, both being recorded on 20.02.2019 and 26.01.2020.

In the breeding periods, it was determined that the female/male composition in the flock has changed over the years. This change is summarized in Figure 8.

This species is present all year around in Area 1 and utilizes the area for breeding, summering, and wintering (Fig. 9). As a result, this area hosts about 50-55 males and 11-17 females during the breeding period, around 25-35 individuals during the wintering period, and around 10-15 individuals during the summering period.

In Area 2, between July 2017 and January 2020, the greatest number of the recorded individuals in the breeding periods was 26, on 02.03.2019. However, 1 nest with 2 eggs in May 2019 and 1 nest with 3 eggs in May 2020 were recorded in the study area. The greatest number of the recorded individuals in the summering periods was 80, on 26.11.2018. The greatest number of the recorded individuals in the wintering periods was 130, on 26.01.2020. This species generally prefers this area as a summering and wintering location between September-February (Fig. 10). As an exceptional case, this species used this area as a nesting area in May 2019 and May 2020. As a result, this area hosts about 90-100 individuals during the wintering period and about 15-20 individuals during the summering period.

In Area 3, between April 2017 and February 2020, the greatest number of the recorded individuals in the breeding periods was 19, on 15.04.2017. The greatest number of the recorded individuals in the summering periods was 17, on 29.10.2018. The greatest number of the recorded individuals in the wintering periods was 30, on 03.02.2020. This species generally prefers this area as a breeding and summering area, except for exceptional wintering cases (Fig. 11). As a result, this area hosts about 10-19 males and 2-3 females during the breeding period, about 15-20 individuals during wintering period, and about 11-17 individuals during the summering period.

In Area 4, between July 2018 and October 2019; the greatest number of the recorded individuals in the breeding periods was only 1, on 04.05.2019. Also, in April 2017, a nest with 2 eggs was spotted and photographed by the villagers. The greatest number of the recorded individuals in the summering periods was 7, on 28.10.2018. No individuals in the wintering periods were observed. This species generally prefers this area as a summering area between September-November and a nesting area between April-May (Fig. 12).

In Area 5, between March 2016 and October 2019, no individuals were encountered by our research teams at the study site. However, according to the inventory count studies carried out between March 2016 and October 2019 by The Nature Conservation and National Parks Kütahya Branch Directorate, the greatest number of the recorded individuals in the breeding periods was 43, in May 2016. The greatest number of the recorded individuals in the summering periods was 18, in September 2016. No individuals in the wintering periods were observed (Fig. 13).

In Area 6, between March 2016 and November 2019, the greatest number of the recorded individuals in the breeding periods was 4 males, on 06.03.2016. The greatest number of the recorded individuals in the summering periods was 10, on 31.10.2018 and 25.11.2018. The greatest number of the recorded individuals in the wintering periods was 3, on 05.12.2018. This species generally prefers this area as a summering area, except for exceptional cases during breeding and wintering (Fig. 14).

In Area 7, between July 2016 and January 2020, the greatest number of the recorded individuals in the breeding period was 40, on 15.03.2019. In addition to this, 3 different areas were identified as the *Lek area* of this species in April 2019 and the female/male composition of this flock is 11 females/13 males. 2 nests with 2 eggs were detected in May 2019. The greatest number of the recorded individuals in the summering periods was 30, on 24.11.2018. The greatest number of the recorded individuals in the wintering periods was 105, on 20.12.2019. This species is present all year round in Area 7 and utilizes the area for breeding, summering, and wintering (Fig. 15). As a result, this area hosts about 16-26 males and 7-11 females during the breeding period, about 60-70 individuals during wintering period, and about 10-20 individuals during the summering period.

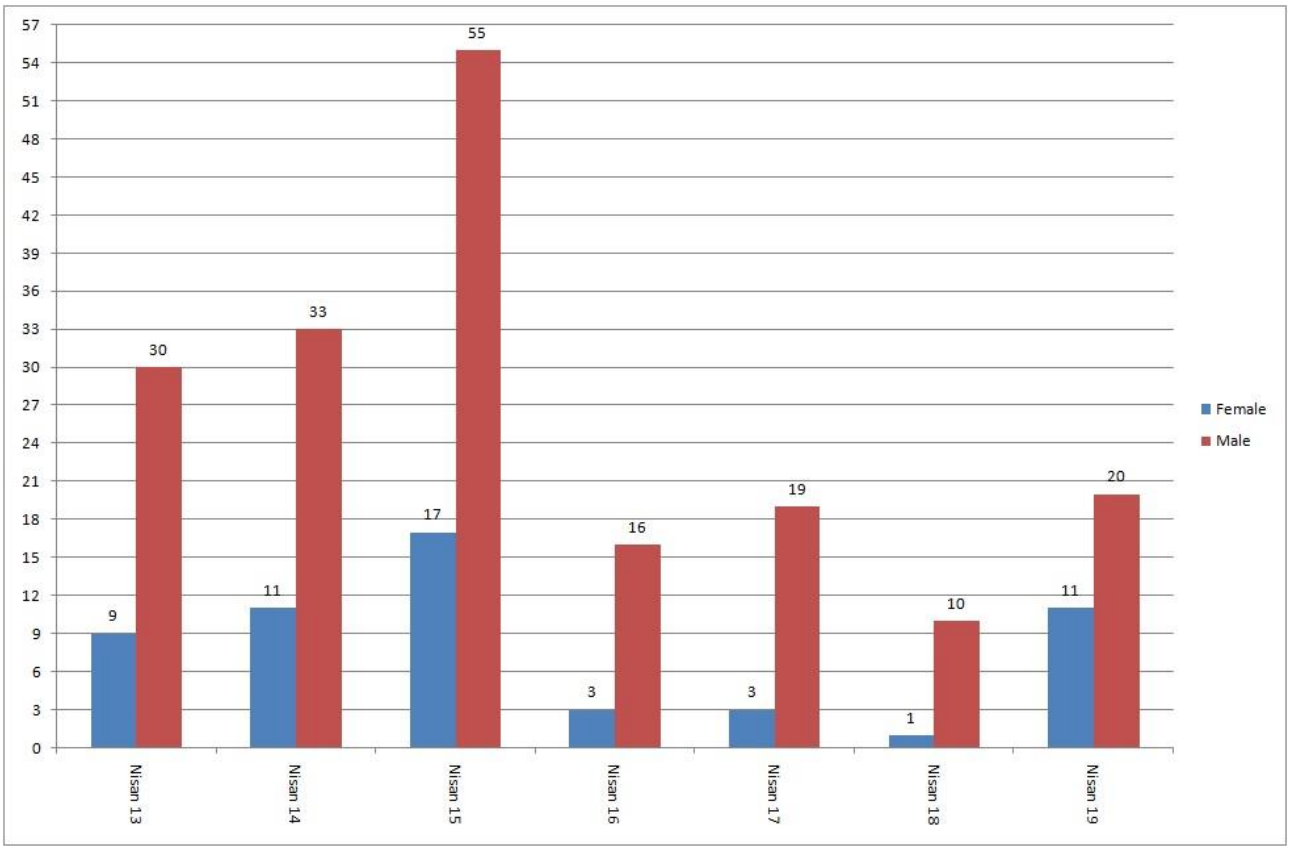

Figure 8. The change over the years of female/male composition in the flock.

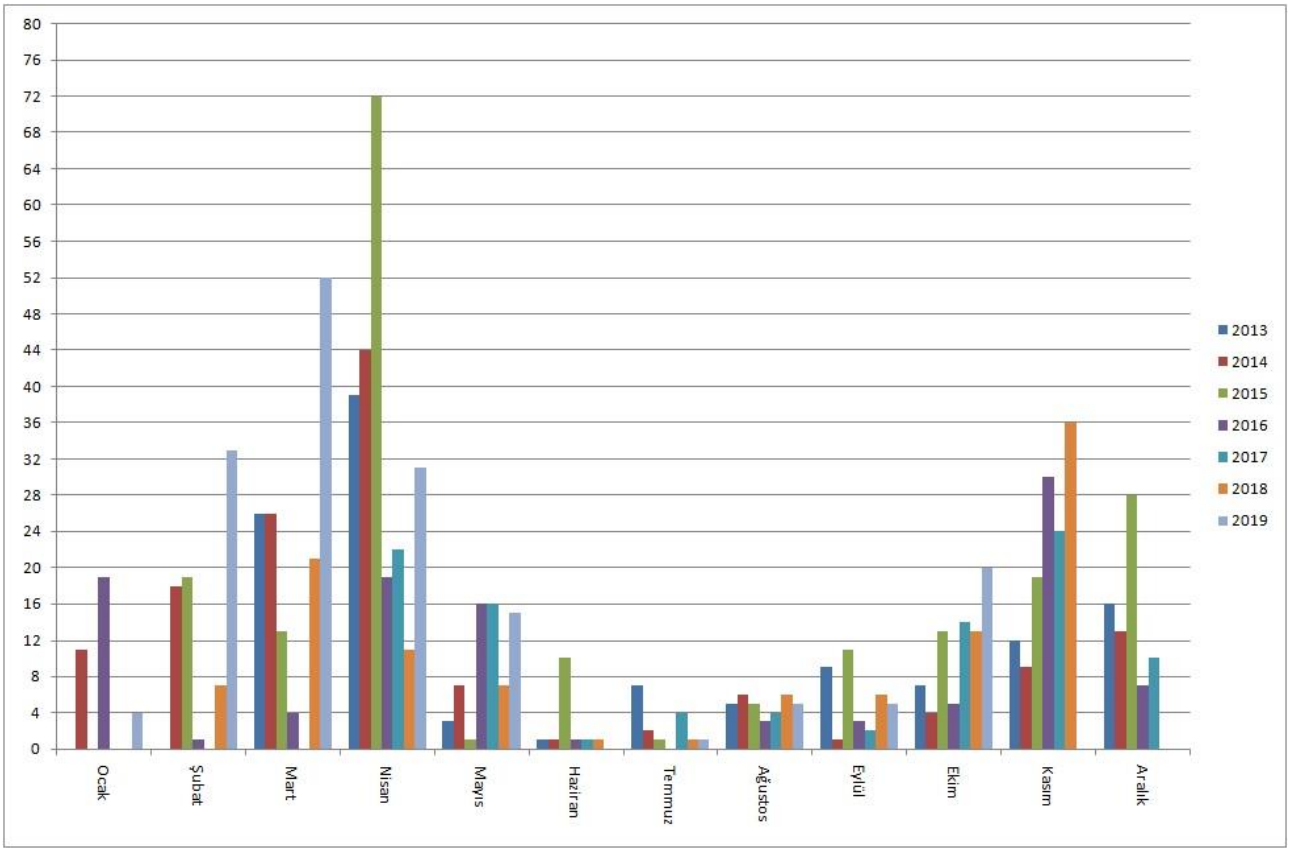

Figure 9. Annual and monthly changes in the number of individuals in Area 1.

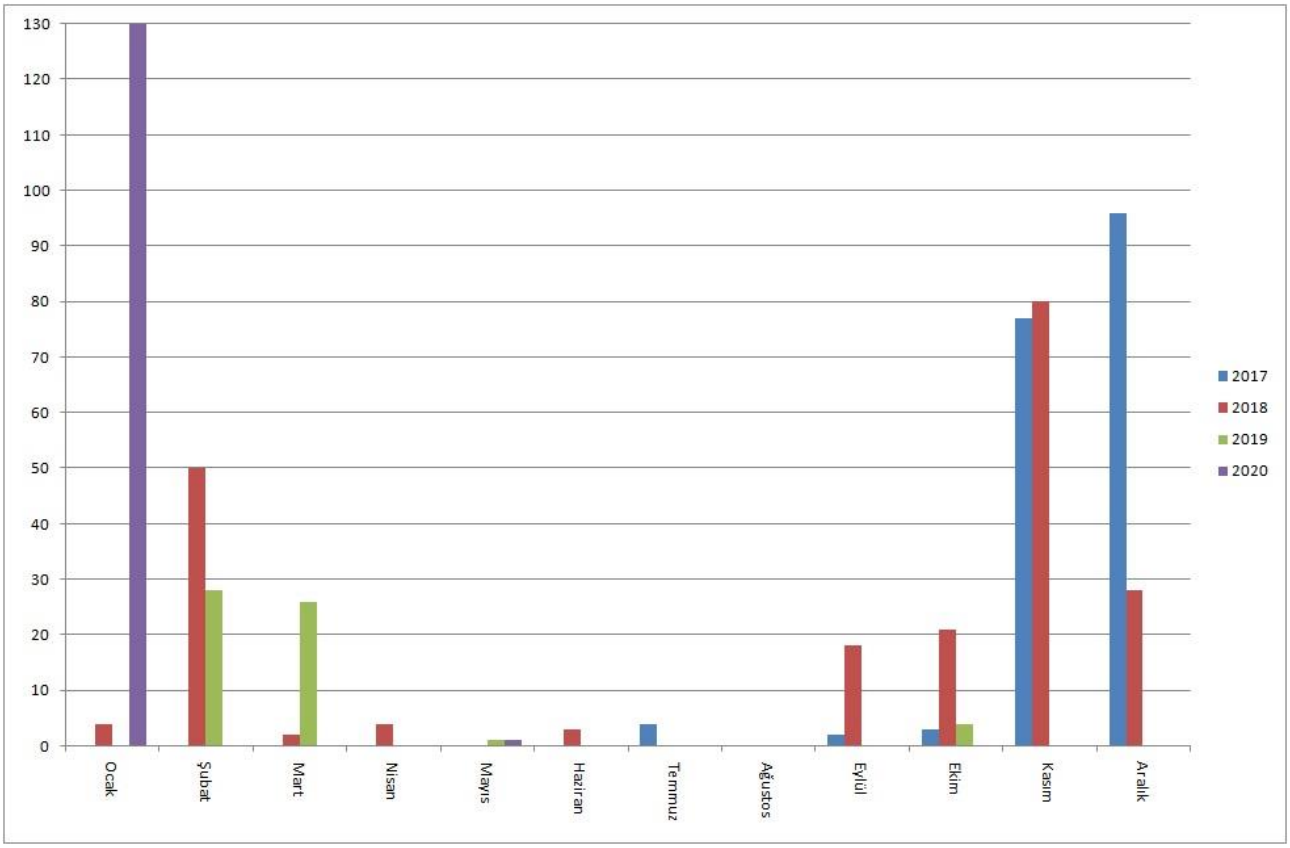

Figure 10. Annual and monthly changes in the number of individuals in Area 2.

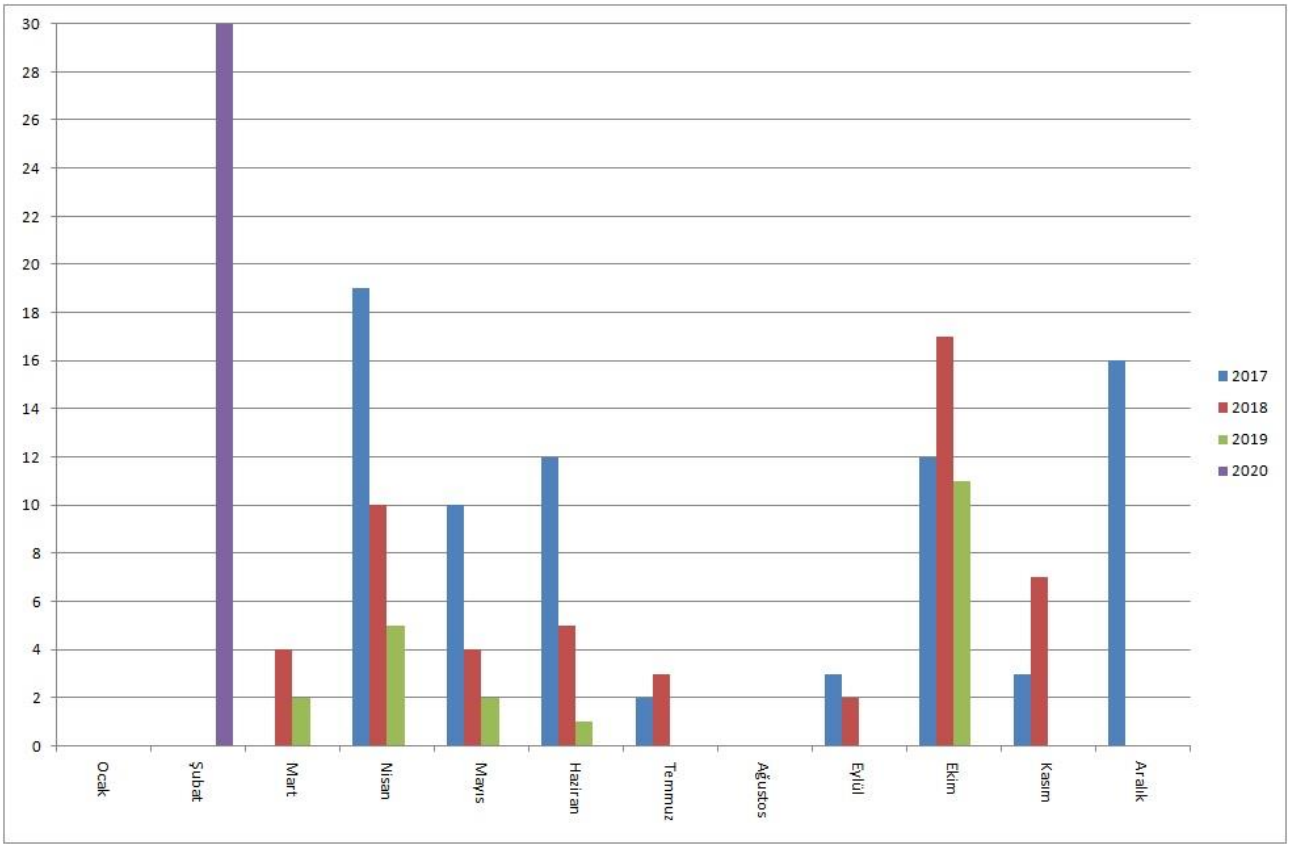

Figure 11. Annual and monthly changes in the number of individuals in Area 3.

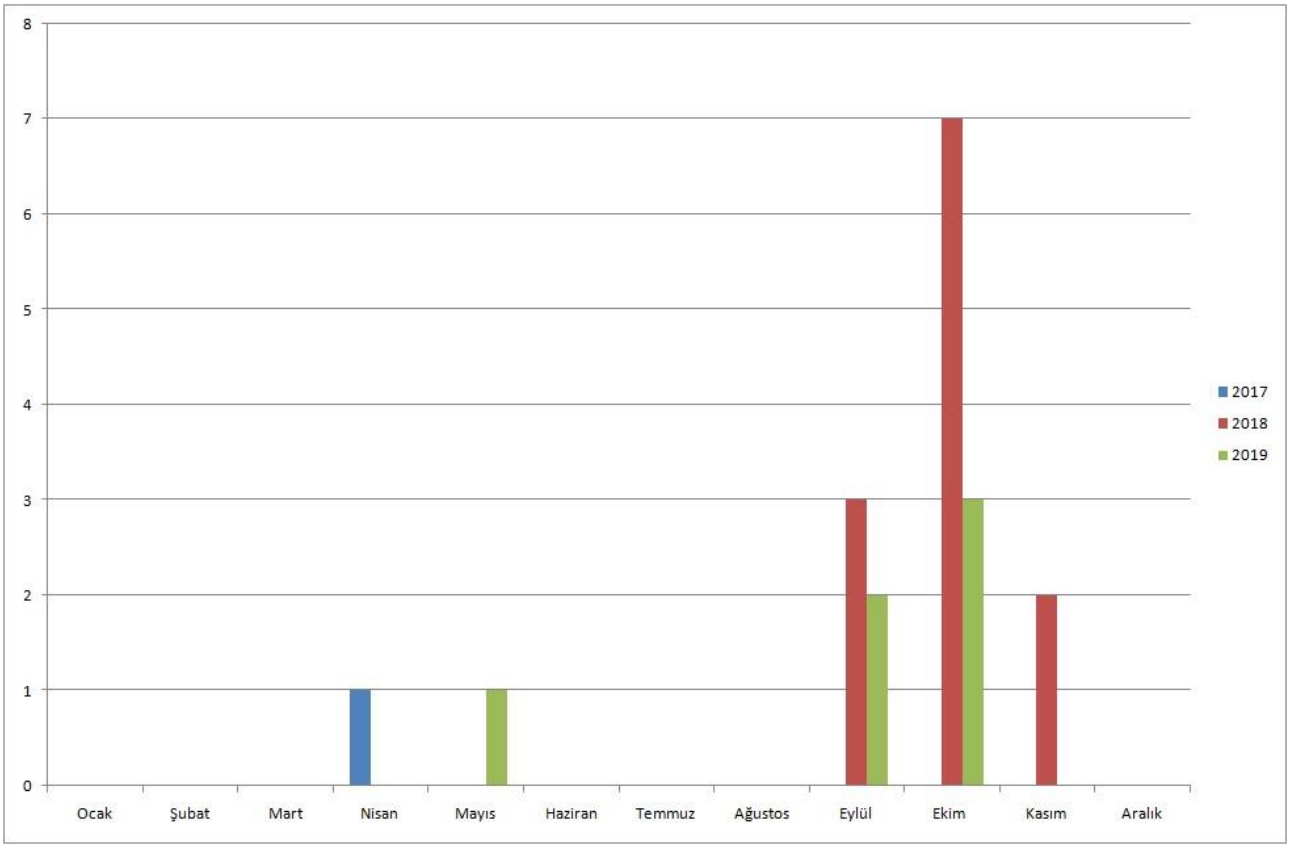

Figure 12. Annual and monthly changes in the number of individuals in Area 4.

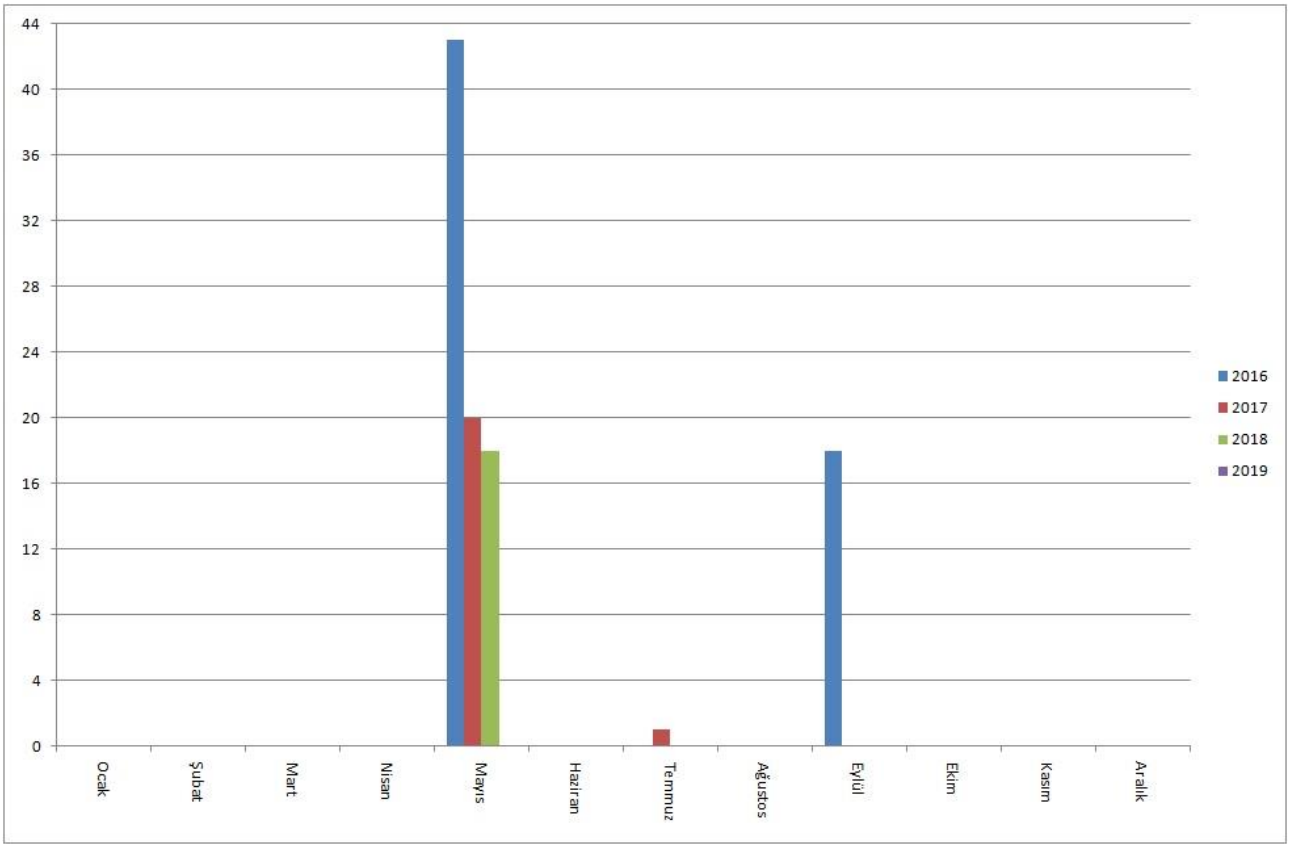

Figure 13. Annual and monthly changes in the number of individuals in Area 5.

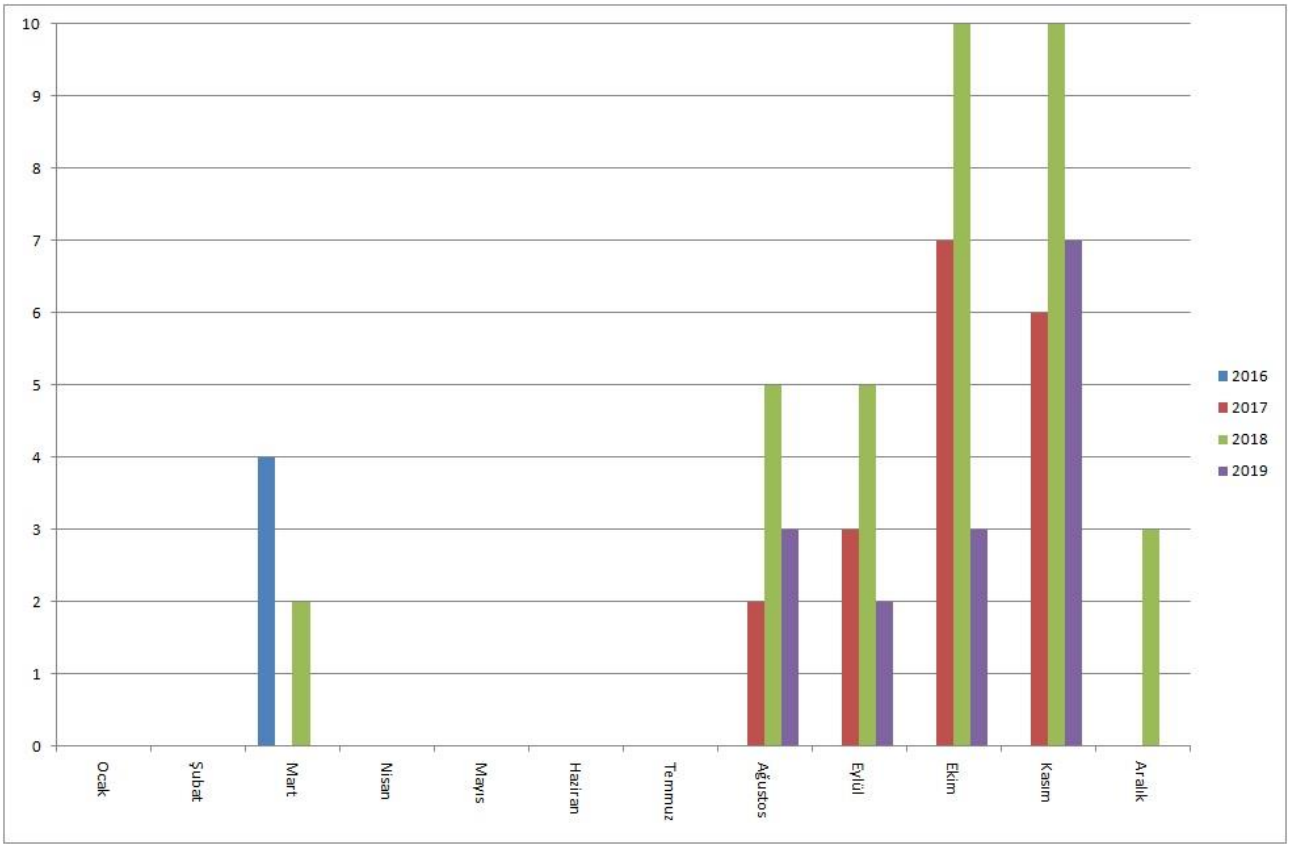

Figure 14. Annual and monthly changes in the number of individuals in Area 6.

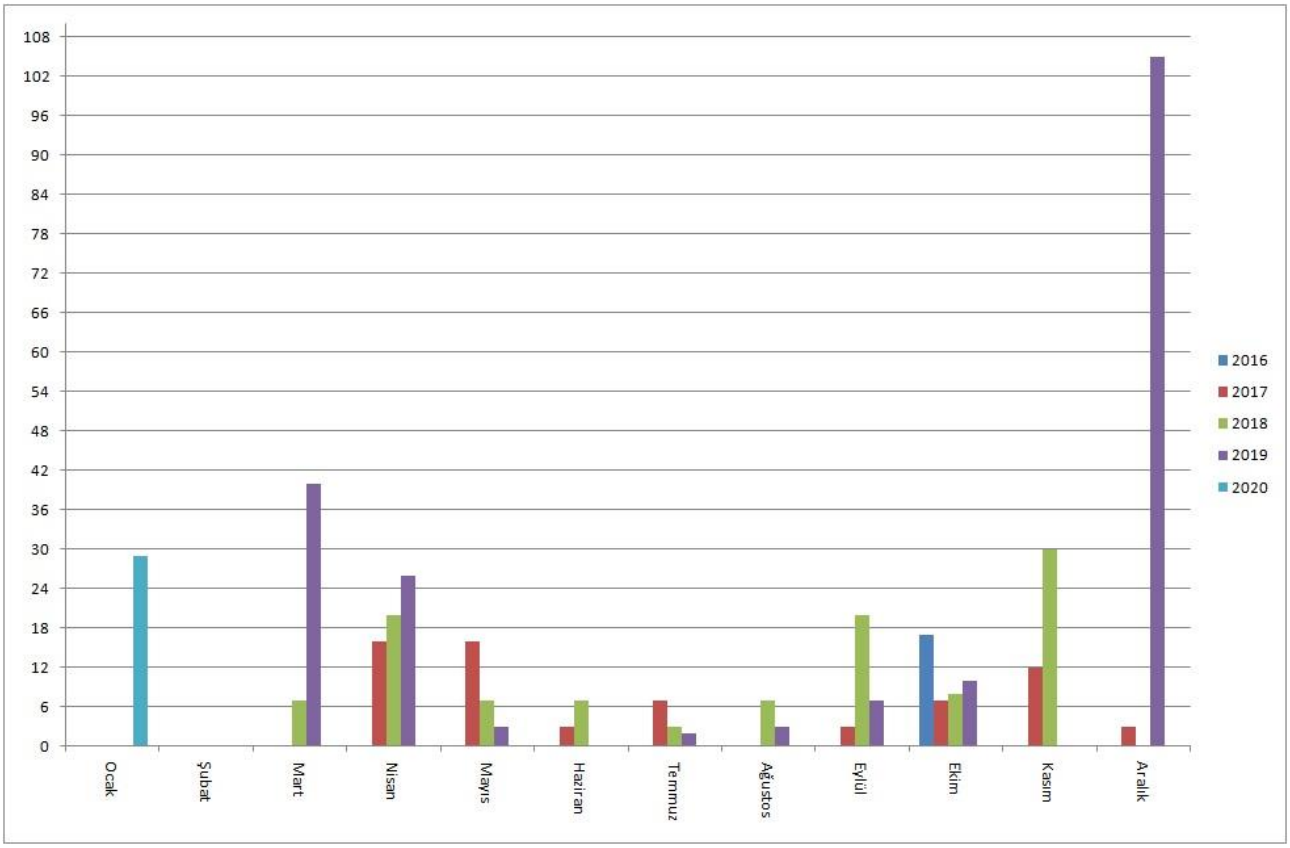

Figure 15. Annual and monthly changes in the number of individuals in Area 7.

Finally, it has been determined by the team involved in this study that individuals belonging to this species are encountered irregularly during some periods of the year in Ablak – Ümraniye – Camili – Aydınyaka and Yenikapı – Kılıçlar villages of Emirdağ district of Afyonkarahisar province and the villages of Benlikuyu – Eskiakören,

Paşakadın, and İlyaspaşa in Sivrihisar District of Eskişehir province. Also, due to the fact that this species could not be caught and marked in some way to distinguish from each other, the population number that we declare as the study results is the maximum number of individuals observed at one time.

4. Discussion

The number of Great Bustards Turkey accounts for only 1-2% of the Great Bustard world population and consists of approximately 700-1200 individuals (Morales & Martin, 2002; Kılıç & Karakaş, 2005; Palacin & Alonso, 2008; Karakaş & Akarsu, 2009; Alonso & Palacin, 2010; Birdlife International, 2017). In summary, 7 surveyed areas were recorded as having a total breeding population of 100 individuals, a total summering population of 70 individuals, and a total wintering population of 205 individuals. The surveyed areas represent an average of 8-14% of Turkey's population in breeding period, an average of 6-10% of Turkey's population in summering period, and an average of 17-29% of Turkey's population in wintering period.

Area 1 holds about 50-55 males and 11-17 females during the breeding period, about 25-35 individuals during the wintering period, and about 10-15 individuals during the summering period. Area 7 holds about 16-26 males and 7-11 females during the breeding period, about 60-70 individuals during the wintering period, and about 10-20 individuals during the summering period. Area 3 holds about 10-19 males and 2-3 females during the breeding period, about 15-20 individuals during the wintering period, and about 11-17 individuals during the summering period. Area 2 holds about 90-100 individuals during the wintering period and about 15-20 individuals during the summering period. These areas are the most important areas among seven study areas where this species has spread and these areas should absolutely be protected.

When the study areas are compared with each other in terms of the number of individuals they host, Area 1 during the breeding period, Area 2 during wintering period, and the Area 2 during the summering period are the most important ones. However, Area 7 is as important as Area 1 and Area 2 in all three periods: breeding, wintering, and summering due to its location. There are fewer settlements around the Area 2 study area and there is no sheep farming. Therefore, they encounter fewer uneasiness factors during the winter and summer periods when compared to other areas. The main reason for the increased number of individuals during the breeding period of the Area 1 study area is that this species is loyal to the lek areas and comes to the same spot during the breeding period every year. The reason for the difference in the number of individuals between the same months in different years is that our species moves to alternative areas inside or outside the area when nutrients are lacking. The increased unrest of the species is due to the agricultural activities in the area and unsuitable climatic conditions such as heavy snow cover. Also, since the species perceive incoming danger from distances as far away as 1 km and flies away or hides itself, it cannot be observed and is therefore not included in the census results. These factors may account for the difference in the number of individuals.

Karataş (2012) reported the population figure recorded in the Altıntaş Wildlife Development Area during the study period between 2010 and2012 as 8 individuals, consisting of 5 females and 3 males. This species may become extinct within the Altıntaş Wildlife Development Area within the next 10 years if protection measures against the factors threatening the species within the study area are not implemented immediately. Mainly during the breeding period, only 1 male and 1 female individual were observed in individual field studies conducted between 2015-2019 in and around the location where individuals are regularly seen. This situation suggests that this species' population within the Altıntaş Wildlife Development Area may become extinct. Continuing to carry out more detailed field studies including the areas outside the current study area is imperative and necessary for the future of this sensitive species in Altıntaş WDA.

Populations globally and in Europe are defined as completely immigrant (Birdlife International, 2017). However, among the populations; highly variable migration behaviors are exhibited including forced winter migrants (Asian and Russian populations), partial summer migrants and winter visitors with a gender differential model, and facultative migrants (central European population) (Morales et al., 2000; Alonso et al., 2000, 2001; Palacin et al., 2009, 2011; Birdlife International, 2017). Most western subspecies are partially immigrants but eastern subspecies in eastern Russia, northeast China, and Mongolia are completely immigrants (Kessler et al., 2013; Birdlife International, 2017). This species is tolerant of cold, but in some cases, regular or irregular displacements can be seen in indigenous populations during severe and heavy winters (Morales & Martin, 2002). In addition, sometimes successful wintering in advantageous areas may cause these areas to become a traditional winter area in the next period (Morales & Martin, 2002). Many populations are indigenous to Turkey but some populations perform seasonal movements within the country. For example, eastern populations descend southward in the winter period November-December in Turkey during which they are regularly found near Şanlıurfa (Tanriverdi, 2015). The Great bustard was defined as "local" as they were observed throughout the year in Area 1 and Area 7. However, in Area 2, it is thought that this area is a "wintering area" that is visited regularly due to the increasing population, especially in November-January compared to other months. In addition, in this area which is defined as a wintering area, it is necessary to investigate in a more detailed study using marking and satellite tracking systems to determine where the wintering individuals come from.

The Female/male ratio was 1.10/1 and 1.41/1 in Spain's Entradas and Marcos regions (Morgado & Moreira, 2000), the female/male ratio was 2.42/1 in Madrid (Alonso et al., 2003a), it was 1.6/1 in Villafáfila (Alonso et al., 2003b), 2.99/1 in 2003, 3.12/1 in 2004 in Osuna (Alonso et al., 2005a). The female/male ratio was 2.4/1 in 2007 in Madrid (Martín et al., 2007), it was 3.29/1 in 2002, 5.67/1 in 2005, (Alonso et al., 2005b) and 3.80/1 in 2016 in Morocco (Palacin et al., 2016), The 3-year average female/male ratio is 2.57/1 in China's Tacheng and Qapqal regions (Wang et al., 2018). In addition, the female/male ratio was determined as 1.6/1 during the

breeding periods of 2010-2012 in Altıntaş WDA (Karataş, 2012). On the other hand, the female/male ratio was determined as 1/3.3 in 2013; 1/3.0 in 2014; 1/3.2 in 2015; 1/5.3 in 2016; 1/6.3 in 2017; 1/10 in 2018 and 1/1.8 in 2019 in Area 1. Also, the female/male ratio was determined as 1/1.2 in 2019 in Area 7. The fact is that while more females were observed than males in a global scale, in Area 1 more males were observed than females. This may be due to the fact that no matter how detailed we make our field studies, we cannot observe very well camouflaged female individuals, which means that their population situation may be approximately 1.5 times more than our estimates. This shortfall in our estimates may be caused by a special situation specific to our work areas. In both possibilities, this situation should be investigated in detail with branding and satellite tracking systems.

Magana et al. (2010) found out that most of Spain's nesting areas were in the cultivation and fallow areas, there were only a few nests in the ploughed areas. Tanrıverdi (2015) found out that in the Muş Plain, all of the nesting areas were in the cultivation areas. In this study, although almost all of the nests are in the cultivation areas, the nest in the Area 7 in 2019 was found in the Garden rocket (*Eruca vesicaria*) fields. Although it has been reported in the literature that this plant species was included among the feeding preferences in Portugal's spring season (Morales & Martin, 2002), no nest registration was reported for this plant species. However, records show that a nest was built in a weedy or meadow area. Rather than the type of plant in the area where the nest is built, it is thought that the nest must be built in a uniform and non-segmented pattern to protect the nest against dangers. Of course, this prediction needs to be investigated in detail with satellite tracking systems.

Although the Great Bustard prefers different areas in different seasons as their habitat preferences, they generally avoid small clustered or isolated park areas of forests and trees. On the other hand, it is known that they use open oak forests and olive groves in the Iberian Peninsula. In Turkey, only in Kars, 1 female and 3 cubs were observed on the edge of pine forests (*Pinus sylvestris*) in 2011 (Per et al., 2012). In our field studies, it is determined that uninterrupted mobility in all directions on the ground and having a clear field of view over 1 km is absolutely necessary for the Great Bustard to choose an area. Although individuals of this species prefer treeless areas with at least 1 km uninterrupted visibility in Eskişehir province every season, individuals who live in Area 6 and Area 7 prefer Hawthorn (*Crataegus pseudoheterophylla*) areas to rest in the summer months unlike the individuals in Eskişehir. It is thought that the birds choose the areas which contain these plants to protect themselves from extreme temperatures in summer months.

The threat factors are reported for the Great Bustard are: human presence and disturbance during agricultural activities especially during the breeding season, pesticide usage, habitat loss due to infrastructural changes such as the construction of village roads and electric transmission lines, leakage hunting, collisions with electric transmission lines, and expansion of irrigation systems used in agriculture (Hellmich & Idaghdour, 2002; Alonso et al., 2005b; Pinto et al., 2005; Sastre et al., 2009; Abdulkarimi et al., 2010; Lemus et al., 2011; Bravo et al., 2012; Horreo et al., 2013; Karataş et al., 2015; Karakaya et al., 2017). The threats mentioned by these authors have been observed in all our fields of study and they affect this species with a high degree of importance. Electricity transmission lines are especially a threat that needs to be solved first. Studies on electrical transmission line collisions in Spain, Portugal, Hungary, and Norway have shown that birds such as the Great Bustard, which are described as "weak fliers" which have particularly short wings and tails and high body mass, the risk of collisions with human-made structures such as electric transmission lines or wind turbines are extremely high and these collisions often result in death (Alonso et al., 2003a; Martin et al., 2007; Raab et al., 2010, 2012; Bernardino et al., 2018). The most serious threat is electricity transmission lines in Eskişehir, Kütahya, and Afyonkarahisar provinces where this species is spread. Electricity transmission lines pass around the lek areas in all three provinces. During our working period, electricity transmission line collisions occurred in Eskişehir on different days in May 2019 resulting in the death of two male individuals older than 8 years old. Collisions with electric wires can cause direct death of the species as well as the lesions caused by the collision and the effect of electric current on the body of this species. In order to prevent or reduce individuals of this species from colliding with electrical transmission lines, electrical wires may be marked to allow them to be seen from a distance or electric wires can be covered with insulating material to protect the electricity from being transmitted to the bird's body in the event of a collision. By marking the power lines, birds can reduce the risk of collisions by directing their flight around marked power lines, thereby reducing losses. However, marked power lines seriously affect the flight direction of birds. This causes birds to spend more energy. For this reason, it is recommended that the cables be underground rather than marking the power lines. In this way, all adverse effects of power transmission lines on this species will be eliminated (Raab et al., 2010).

Due to the high mortality rates in adult individuals from hunting in Central and East Asia and habitat loss, degradation, and fragmentation caused by land-use changes in Russia, central Asia, Morocco, and eastern Europe, the population of this species may rapidly decrease over the next three generations. For these reasons, this species has been classified as Vulnerable worldwide. However, with the successful conclusion of studies on the protection of this species and its habitats in Europe, especially in Spain in recent years, it is thought that the population of this species is unlikely to decrease rapidly over the next three generations; therefore, it has been classified as the Least Concern on the European scale. Unlike Europe, in Turkey, the population status of this species is adversely affected by habitat fragmentation resulting from the transition from dry farming practices such as wheat and barley to irrigated agriculture practices such as the production of sugar beet and corn. It is also negatively affected by the habitat destruction caused by infrastructural changes such as drilling for these irrigated farming practices, electrical lines, and by direct individual deaths such as collisions with electricity transmission lines and hunting. In addition, given the insufficient protection measures taken against the aforementioned adverse factors, it is considered likely that this species' population

will decline rapidly over the next generations.

Creating a research and monitoring plan, a habitat management plan, a species management plan, laws and regulations, and raising the awareness and education of local people are recommended as protection measures. *With the creation of an effective research and monitoring plan*; every year spring, summer and winter censuses should be carried out in detail and the population status of the species should be checked continuously. Age and sex composition, population size, and the trends of populations should be observed. Habitat use, distribution patterns, movements, and factors causing death should be investigated. Considering all the areas where this species has spread to, the existence of key areas should be absolutely necessary for their vital activities such as breeding, resting and feeding, and whether key areas are needed should be investigated. Detailed ethological studies should allow an understanding of the species' preferences. *With the creation of an effective habitat management plan*; agricultural activities such as plowing, spraying, harvesting should be done while taking into consideration the life cycle of this species. Electricity transmission lines should be run underground to prevent collisions. The use of pesticides in or around the areas where the species lives should be restricted or prohibited with the support of various incentive methods. Within the areas where the species is found, "Key areas" should be created and protected with the help of incentives and rewards but preferably through raising awareness. Agricultural activities in these key areas should be restricted according to the breeding cycles of the species such as courtship, mating, and incubation. Agricultural practices that do not protect the habitats of this species, such as the degradation of fallow lands and the cultivation of crops for the second time should not be supported. Infrastructure and construction activities in or around the areas where the species lives should be restricted or completely banned. This involves taking into consideration the life cycle of the species. *With the creation of an effective species management plan*; local residents should be encouraged and rewarded if they report to the authorities when abandoned hatchlings or eggs or injured individuals are found. Rehabilitation and reproduction centers for the species should be established, and in these centers, wildlife-adapted individuals should be bred and released into the nature. *The creation of an effective awareness raising campaign and a plan for the education of local people is required* in order to protect the habitats of this species and to raise awareness about the conservation of this species. Meetings and interviews should be held among the people living in villages close to the Great Bustard habitats and training should be given to the students in schools within the local region. Finally, *with the creation of effective laws and regulations*; for the purpose of deterring and preventing the illegal hunting of the Great Bustard need to be implemented, instead of a rather insufficient and non-deterrent fine such as 8.679 Turkish Liras, a prison sentence should be imposed. In conclusion, we think that making the recommendations outlined in these plans will increase the success rates of regaining a healthy level of population.

Among the areas that have been identified in the National Bustard Action Plan, the current status of the Area 5 is Wildlife Development Area, and there are approximately 12 villages within the area with a total population of 5000 people. However, Area 1 ranks as one of the important bustard breeding areas, although there is not a recognized status for it. This population is the western part of Central Anatolia subpopulation. It is the biggest one in the Central Anatolia subpopulation with regard to breeding. For this reason, regardless of the area's protected status, protecting these 7 areas and supporting the Bustard studies in the area have great importance for the conservation of the Great Bustard on both a local and global scale. Also, detailed bio-ethological studies should be performed. According to the findings and results, new measures should be taken both in this local area and country-wide to enable the species to reach a healthy level of population.

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Conflict of interest: The authors declares that there is no conflict of interest.

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Research Article / Araştırma Makalesi

Carbon Footprint Analysis of Ege University within the Scope of Environmental Sustainability

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Abstract: Universities play a leading role in promoting sustainability in society. Besides their technical roles, they have important social responsibilities and should be a leader for their stakeholders. Therefore, there is an important need to manage and reduce carbon emissions in universities to ensure environmental sustainability. Ege University (EU) has the vision of being a sustainable campus. In this context, in order to reduce carbon emissions, a carbon footprint study has been carried out primarily to determine carbon emission resources. The life cycle assessment (LCA) tool was used to analyze the Ege University carbon footprint. The study was based on the Greenhouse Gas Protocol Corporate Accounting and Reporting Standard and ISO14064/1-2 standards. In the study, the EU campus was evaluated as the main campus and the medical faculty campus. Total carbon emissions of Ege University in 2016 were analyzed as a total of 40.608 tCO_2e within the Scope 1, Scope 2, and Scope 3. As a result of the study, it was revealed that 37% of total emission was caused by constant and mobile combustion, 39% from electricity usage, and 24% from travel and staff transportation. In the discussion, it was emphasized that EU should turn to energy conservation and efficiency, awareness activities, and renewable energy resources in its short, medium, and long term plans in order to reduce carbon emissions.

Keywords: Carbon management, energy management, carbon emission in campus, reducing carbon emission.

Çevresel Sürdürülebilirlik Kapsamında Ege Üniversitesi Karbon Ayak İzi Analizi

Öz: Üniversitelerin toplumda sürdürülebilirliği teşvik etmek için teknik rollerinin yanı sıra önemli sosyal sorumlulukları vardır ve paydaşları için bir lider olmalılardır. Sürdürülebilirliği sağlamak için karbon emisyonlarını yönetmek, azaltmak ve üniversitelerde sürdürülebilirliğin teşvik edilmesine önemli bir ihtiyaç vardır. Ege Üniversitesi (EU), sürdürülebilir ve yeşil bir kampüs olma vizyonuna sahiptir. Bu çalışmada, Ege Üniversitesi'nin kurumsal karbon ayak izi hesaplanmıştır. Yaşam döngüsü değerlendirme (YDD) aracı Ege Üniversitesi karbon ayak izini analiz etmek için kullanılmıştır. Çalışma, Sera Gazı Protokolü Kurumsal Muhasebe ve Raporlama Standardı ve ISO14064 / 1-2 standartları temel alınarak yapılmıştır. Çalışmada EU kampüsü Merkez Kampüs ve Tıp Fakültesi kampüsü olarak değerlendirilmeye alındı. Ege Üniversitesi'nin 2016 yılı toplam karbon salımı Kapsam 1, Kapsam 2 ve Kapsam 3 çerçevesinde toplam 40.608 tCO_2e olarak analiz edildi. Çalışmanın sonucunda %37 sabit ve mobil yanmalardan, %39 elektrik kullanımından ve %24 ise seyahat ve personel ulaşımından kaynaklandığı ortaya konmuştur. Çalışmanın tartışma ve öneriler bölümünde ise EÜ'nün karbon salımını azaltması amacıyla kısa, orta ve uzun vadeli planlarında enerji tasarrufu, bilinçlendirme çalışmaları ve yenilenebilir enerji kaynaklarına yönelmesi gerekliliği vurgulanmıştır.

Anahtar kelimeler: Karbon yönetimi; enerji yönetimi; kampüslerde CO_2 kaynakları; karbon emisyon azaltımı.

1. Introduction

Emission of greenhouse gases, a crucial factor of global warming comes from various type of corporations (Aroonsrimorakot et al., 2013). Consumption of fossil fuels and electricity, transportation, gases used in air conditioning and gases used in laboratory are some activities causing the direct and indirect emissions and need to be managed in environmentally friendly manner in order to reduce the amount of greenhouse gas emissions (GHG) (Aroonsrimorakot et al., 2013). Therefore, many sectors including universities have initiated GHG management systems and focused on reducing carbon emissions (Tatsanawalai, 2015).

Academic sectors have been more conscious and aware of the process and activities which would help reducing GHG after the "Brundlandt Report" and Rio Conference (Gomez et al., 2016). Universities have important roles for sustainable carbon management with their long-short range strategic plans, dense populations, and organizations. A sustainable university campus can be a leader for a sustainable city and change the human behavior (Li et al., 2015).

A sustainable university is an integrated system that should focus on sustainable campus operations such as sustainable transport, sustainable energy management, and etc. (Townsend & Barrett, 2015). Furthermore, the universities that wish to become leaders in sustainability should consider sustainability issues in their operations, strategic road maps, investment, purchasing, and so on. Additionally, they also need instruments to report and assess their actions and achievements (Gomez et al., 2016). In order to build a sustainable campus, actions should be measured and subsequently reported. Measuring the progress of campus actions can help to show opportunities and threats (Townsend & Barrett, 2015). For this purpose, carbon footprint (CF) is most widely used and one of the

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important global tools to recognize the impacts on environment (Gomez et al., 2016). To know the carbon footprint of institutions is also important to control greenhouse gases arising from their activities (Üreden & Özden, 2018). In general, to analyze CF is a way for higher education to monitor sustainability performance and raise awareness among the staff and students. Universities also calculate their CF to respond to the sustainability needs of the society, to perform a sustainability assessment of their operations, to use as an educational tool with students, and to use for policy development (Lambrechts & Liedekerke, 2014). In order to make the calculation more accurate and accurate, it should be taken consideration based on a calendar year or fiscal year (Üreden & Özden, 2018).

Carbon footprint studies have been carried out in many different countries of the world. While these studies were sometimes limited to only one faculty, sometimes the entire campus was evaluated. Alvarez et al. (2014), calculated the carbon footprint (CF) of the School of Forestry Engineering in 2010 and the results showed that the CF was 2147 tons CO_2e, of which 59.0% corresponds to scope 3 emissions. Flores et al. (2019) studied the CF of the Cuajimalpa campus of the Autonomous Metropolitan University. The CF of the campus was calculated as 3000 tons of CO_2 equivalent (CO_2e). Emissions analysis by activity indicated 51% for commuting, 24% for electricity usage, 14% for academic travel, and 11% for other activities. Yañez et al. (2020) studied the CF of five campuses in University of Talca. Results show Scope 3, which measures indirect emissions generated by activities like transportation of people, produced the highest contribution of 0.41 tCO_2e per person to the UT's CF in 2016.

The CF was also calculated in national universities. Binboğa & Ünal (2018) studied the corporate carbon footprint of Manisa Celal Bayar University. They determined that the University emitted 8.954 tons of CO_2 (not considering other greenhouse gases) in 2016 and 87.85% of this was due to electricity consumption. Gökçek et al. (2019) compared the CF of male and female students in Niğde Ömer Halisdemir University and they calculated the CF of male students as 392 kg/year and CF of female students as 359 kg/year. The reason that male students have more carbon footprints has been identified as using more electronics and staying at student homes instead of dormitories. The CF was also calculated in Sakarya University, Akdeniz University, and Boğaziçi University. Esentepe Campus of Sakarya University released 12,330 tons of CO_2e. In the study, scope 2, purchased electricity is the most important emission source, followed by emissions from student and employee commuting (Sreng & Gümrükçüoğlu Yiğit, 2015). In Akdeniz University Health Services Vocational School, the carbon emission from the personnel transportation and electricity consumption was calculated. The annual carbon emission is determined as 98.307 kg CO_2e (Yaka et al., 2015).

In this study, the corporate carbon footprint was calculated in order to determine the carbon emission sources of Ege University. The calculations were carried out within the scope of ISO 14064/1 standards. It is considered that determining carbon emission sources within the framework of environmental sustainability steps are very important in determining a roadmap to reduce carbon emissions. It has been observed that the CF analysis of national universities were mostly on the basis of departments. In this study, it was also aimed to present data of the corporate campus which was located in the city, had a large medical campus, and subway in the concept of holistic approach. On the other hand, in the scope of climate change and the EU Green Deal, it is believed that to deliver data from universities in Turkey is significant to take place among world universities.

2. Material and Methods

2.1. Ege University (EU)

Ege University (EU) consists of two main campuses covering most of the activities and is located in Bornova/İzmir. There are 66.764 students and 6897 employees (academic and administrative) in the campuses. 14 Faculties, 9 Institutes, 6 High Schools, 1 Conservatory, 8 Vocational Training Schools, 27 Research and Application Centers are divided into two main campuses.

2.2. Research Method

Life cycle assessment (LCA) based on the international standard of ISO 14064/1 was used for the purpose of analyzing corporate carbon footprint of EU. This standard specified the requirements for the design, development, management, reporting, and verification of an organization's greenhouse gas inventory. According to the standard, both direct and indirect greenhouse gas emission sources were identified within the context of Scope 1, 2, and 3 (Fig. 1). Direct emissions from constant and mobile consumption of liquefied petroleum gas used on campus are covered in Scope 1. Scope 2 includes indirect emissions by generation and transmission of electricity. Scope 3 covers other indirect emissions and this is an optional category. Scope 3 activities are a consequence of activities of the organization that occur in locations or from sources that are not owned or controlled by the university (Fig. 1) (Yañez et al., 2020).

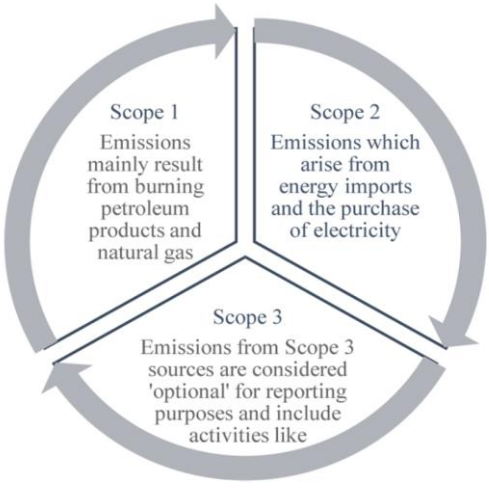

Figure 1. Direct and indirect emissions sources

The basis of the study was determining CO_2 equivalent of each activity. The Greenhouse Gas Protocol, Corporate Accounting and Reporting Standard, and ISO14064/1-2 standards were used for analysis.

In the EU corporate carbon footprint calculation study, the data obtained from the campus were multiplied

with the relevant emission factors and the emission data according to the activities were obtained in terms of carbon dioxide equivalent (CO_2e). The CO_2e was obtained by multiplying the mass of the supplied greenhouse gas and its global warming potential (for methane 28 times, for N_2O 256 times). The greenhouse gases were determined as carbon dioxide (CO_2), nitrous oxide (N_2O), methane (CH_4), hydrofluorocarbons (HFC), sulfur hexafluoride (SF_6), perfluorocarbons (PFC), and refrigerant gases in the Kyoto Protocol and in the study, global warming potential of GHGs were used as Intergovernmental Panel on Climate Change (IPCC, Climate Change-5th Assessment Report (2013) 1 and DEFRA2 British Government Ministry of Environment published guide.

2.3. Data collection

For the base year 2016, all data were collected from each academic unit (faculties, research centers, etc.) and administrative institutions. Fuel consumption used for heat production, generator usage, vehicle fuel consumption, gas purchased for air conditioner and cooling systems were collected as Scope 1 emissions. The annual amount of natural gas (Sm^3) and fuel consumption in generators were separated for EU medical and main campus. Emission factors were provided from the "Greenhouse Gas Calculation Inventory Manual" published by the IPCC in 2006 (Table 1) (GHG Inventory Manual, www.climate.emb.gov). In order to calculate emissions from the purchased electricity (Scope 2), it is necessary to know the value per kWh electricity generation by country. In Turkey, the amount of emissions released during electricity generation is 0,496 kg CO_2e / kWh. Domestic and international flight data were used for Scope 3. Emissions from staff transportation were also calculated within Scope 3. 3 types of transportation were taken into consideration in staff transportation calculated within the Scope 3. EU private vehicle entry was 15% according to the information obtained from the security data. The arrival of other staff to the campus was considered as 35% by subway (rail, subway, GHG transport tool) and 65% by bus and minibus (road, local bus, GHG transport tool) according to Izmir public transportation rates (Pektaş, 2017; www.ghgprotocol.org). The distance to the campus was accepted as 25 kilometers (between Konak-Bornova, round-trip) for all transportation types.

Greenhouse gas potentials were taken from DEFRA Guide (UK) (Defra Guide, www.gov.uk). All data are given in Table 2.

3. Results

Greenhouse gas emissions by Ege University in the year 2016 are shown in Table 3. The total greenhouse gas emissions that resulted from the activities of Ege University in the year 2016 are equal to 40.608 tCO_2e as presented in Table 3 and Figure 2 shows the ratio of GHG emissions. The total carbon footprint of the EU Main Campus in 2016 was 11.897 t CO_2e within Scope 1 and 2. GHG caused by air conditioning and refrigerant leaks took the 3% share in Scope 1. Emissions caused by the use of vehicles (mobile combustion) connected to the university the emissions caused by these activities covered the part of 1%. P-10, acetylene and CO_2 gases used in laboratory studies had 1% effect on total greenhouse gas emissions. According to the greenhouse gas resources used in the carbon footprint calculation within the Medical Campus, 46% of the 19,132 tons of CO_2e carbon footprint was caused by natural gas used in heating activities. Emissions from indirectly purchased electricity accounted for 49%. In addition, emissions of refrigerant gas R410 and gasoline used in vehicles were calculated less than 5%. The contribution of other refrigerant gases such as R407 and R134a to the total greenhouse gas emission was less than 1%. CO_2 used in medical activities made 1% effect on total greenhouse gas emissions.

It is demonstrated that the staff transportation within Scope 3 had an important ratio of 24% within the total carbon emission of the EU. 15% private vehicle use caused 73% of transportation emissions. When considering that 35% of staff come by subway and 65% by road; emission rates for transportation were analyzed as 11% and 16%, respectively.

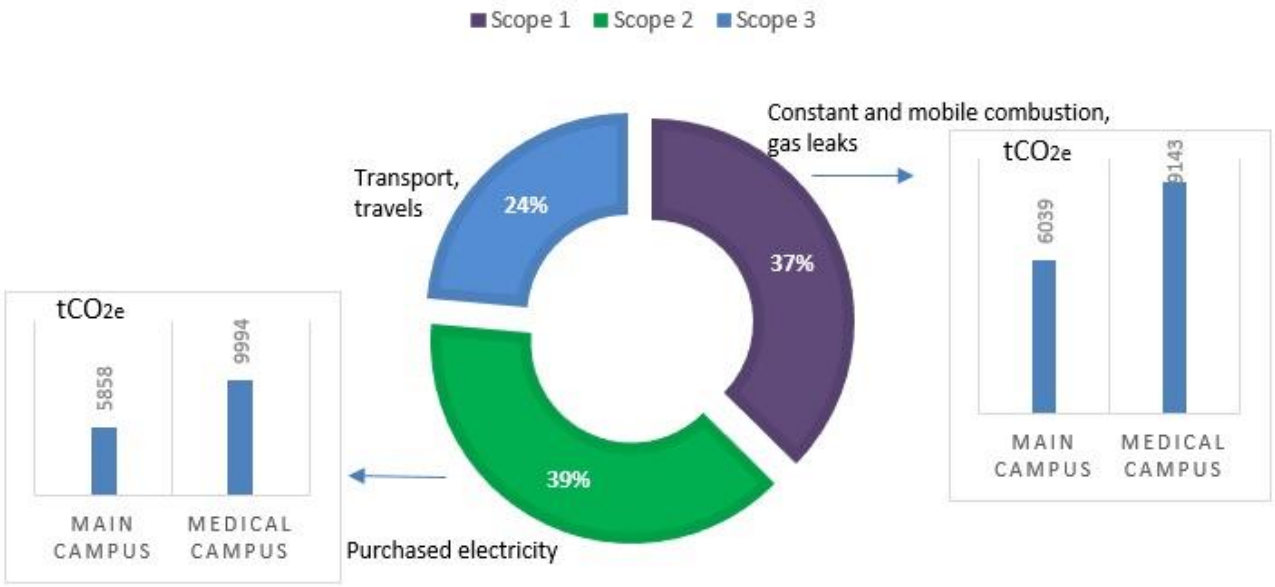

Figure 2. EU Carbon Footprint Analyses- GHG sources

Table 1. Emission factors of greenhouse gases

Operation (constant combustion)	Emission Factor (kg/TJ)			Operation (constant combustion)		Emission Factor (kg/TJ)		
	CO_2	CH_4	N_2O			CO_2	CH_4	N_2O
Heating (Natural Gas)	56.100	5	0.1	Off-road vehicles (tractor, lawnmower, etc.)	Gasoline	69.300	25	8
Generator (Diesel)	74.100	10	0.6		Diesel	74.100	4.15	28.6
LPG	63.100	5	0.1	Vehicle use	Gasoline	69.300	25	8
					Diesel	74.100	3.9	3.9
				Boat use	Diesel oil	74.100	7	3

Table 2. Emission sources of EU

	Operation	Emission Sources	Total	Unit	Main Campus	Medical Campus
1	Boilers	Natural gas	7.141.562	Sm3	2.840.814	4.300.748
2	Generator	Diesel	43.500	L	7.500	36.000
3	LPG	Natural gas	2.497	m3	2.497	-
4	Industrial and domestic energy	Purchased electricity	31.743.251	kWh	11.730.129	20.013.122
5	Vehicle use (tractor, lawnmower) off-road	Gasoline	34.125	L	34.125	-
		Diesel	2.013	L	2013	-
6	Vehicle use	Gasoline	4.814	L	4.814	
		Diesel	16.063	L	2.713	13.350
7	Truck, transport vehicle	Gasoline	37.583	L	10.883	26.700
		Diesel	1.675	L	1.675	-
8	Boat	Diesel	1.000	L	1.000	-
9	Air conditioner gas leakage	R407	300	kg	0,01	300
		R410	36	kg	18	18
		R134A	27	kg	27	-
		R22	2.044	kg	234	1.810
10	Laboratory gas	N2O	14	kg	14	-
		Acetylene	92	kg	46	46
		CO2	5.316	kg	869	4.447
		P-10	2.863	kg	2.863	-

Table 3. GHG emissions and sources

Scope	Operation	Main Campus Carbon Footprint (tCO_2e)	Medical Campus Carbon Footprint (tCO_2e)
Scope 1	Constant combustion (natural gas)	5.512	8.345
	Constant combustion (diesel)	19.8	27.7
	Constant combustion (LPG)	7.23	-
	Mobile combustion (diesel)	23.6	35.5
	Mobile combustion (gasoline)	135	61.5
	Cooling / air conditioner gas leaks & leak gases- R410	221	85.5
	Cooling / air conditioner gas leaks & leak gases- R407	0.0211	583
	Cooling / air conditioner gas leaks & leak gases- R134a	35.1	-
	Other gases (Laboratories) - CO_2	85.0	4.60
	TOTAL	6.039	9.143
Scope 2	Purchased electricity	5.858	9.994
	TOTAL	5.858	9.994
	TOTAL (SCOPE 1 + SCOPE 2)	11.897	19.136
Scope 3	Travels	315	
	*Staff transportation (private vehicle)	6.752	
	*Staff transportation (rail-subway)	996	
	*Staff transportation (road)	1.512	
	TOTAL (SCOPE 1 + SCOPE 2+SCOPE3)	21.472	19.136

* This calculation includes the total emission of medical and main campus staffs. The distance between the medical and main campus is not taken into account

The results clearly highlighted that purchased electricity in the context of Scope 2 was the highest GHG emission source with 39%. The Medical campus with 19.132 tCO_2e GHG emission should be taken into consideration primarily. The second highest GHG emissions result from constant combustion (burning

natural gas, fuel oil, *etc.*) with 37%. Emissions from mobile combustions and leakages are approximately 4% for 2016 year of Ege University and 0,55 tCO_2e carbon were emitted per capita.

4. Discussion

In this section, the results are compared with the other studies and the recommendations to reduce the carbon footprint are discussed.

In comparison to the universities in Europe, Asia, and America, it is clear that universities in Turkey has much less emissions (Fig. 3) (Boğaziçi University Report, 2014, Tokio University Report, 2012; Larsen et al., 2013; Hong Kong University Report, 2013; Cambridge University Report, 2013; Cape Town University Report, 2014; California University Report, 2007; Meida et al., 2013; Queen University Report, 2015; College Cork, 2012; Nottingham University Report, 2015). However, it could be seen that energy consumption was the most significant emission factor which should be taken into consideration in universities all over the world (Aroonsrimorakot et al., 2013, Tatsanawalai, 2015, Gomez et al., 2016, Li et al., 2015, Townsend & Barrett 2015). Yañez et al. (2020) compared the carbon footprints of different universities from different parts of the world such as the USA, China, and Europe. In the study, it was seen that the highest carbon footprint was in the USA. In different universities of the USA, their footprints per student varied between 7.9 and 36.4 tCO_2e. The CF was determined as 4.6 tCO_2e per student and 16.7 per employee tCO_2e for Norway. In Spain, 0.31 tCO_2e was calculated per student within only Scope 1 and 2 and 3.84 tCO_2e was analysed in Tongji University from China. In Ege University study 0.55 tCO_2e was calculated per capita. The USA universities have the highest CF on the basis of countries, it is in parallel with the fact that the USA has one of the highest CF's with 16 tCO2e per capita. While this value is 6-7 tCO_2e for China and the European Union, it varies between 0.3 and 8 in the underdeveloped countries of Africa (www.data.worldbank.com). With regard to other universities in Turkey, it was seen that the analysis were carried out only for specific buildings; Boğaziçi, Sakarya, and Akdeniz universities reported limited case studies in their campuses. While GHG emissions from purchased electricity were major reasons for both Sakarya and Akdeniz universities, natural gas burning was the highest GHG emissions sources for Boğaziçi University (Yaka et al., 2015; Sreng & Gümrükçüoğlu Yiğit, 2017; Boğaziçi University Report, 2014). Binboğa & Ünal (2018) studied the corporate carbon footprint of Manisa Celal Bayar University. As a result of the calculation, they determined that the University emitted 8.954 tons of CO_2 (not considering other greenhouse gases) in 2016 and 87.85% of this was due to electricity consumption. While purchased electricity in Scope 2 appeared as a hot spot in national universities, it was observed that Scope 3 and transportation were hot spots in studies in different countries. Alvarez et al. (2014), Flores et al. (2019), and Yañez et al. (2020) reported that transportation and commuting in Scope 3 were crucial emission factors. Therefore, it was found that some points should be paid attention to make comparisons. In order to make an accurate comparison, it should be observed which method is used in the study, especially which parameters are in operation in Scope 3 which is optional.

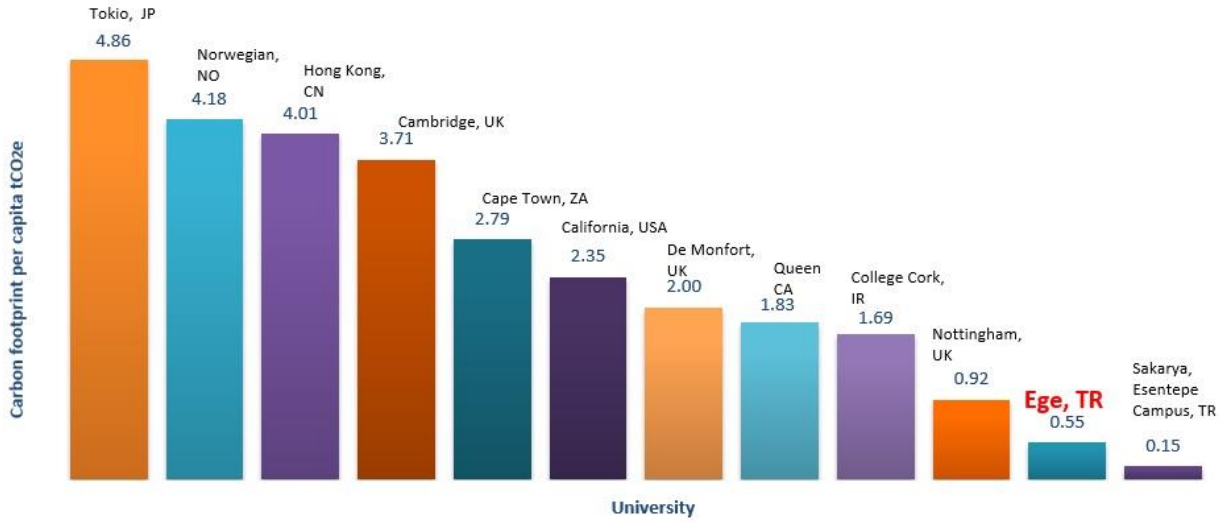

Figure 3. Carbon footprint per capita in different universities

In this study, the hot spot of Ege University was determined as the electricity consumption of the Medical Campus. When Scope 1 and 2 considered, the emission of the Medical Campus had an emission rate of 61%. In the whole campus, the first emission source was determined as electricity consumption. On the other hand, staff transportation accounted for 24% of all emissions. The crucial point to note here is that only 15% of the staffs with their private vehicles occupied 73% of the transportation emissions.

These results showed the important emission sources of Ege University within the scope of determining the hot spots, which was the main purpose of the study. As the next step, recommendations for Ege University were researched. In this context, in order to monitor the sustainability steps of the campus, the globally accepted green measurement rankings and the recommendations of the United Nations were examined. There are many different evaluation methods and green campus tools in the world for universities to make their own situation

evaluations. The Greenmetric from Indonesian Universities is one of these evaluation methods which Ege University has applied. Greenmetric ranks universities by scoring on energy, water, transportation, infrastructure, education, and waste under environmental sustainability titles. In fact, this ranking systems provides an important step in evaluating the scores of universities within themselves and being a road map for sustainability steps. Carbon footprint is also included in these evaluations under the heading of energy. In this system, which Ege University has applied since 2016, the heading of "energy" and the necessity to reduce the carbon footprint stand out. At this point, lower scores obtained from the energy category also support the transition from fossil fuels to cleaner energy sources and the need to use energy efficient devices.

One of the other important resources for universities to become more sustainable is the Greening Universities Toolkit prepared by the United Nations. The toolkit offers 3 main topics to universities in order to reduce their carbon footprint. Energy conservation is one of the topic which includes staff energy conservation training; energy awareness programs, such as campus posters; improved space utilization; and energy efficiency standards for new construction and refurbishments (www.unep.org). At this point, Ege University should reach all students and staff to raise awareness in energy saving, just like in the Zero Waste System of EU. These trainings will also provide an important step towards obtaining ISO: 50001 Energy Management Certificate. In Turkey, there were not yet ISO: 50001 Certification in public universities but Nişantaşı and Yaşar University have been awarded ISO: 50001 Certificate. In Ondokuz Mayıs University, Energy Management Directive has been prepared within the scope of energy studies and put into practice as an accepted directive (OMU, 2020). According to Yañez et al. (2020) adopting an energy management system such as ISO 50001 could provide 6% energy consumption and it would mean 2.1% total GHG emission reduction. Ege University should prioritize energy efficiency for the Medical Campus among short-term goals to reduce GHG emission. Besides, energy efficiency studies of buildings should be carried out for the whole campus to get ISO: 50001 certificate.

According to Tatsanawalai (2015), the most important method for reduced GHG is to change the behavior through focusing on carbon footprint. Gökçek et al. (2019) investigated the relationship between behavior patterns of students and carbon footprint at Niğde Ömer Halisdemir University. It was observed that the highest carbon footprint was found in the medical faculty students with an average of 433 kg CO_2 / year. Also, in this study, it was determined that male students had higher carbon footprint. The reason for this was determined to be staying at student houses rather than dormitories and using electronic devices more than females (Gökçek et al., 2019). Therefore, educational programs in universities have enormous potential to reduce the power consumption of students and staff. Projects and courses for changing behaviors, reducing fossil fuels should be supported by the university. Media organs of the university and the university webpage could be used for communication and awareness purposes.

The second proposal of the United Nations Greening Universities Toolkit is energy efficiency. The steps include building retrofitting; heating; ventilation and air-conditioning; and periodic recommissioning and building tuning to optimize energy efficiency. This steps need technical operation and budget. It will be necessary to make energy efficiency analyses in the buildings and to create a road map according to the results. The current status of Karamanoğlu Mehmetbey University (KMU) in terms of energy was revealed and its energy efficiency potential was examined. The energy consumption values used in the buildings belonging to the KMU campus were determined, regular measurements were taken using appropriate devices (thermal camera, flue gas analyzer, ultrasonic flowmeter, etc.) at energy consumption points, and efficiency increasing projects were proposed at these points. According to the results, it is determined that the energy consumption value of the university in 2016 reached 1422 tons of equivalent oil (TEP) and has an energy saving potential of up to 18% (Rüşen et, 2018). Üreden & Özden (2018) suggested that the external insulation of the buildings could reduce the emission due to heat. Providing the building ventilation over the heating system especially in winter months would prevent sudden temperature drops in the building and would not cause unnecessary carbon emissions.

Biomass of the campus is also important for carbon absorption potential. One ton of carbon storage in a tree represents the removal of 3.67 t of carbon from the atmosphere and the release of 2.67 t of oxygen back into the atmosphere (Ugle et al., 2010). The carbon storage capacity of different species was reported in Erdoğan et al. (2020) study (www.tmmobizmir.org). In this context, it is recommended to reveal the flora biodiversity in the campus and analyze the carbon storage capacities on the basis of species. In addition, increasing the biomass of the campus will reduce GHG emissions.

Private vehicle use accounted for the largest share of transport emissions. In this context, staff buses between campus and residents are offered to stop the use of private vehicles. It is recommended that the staff in units that are far away from the campus entrance such as Faculty of Engineering, Ege Vocational School, Faculty of Education, are directed to low-priced services provided by the management of the EU. On the other hand, to provide a safe bicycle path to the campus entrance for staff and students from districts such as Bornova and Bayraklı can also reduce the private vehicle use.

Another important step is to turn to renewable energy sources and installation of solar cells, wind, biomass, and other renewable energy systems. As mentioned before; EU, which was founded in 1955, is not in a position where all these transformations can happen at once with its 90 buildings and 70.000 population. Therefore, as a result of this study it is suggested that EU should determine and follow-up of numerical targets to reduce its carbon footprint within the short, medium, and long term periods.

In terms of accelerating the efforts of universities to reduce GHG emissions, universities should be supported financially. TUBITAK, the Ministry of Environment and Urbanization, the Ministry of Development, and the Ministry of Energy can encourage the national universities with grant applications. These grants will support the

GHG reduction targets of Turkey by 2030 and the EU Green Deal targets.

5. Conclusion

In conclusion, GHG emissions of EU in 2016 were 40.068 tCO_2e. These results suggest that "Energy Policy and Action Plan" should be urgently prepared by Ege University. Use of fossil fuels and purchased electricity and use of constant combustion sources which come from burning of fossil fuels were main sources and should be reduced. In order to realize an efficient energy management system in EU, energy-saving technologies and/or energy saving campaigns should be put in practice. The summary of recommendations is;

- Energy efficiency studies, with priority in Medical Faculty,
- To provide staff transportation service to campus and safety bicycle way to campus,
- To increase education, courses, activities to raise awareness,
- To start using renewable energy sources at a certain rate,
- To increase biomass in the campus for storing the carbon.

Universities need to become more proactive in sustainable development actions for helping societies to become more sustainable. The most successful universities on the way to becoming a green campus will be universities that accept these changes and take action. Although there are carbon footprint studies in some universities in our country, it is necessary to spread these studies throughout the country and to implement energy policies aimed at reducing the carbon footprint.

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Research Article / Araştırma Makalesi

A Preliminary Study on Age Determination and Examination of Some Growth Parameters in Agile frog (*Rana dalmatina* Bonaparte, 1839) (Anura: Ranidae) Specimens

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Abstract: In this study, a total of 16 Agile frog, *Rana dalmatina* species preserved in the ZDEU-COMU collection and collected from Çanakkale (4 ♂♂, 4 ♀♀) and Kırklareli (7♂♂, 1♀) were examined to estimate their age and the relationship between age and body size using the skeletochronology method. In addition, both femora and phalanx of the samples were evaluated together. Sexual maturity ages were found to be 2-3 in male and female individuals of both populations. The age ranges from 2-4 in males and 3-5 in females. According to this study, there was a positive correlation between length and age (r=0.740). In addition, no difference was observed in terms of LAG numbers compared to the age rings in the femora and Phalanx bones of the samples.

Keywords: Skeletochronology, Thrace, Çanakkale, Kırklareli.

Çevik Kurbağa (*Rana dalmatina* Bonaparte,1839) (Anura: Ranidae) Örneklerinde Yaş Tayini ve Bazı Büyüme Parametrelerinin İncelenmesi Üzerine Bir Ön Çalışma

Öz: Bu çalışmada, ZDEU-ÇOMÜ koleksiyonunda bulunan Çevik kurbağa *Rana dalmatina* türüne ait toplam 16 örneğin iskelet kronolojisi yöntemi kullanılarak yaşları ve yaş ile vücut büyüklükleri arasındaki ilişkiler ortaya konmaya çalışılmıştır. Ayrıca örneklerin hem femur hem de falanj kemikleri birlikte değerlendirilmiştir. Eşeysel olgunluk yaşları her iki populasyonun erkek ve dişi bireylerinde 2-3 olarak bulunmuştur. İncelenen örneklerde yaş erkeklerde 2-4, dişilerde ise 3-5 arasında değişmektedir. Yapılan bu çalışmaya göre boy ile yaş arasında olumlu bir korelasyon olduğu görülmüştür (r=0.740). Ayrıca örneklerin femur ve falanj kemiklerindeki yaş halkaları karşılaştırıldığında LAG sayıları açısından bir farklılık gözlemlenmemiştir.

Anahtar kelimeler: İskelet Kronolojisi, Trakya, Çanakkale, Kırklareli.

1. Giriş

Canlıların yaşlarının tayin edilebilmesi için günümüze kadar çeşitli yöntemler kullanılmıştır. Bunlardan yakala-tekrar yakala yöntemi (Durham & Benett, 1963) güvenilir bir yöntem olmasıyla birlikte birtakım zorlukları da bulunmaktadır. Doğada markalanıp bırakılan canlıları tekrar yakalamanın güçlüğü, daha uzun bir zamana yayılması vb. gibi durumlar söylenebilir. Bununla birlikte canlılığın devamı için önem arz eden bu yöntem sıklıkla kullanılmıştır. Önceki çalışmalarda, yaşları bilinen örneklerle karşılaştırması yapılan morfometrik ölçümlerdeki farklılıkların belirlenmesi, sert kemik dokulardaki değişimlerin karşılaştırılması gibi yöntemler de kullanılmıştır (Petersen, 1892; Senning, 1940; Tanaka, 1956; Peabody, 1961; Kleinenberg & Smirina, 1969; Castanet, 1994). İskelet kronolojisi yöntemi amfibi ve sürüngenlerde sıklıkla kullanılmaktadır. Bu yöntem, bireylerin yaşlarının belirlenmesi, yaşam uzunlukları, olgunlaşma zamanları, eşeysel dimorfizm, büyüme ve üreme zamanları, kemik yapısı hakkında bilgi edinilmesini sağlar (Castanet et al., 1993). Aynı zamanda populasyonu etkileyen çevresel varyasyon ve seçilimler için canlının yaşam öyküsünün bilinmesi önemlidir (Miaud et al., 2003). Amfibi ve sürüngen örneklerinden alınan uzun kemik enine kesitlerinde birbirine paralel halkalar şeklinde görülen büyüme izleri sayılarak yaş tahmininde bulunulmaktadır. Tespit yapılırken kemiğin diyafiz bölgesi kullanılmalıdır (Rozenblut & Ogielska, 2005).

Doğadaki canlılar, özellikle amfibiler çoğu zaman insan kaynaklı etkilerden ötürü büyük bir yaşam mücadelesi içindedirler ve tehdit altında yaşamak zorunda kalmaktadırlar (Wake, 1991; Pechmann et al., 1994). Populasyonlara ait bireyler üzerinde yaşam sürelerini belirleme amacıyla yapılan bu çalışmalar, çalışılan türün doğadaki yaşam uzunluğu, tehditleri vb. gibi durumlarının bilinmesi için önemlidir. Bununla birlikte yaş çalışmaları, amfibilerin ekolojisi, büyüme ve gelişmesi ile ilgili yapılacak çalışmaların temelini oluşturacak niteliktedir (Smirina, 1994).

Türkiye'de, amfibilerde yaş çalışmaları 2000'li yıllardan itibaren başlamıştır. Erişmiş et al. (2002) *Rana bedriagae* örneklerinde ikinci parmağın falanjı ile çalışmışlardır. Bu kemiğin iskelet kronolojisi yöntemine uygun olduğunu göstermişlerdir. Yılmaz et al. (2005) Trabzon Yıldızlı deresinde bulunan *Pelophylax ridibundus* populasyonlarıyla çalışmışlardır. *P. ridibundus* falanj kemikleri çıkarılıp, iskelet kronolojisi yöntemi kullanılmıştır. Dişilerde maksimum yaş 6, erkeklerde 7 olarak bulmuşlardır. Ayrıca erkeklerin dişilerden daha

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küçük olduğunu belirlemişlerdir. Olgun et al. (2005) *Triturus karelinii* örnekleriyle çalışmışlardır. Erkek ve dişilerin vücut uzunluğu, yaş ve büyüme oranları arasında benzer ilişki bulmuşlardır. Eşeysel olgunluğa 3 ile 4 yaşında ulaştıklarını, maksimum yaşlarını ise erkek ve dişilerde sırasıyla 8 ve 11 olarak belirlemişlerdir. Kutrup et al. (2011) yapmış oldukları çalışmada farklı rakımlarda, Dörtyol (6 m yükseklik) ve Karagöl'de (1480 m yükseklik) yaşayan iki *Pelophylax ridibundus* populasyonunun, vücut büyüklükleri ve yaş yapılarındaki farklılıkları tahmin etmeye çalışmışlardır. Karagöl'de erkekler 2-8, dişiler 2-7 yıl arasında iken Dörtyol'da erkeklerin yaş dağılımını 4-11, dişilerin yaş dağılımını 3-7 yıl arasında bulmuşlardır. Eşeysel dimorfizm sadece Dörtyol populasyonunda görülmüş, dişilerin erkeklere göre daha büyük olduğunu tespit etmişlerdir.

Rana dalmatina batıda Fransa ve Kuzeydoğu İspanya'dan başlayarak Orta ve Güney Avrupa üzerinden Kuzeybatı İran'a kadar uzanan bir dağılış alanına sahiptir. Türkiye'de ise Kuzey Anadolu bölgesi boyunca ve Trakya bölgesinde dağılış gösteren bir dağ kurbağası türüdür (Başoğlu et al., 1994). Türkiye'de dağılış gösteren anurlar üzerine yapılmış birçok yaş çalışması [*R. holtzi* Guarino & Erişmiş (2008), *R. macrocnemis* Çiçek (2011*), Bufo viridis* Kutrup et al. (2011), *Hyla arborea* Özdemir et al. (2013), *Pelophylax caralitanus* Erişmiş (2018), *Bombina bombina* Bülbül et al. (2018), *P. bedriagae* Başkale et al. (2018)] olmasına karşın, literatür taramasında *R. dalmatina*'nın Türkiye populasyonları ile ilgili bir çalışmaya rastlanmamıştır. Tür ile ilgili Sarasola-Puente et al. (2011) İber yarımadasında dağılış gösteren *R. dalmatina* populasyonlarında yapmış olduğu araştırmada 58 dişi, 118 erkek bireyle çalışılmış maksimum yaş dişilerde 6, erkeklerde 8 olarak bulunmuştur. Maksimum SVL dişilerde 73.00 mm, erkeklerde 59.50 mm ölçülmüştür.

Rana dalmatina türü IUCN verilerinde "LC" (Düşük Riskli) olarak verilmiştir (IUCN, 2009). Çanakkale ve Kırklareli civarından yakalanan *R. dalmatina* örneklerinde vücut ölçümleri ve yaş değerleri karşılaştırılarak, populasyonlar arasında bir karşılaştırma yapılması, femur ve parmak kemiklerinde yaş halkalarının karşılaştırılması, incelenen bireylere ait eşeysel olgunluk yaşının ortaya konulması ve böylelikle daha sonraki çalışmalar için altlık oluşturması amaçlanmıştır.

2. Materyal ve Metot

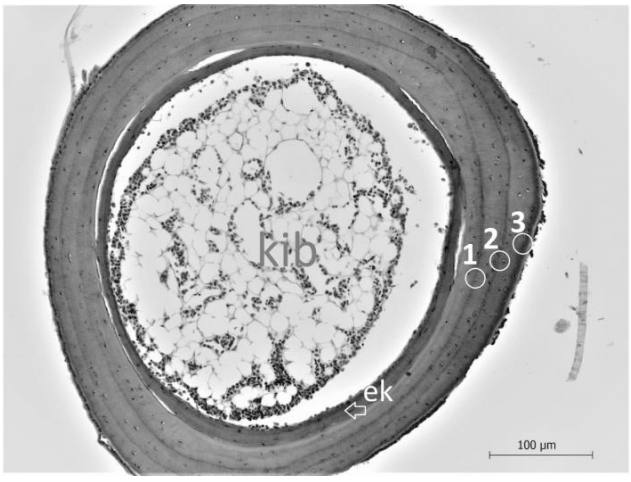
Çalışmada, Çanakkale (4 ♂♂, 4 ♀♀) ve Kırklareli (7 ♂♂, 1 ♀) civarından daha önceki çalışmalarda toplanmış ve ZDEU-Çanakkale Onsekiz Mart Üniversitesi koleksiyonunda bulunan, toplam 16 *Rana dalmatina* örneğinin vücut ölçümleri yapılmış ve tahmini yaşları hesaplanmıştır. Çalışmada Kırklareli ve Çanakkale'den incelenen *R. dalmatina* örneklerinin Trakya kesiminden olması ve örneklerin toplandığı lokaliteler arasında yükseklik farkının (yaklaşık 200 m) çok olmaması nedeniyle birlikte değerlendirilmiştir. Ancak literatürde dişilerin erkeklerden daha büyük boylu olduğu belirtilmiş olması nedeniyle yaş gruplarına göre erkek ve dişiler ayrı ayrı değerlendirilmiştir (Başoğlu & Özeti, 1973). Tahmini yaşları hesaplanan bu örneklerde, vücut uzunluğunun yaş ile ilişkili olup olmadığı araştırılmıştır. Tüm istatistiksel analizler IBM SPSS Statistics 20.0 programı kullanılarak yapılmıştır.

2.1. Morfolojik ölçümler

Çanakkale ve Kırklareli populasyonlarına ait örneklerdeki yaş değerlerinin boy ile olan ilişkisini ortaya koymak adına çeşitli morfolojik ölçümler yapılmıştır. Burun ucu-kloak arası mesafe (SVL), femur uzunluğu (FU), tarsus uzunluğu (TU), femur+tibia mesafesi (FTM), aksillar ve inguinal arası mesafe (AİM), baş uzunluğu (BU) 0.01 mm hassasiyetli Mitutuyo marka dijital kumpasla yapılmıştır.

2.2. Parmakların iskelet kronolojisi için hazırlanması ve histolojik incelemeler

Vücut ölçümleri yapılan örneklerin femur ve ayak ikinci falanj kemikleri alınmıştır. Alınan kemiklerin üzerindeki deri ve kas dokuları temizlendikten sonra hem femur hem de falanj örnekleri %5'lik nitrik asit (HNO_3) ile dokunun boyutuna göre 2-7 saat aralığında bekletilip dekalsifikasyon (kalsiyumdan arındırma) sağlanmıştır. Dokulardan asidi arındırabilmek için, örnekler bir gece boyunca akarsu altında bırakılmıştır. Sonrasında dehidratasyon işlemi için dokular 1'er saat artan alkol serilerinden geçirilmiştir. Sonrasında ksilen-parafindeki dokular, saf parafin banyosuna alınarak ksilen uzaklaştırılıp parafin içerisine gömülmüştür. Dokulara ait parafin bloklardan Leica 2125 RT marka mikrotom kullanılarak 12-14 µm kalınlığında kesitler alınmıştır. Boyamadan önce etüvde 20-30 dk. bekletilmiş, fazla parafin alınarak Hematoksilen & Eosin (H&E) ile boyama yapılarak yaş halkalarının belirginleşmesi sağlanmıştır. Olympus CX21 marka ışık mikroskobunda yorumları yapılıp Olympus BX51 marka ışık mikroskobunda Olympus Analysis LS programı kullanılarak fotoğrafları çekilmiştir (Şekil 1).



Şekil 1. Çanakkale üç yaşındaki dişi örneğe ait femur enine kesiti (SVL: 43.55 mm) (O: LAG çizgileri kib: kemik iliği boşluğu, ek: endosteal kemik)

Figure 1. Femoral cross-section of a three years old female specimen from Çanakkale (SVL: 43.55 mm O: LAG lines, kib: marrow cavity, ek: endosteal bone)

3. Bulgular

İncelenen örneklerde yaş; erkeklerde 2-4, dişilerde ise 3-5 arasında değişmektedir. İki yaşındaki bir erkek örnek cinsel olgunluğa erişmiştir ve SVL değeri 34.5 mm'dir. Üç yaşındaki 6 erkek örnekte SVL değerleri 42.73-47.91 mm arasında değişmektedir. Dört yaşındaki 4 erkek örnekte ise SVL değerleri 47.80-53.53 mm arasında bulunmuştur. Üç yaşındaki 3 dişi örnekte ise SVL değeri 40.34-45.73 mm

arasında bulunmuştur. Dört yaşındaki bir dişi örnekte 52.47 mm, 5 yaşındaki 1 dişi örnekte ise SVL değeri 65.66 mm olarak ölçülmüştür (Tablo 1). Örneklerde yapılan Spearman korelasyon analizine göre SVL ile yaş arasında pozitif yönde anlamlı bir ilişki bulunmuştur (r=0.740) (Tablo 2). Eşeysel olgunluk dişi ve erkeklerde 2-3 olarak tespit edilmiştir. Femur ve parmak ucu kemiklerinde aynı hayvanda yaş halkaları benzer bulunmuştur.

Tablo 1. *Rana dalmatina* örneklerinde SVL ve yaş değerleri

Table 1. SVL and age values in *Rana dalmatina* specimens

	Çanakkale								Kırklareli							
Cinsiyet	♂♂				♀♀				♂♂							♀
Yaş	2	3	3	3	3	3	4	5	3	3	3	4	4	4	4	3
Örnek No	2012	2004/15A	2004/15C	2019	2004/15B	2003/14	2008/1B	2008/1A	2011/22A	2010/52A	2010/52B	2011/22C	2011/22B	2010/55	K.Örnek	2009/41
SVL	34.5	42.99	43.63	47.91	43.55	45.73	52.47	65.66	42.73	45.71	44.73	47.8	49.48	53.53	48.79	40.34
FU	17.79	22.39	22.28	24.3	23.18	23.16	25.81	35.88	25.22	24.97	24.74	29.49	27.82	28.95	25.35	24.74
TU	10.11	13.54	12.3	12.89	13.98	14.45	16.1	19.47	14.03	13.75	14.66	14.44	15.16	15.76	15.44	13.15
FTM	38.71	45.1	47.6	46.92	46.5	50.59	54.28	74.3	46.61	52.08	50.75	55.77	53.65	53.75	57.43	47.75
BU	12.69	12.89	13.13	15.03	11.71	13.23	12.8	22.29	15.22	15.66	12.3	16.94	16.74	18.05	16.27	13.54

Tablo 2. *Rana dalmatina* örneklerinde ortalama değerler

Table 2. Average values in *Rana dalmatina* specimens

Çanakkale	Yaş	N	SVL				
			Min. (mm)	Ort. (mm)	Maks. (mm)	SH	SS
♀♀	3	2	43.55	44.64	45.73	1.09	1.54
	4	1	52.47	52.47	52.47		
	5	1	65.66	65.66	65.66		
♂♂	2	1	34.50	34.50	34.50		
	3	3	42.99	44.84	47.91	2.18	2.67
Kırklareli							
♀	3	1	40.34	40.34	40.34	-	-
♂♂	3	3	42.73	44.39	45.71	1.24	1.51
	4	4	47.8	49.89	53.53	2.18	2.51

SVL: Burun ucu-kloak arası mesafe, N: örnek sayısı, maks: maksimum değer, min: minimum değer, ort: ortalama, SH: standart hata, SS: standart sapma

4. Tartışma ve Sonuç

İncelemelerde endosteal kemik ile periosteal kemik bazen birbirinden bariz şekilde ayrılmışken bazen ise bazıları neredeyse iç içe olarak görülmüştür. Histolojik boyama ile endosteal kemik biraz daha koyu gözükse de endosteal rezorbsiyon halka sayımlarında zaman zaman yanılgıya yol açabilmektedir (Rozenblut & Ogielska, 2005). Femur ile falanj görüntüleri incelendiğinde ikisinde de LAG sayıları aynı sayılmıştır, nadiren de olsa femur ve falanjda ±1 LAG görüldüğü, bu farklılığının histolojik kesitlerdeki hatadan kaynaklı olabileceği düşünülmektedir.

Sarasola et al. (2011) İber yarımadasında *Rana dalmatina* üzerine yaptıkları çalışmaya göre eşeysel olgunluk yaşını dişi ve erkeklerde genellikle 2 olarak tespit etmiş, bazı erkeklerin 1, bazı dişilerin ise 3 yaşından sonra ergenliğe ulaştıklarını bulmuşlardır. Bu çalışmada da genelde 2 yaşında olgunluğa ulaştıkları görülürken, bazılarının 3 yaşında olabileceği yorumuna varılmıştır. 3 yaşındaki erkeklerde ortalama SVL 44.61 mm olarak ölçülmüş, İber yarımadasında yapılan çalışmada ise 3 yaşındaki erkek bireylerin ortalama boyu 54.73 mm ölçülmüştür. Aynı zamanda 4 yaşındaki erkek bireylerde ortalama SVL 49.89 mm iken İber yarımadasında 4 yaşındakilerin ortalaması 57.37 mm olarak belirlenmiş, aynı yaşlara oranla vücut uzunluğu İber yarımadasında daha fazla olduğu tespit edilmiştir. Trakya bölgesinde ortalama SVL 44.61 mm sahip dişi ve erkeklerde 3 yaş halkası sayılırken, Sarasola et al. (2011)'ın verilerine göre 43.07 mm ortalaması olan 20 adet erkek bireyde henüz sadece 1 yaş halkası sayılmıştır. İber yarımadasında dişilerin erkeklerden daha uzun olduğu ortaya konulmuştur. Trakya bölgesi ve İber yarımadasındaki örnekler karşılaştırıldığında, Trakya bölgesininkiler İber yarımadası örneklerinden daha küçüktür ve yaş-SVL arasındaki orantı açısından da farklılık göstermektedir. Bu durumun şimdiki çalışmada incelenen örnek azlığından da kaynaklanabileceği kanısındayız. Zira Başoğlu & Baran (1973)'e göre normal olarak boyları 5-6 cm arasında değişirken, erkeklerde 7 cm, dişilerde ise nadir olarak 9 cm olarak görülmektedir. Terentjev & Chernov (1949)'a göre dişiler 12 cm kadar olabilmektedir. Ayrıca femur ve parmaktan elde edilen yaş halkalarının aynı olması nedeniyle bu tür ile yapılacak daha kapsamlı bir çalışmada farklı populasyonlardan elde edilen örneklerden 1-2 örnekte yaş halkalarının benzerliği test edildikten sonra sadece parmak ucundan parça alınarak yaş çalışmasının yapılması söz konusu türün böyle çalışmalardan daha fazla zarar görmesinin önüne geçilmiş olacaktır.

Daha fazla örnek ile yapılacak bir yaş çalışması türün büyüme ve gelişmesi hakkında daha detaylı bilgiye ulaşılmasına ve ileride yapılacak olan koruma ve izleme çalışmalarına altlık oluşturacaktır.

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Research Article / Araştırma Makalesi

Morphology and Histology of Male Reproductive System of *Gryllus campestris* Linnaeus, 1758 (Orthoptera: Gryllidae)

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Abstract: The morphological and histological structures of male reproductive system of adult *Gryllus campestris* Linnaeus,1758 (Orthoptera: Gryllidae) have been defined by using stereo microscope, light microscope, and scanning electron microscope. The male reproductive system of *G. campestris* is formed as a couple of testes, a pair of vas defence, two seminal vesicles, accessory gland, a single muscular ejaculator bulb and ejaculator duct which opens the aedaegus and spermatophore. The mature *G. campestris* has two nearly uniformly broad testes. Spermatozoa are produced in the testes. Each testis is formed as series of slender tubules or follicles in which disparate stage of spermatogenesis (spermatocytes, spermatids, and spermatozoa) and spermatozoa develop. Initially, the germ cells at the proximal end of the testicular follicle undergo mitosis to form spermatocytes. Later, spermatids are formed from the spermatocytes in the middle region of the follicles through meiosis. At last, spermatids, the proximal region of the follicle differentiates into spermatozoa. Every follicle is connected to the vas deferens via vas efferens to transfer spermatozoa. Vas efferens is surrounded by a single layer of cubic epithelium containing an oval core. Ejaculatory duct opens and a large ventral male mating organ opens the penis or the end of aedeagus. The accessory glands is a liquid to aid in the transfer during mating female spermatozoa secretes. Spermatophore or sperm ampulla is a capsule or mass containing spermatozoa formed by males. Spermatophore is synthesized by the male accessory glands. Before the sperm is transposed to the female, the spermatophore is located in the sac like the ampulla. In this study, the morphology and histology of the male reproductive system of *G. campestris*, which is an economically important species for our country, was examined and illustrated by stereo microscope, light microscope and scanning electron microscope (SEM). Our findings, characterizing the structure of the male reproductive system of *G. campestris*, form the basis of future studies including technological approaches to control this pest in agriculture.

Keywords: Testes, vas deferens, spermatogenesis, spermatophore, electron, light and stereo microscopy.

Gryllus campestris Linnaeus, 1758 (Orthoptera: Gryllidae)' in Erkek Üreme Sisteminin Histolojisi ve Morfolojisi

Öz: Ergin *Gryllus campestris* Linnaeus,1758 (Orthoptera: Gryllidae)'ın erkek üreme sisteminin histolojik ve morfolojik yapısı stereo mikroskobu, ışık mikroskobu ve taramalı elektron mikroskobu ile tanımlandı. Erkek üreme sistemi bir çift testis, bir çift vas deferens, iki seminal kese, yardımcı bezler ve kaslı bir ejakulatör kese, aedeagusa açılan bir ejakulatör kanal ve spermatofordan meydana gelmektedir. Olgun *G. campestris* erkek bireyleri spermatozoa oluşturan bir çift testise sahiptir. Her testis çeşitli spermatogenez aşamalarına (spermatosit, spermatid, spermatozoa) sahip bir dizi ince silindir tübüller veya folikülden oluşur. İlk olarak spermatositler foliküllerin proksimal uçlarında germ hücrelerinin mitoz bölünmesi ile oluşur. Ardından foliküllerin orta bölgesinde mayoz bölünme ile spermatidler meydana gelir. Son olarak foliküllerin proksimal bölgesinde spermatidler spermatozoaya farklılaşır. Her folikül spermatozoayı aktarmak için vas eferens aracılığı ile vas deferense bağlanır. Vas eferens, tek katlı kübik epitel ile çevrilidir ve oval çekirdeklidir. Ejakulator kanal ventralde genişleyerek erkek üreme organı penis veya aedeagusa açılır. Yardımcı bezler çiftleşme sırasında spermatozoanın dişiye aktarılmasına yardımcı olan bir sıvı salgılar. Spermatofor, erkek bireylerin yardımcı bezleri tarafından sentezlenen, spermatozoanın kapsül veya kitle halinde aktarılmasını sağlayan yapılardır. Spermler dişiye akatarılmadan önce bir kese şeklinde spermatofor içinde bulunur. Bu çalışmada ülkemiz için ekonomik açıdan önemli bir tür olan *G. campestris* erkek üreme sisteminin morfolojisi ve histolojisi stereo mikroskop, ışık mikroskobu ve taramalı elektron mikroskobu (SEM) ile incelenmiş ve gösterilmiştir. *G. campestris*'in erkek üreme sisteminin yapısını karakterize eden bulgularımız, tarımda bu zararlıyı kontrol etmek için teknolojik yaklaşımlar da dahil olmak üzere gelecekteki çalışmaların temelini oluşturacaktır.

Anahtar kelimeler: Testis, vas deferens, spermatogenez, spermatofor, elektron, ışık ve stereo mikroskop.

1. Introduction

Represented by approximately 26000 species in the world, the Orthoptera order has a wide place among insect orders (Çıplak & Demirsoy, 1996; Çıplak, et. al., 2002; Demirsoy et.al., 2002; Gullan & Cranston, 2010). Orthoptera such as katydids are a range of insects that include grasshoppers and crickets, including closely related insects**.** The order is divided into two subgroups as Caelifera (grasshopper, Locust) and Ensifera (cricket, katydids) (Harz, 1969). *G. campestris* is a species of crickets and is found in the field cricket genus and the Gryllini tribe. This deep colorful insects that can not fly is relatively wide; the males in range of 19 to 23 mm. The females of this group range in size from 17 to 22 mm. *Gryllus* is one of the most widespread genus of field crickets. It is found throughout the Americas, Europe and Africa, extending eastwards into tropical Asia (Harz, 1969; Otte & Cade, 1984; Vrenozi & Uchman, 2020).

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The male reproductive system in insects includes a couple of testes, a couple of sperm channel (vas deferens) coming out of the testes, an ejaculator channel where the vas deferens are opened, and accessory glands opening to the ejaculator channel. (Viscuso et al., 1999, 2014; Liu et al., 2005; Jones et al., 2013). The structure of the testes follicles of male insects can be used to explain taxonomic relationships. In Orthoptera, the testes are made up of 300-400 a thin, tubular follicle, in which spermatogenesis occurs, standing together thanks to the connective tissue. Every follicle is attached to a vas deferens with a very thin vas efferens. (Liu et al., 2005; Jones et al., 2013). In many insect species, spermatozoa are isolated to the genital tract of females by spermatofores with hard and elastic sheath produced by male reproductive acessory glands and transferred in bundles in bulk. (Viscuso & Vitale, 2015). In insects, there are sperm channels that allow spermatozoa to be transported from the testes. The length of the sperm channel varies between species. Each sperm channel is disunited into two ways, one intratesticular channel and the other vas deferens. Vas deferens is the part of the sperm duct outside the testes. (Viscuso et al., 2014). In some insect species, the part of the sperm canal expands to the point where the ejaculator opens into the canal to collect and store sperm before mating. This structure is the seminal sac with mesodermal origin. In some insect groups such as Trichoptera, there is no seminal sac (Viscuso et al., 1999, 2014). The sperm canal and seminal sacs produce secretion. Here, the male gametes will survive until they are sent to the female (Viscuso et al, 2014). The structure and arrangement of the accessory glands can vary in the same family, subfamily, and even in the same breed. This change in the biochemical structure of the outbreak is associated with different functions in the secretions of the glands, such as sperm cell feeding, suppressing the female's acceptance of other insects, or stimulating the laying of eggs (Marchini et al., 2009). *G. campestris* is a species of biological and economical importance both in our country and in the world. This study is important in terms of both providing biological control against this species and contributing to the work to be done later.

2. Material and Methods

2.1. Collection and care of experimentals

The adult male individuals of *G. campestris* (n = 30) were collected from the Kazan, Ankara between June and August 2016. Ten specimens of *G. campestris* were knocked out by the ethyl acetate vapor and broken into pieces 0,1 M sodium phosphate tampon, pH 7.2 analyzed. Finally, samples were photographed under the Leica SZX7 stereo microscope (SM).

2.2. Preparation of samples for light microscopy

To examine male reproductive system samples histologically, mature samples of the species were fixed in 10% neutral formalin liquid for 24 hours. The samples were then cleaned in tap water and passed through a series of 50% to 100% ethanol to remove their water. Next they were cleaned with xylene, filtrated and buried in paraffin wax (65^0C). Using the Microm HM 310 microtome, 5-6 µm thick sections were taken from these paraffin blocks. The sections colored with hematoxylin and eosin (H&E) were imaged under the Olympus BX51 LM brand light microscope (LM).

2.3. Preparation of Samples for Scanning Electron Microscopy

Samples were fixed in 2.5% glutaraldehyde (pH 7.2, phosphate buffer) for examination under scanning electron microscopy. Then they washed out three times in phosphate tampon, dried by following up an ethanol progressive series (50, 70, 80, 90 and 100%). The texture were dried by Hexamethyldisilazane (HMDS). After, the tissues were attached to by double sided tape on the SEM stubs. Then it was covered with gold by spraying gold with the Polaron SC 502 device. Stubs were studied with a JEOL JSM 6060 LV SEM at an acceleration voltage of 5-10 kV. Finally, digital pictures were taken.

3. Results

The adult male reproductive system in *G. campestris* Linnaeus, 1758 is consisted of a pair of whitish, nearly uniformly broad testes, a couple of long vas deferens, seminal vesicles, an ejaculator duct, spermatophore, accessory glands, and aedeagus (Fig. 1 a-d). Each testis consists about 140 follicles which are almost spindle shaped. (Fig. 2 a-b). Testicular follicles have three different developmental zones: growth zone, maturation zone, and differentiation zone. Spermatocytes were observed in the growth zone. (Fig. 3 a-b). The growth zone is followed by the maturation zone and spermatids are formed at this stage. (Fig. 4 a-d). In the differentiation zone, spermatids turn into spermatozoa with prominent head, neck and tail parts (Fig 5 a-d). The testes are surrounded with a white peritoneal lamina. The testes are attached to the seminal vesicle via a pair of vas deferens (Fig. 6 a-f). The outer surface of the testicle is inverted with a dense muscle and trachea network. The cells have a monolayer cubic epithelium. Vas deferens is surrounded by an outer muscle layer and sperm bundles were found inside the lumen (Fig. 6 a-f).

At the end of the seminal vesicles accessory glands were observed. Accesory glands consist of a large number of thin long fingerlike structures. The outer surfaces are quite flat and they have a single-layered cubic epithelium (Fig. 7 a-d). The nuclei of the cells are large and in the midst of the cell. Granules were seen in epithelial cells. Lumen is full of secretion (Fig. 7 a-d). Ejaculator canal consists of a single layer cylindrical epithelial cell and muscle layer surrounding ejaculatory duct. Ejaculator canal has muscle bundle. Ejaculator canal opens into the genital chamber (Fig. 8 a-d)

Except those, the spermatophore structure was observed. The spermatophore was fixed at the level of the female gonophore. They are completely made up of the secretion of the male accessory glands. The spermatophore was flask–shaped with a wider part called ampulla with a neck and attachement plate and with a long sperm tube. The sperm is contained within the sac like ampulla of the spermatophore before it is removed to the female (Fig. 9 a-d).

4. Discussion

The reproductive organ in the insect usually consists of vas deferens, seminal vesicle, ejaculatory duct, and accessory glands; however, some differences can be seen among insect orders. There is morphological diversity in different families in Orthoptera (Snodgrass, 1957). In orthoptera,

generally have as per the number, shape and organizing of sperm tubules (Snodgrass, 1937). The male reproductive systems of *G. campestris* as other Gryllidae species, generally, consist of a couple of testes, vas deferens, and accessory glands. Male reproductive system of *G. campestris* is consists of two whitish, nearly uniformed broad testes. Similarly, male reproductive system of *Gryllodes sigillatus* (Orthoptera: Gryllidae) consists of the testes that are large, white, reversed pear-like organs. Both tests are lying dorso-lateral to the alimentary canal (Nandchahal, 1972).

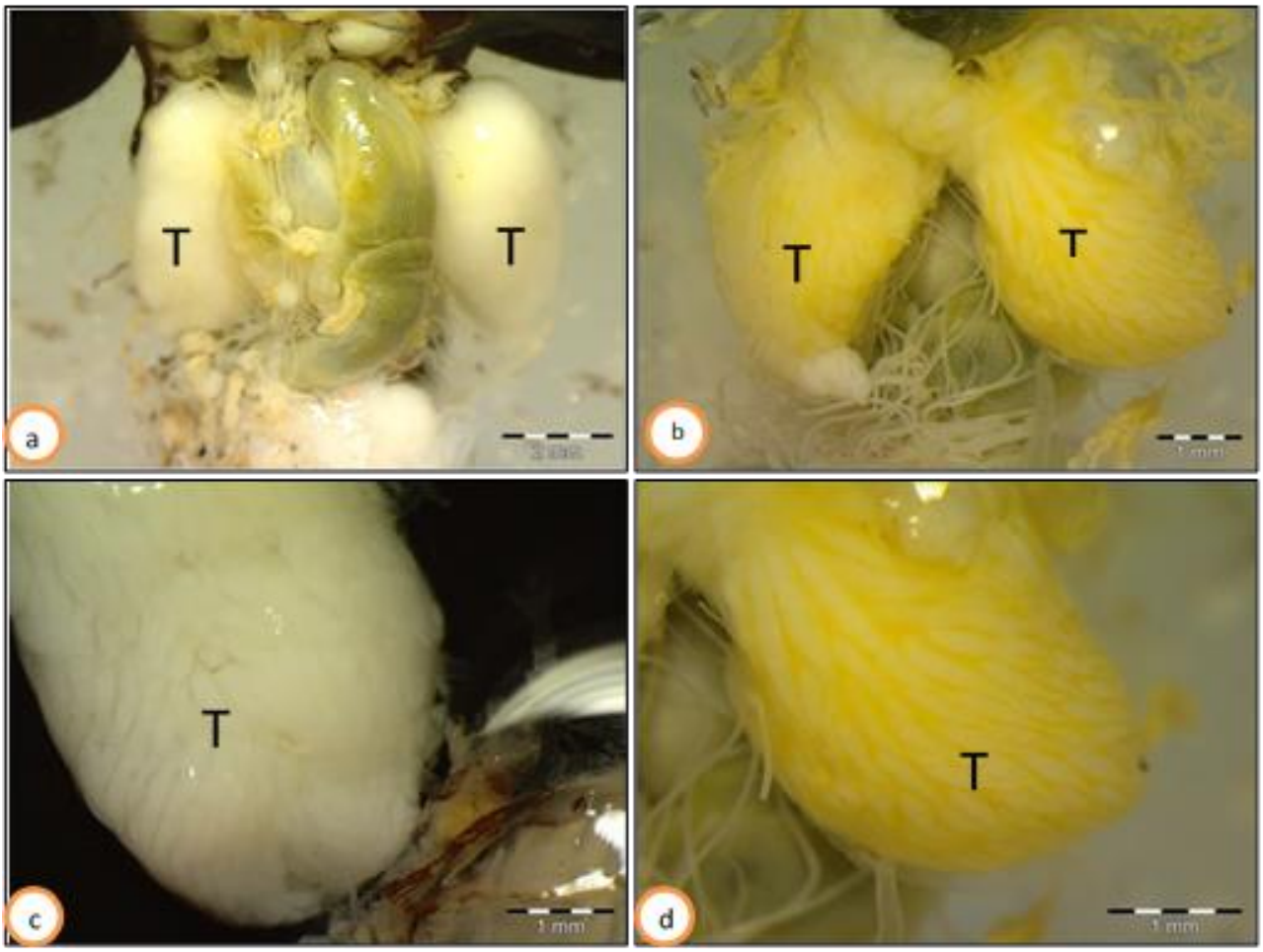

Figure 1. Dissected general morphology of immature and mature male reproductive organs of *G. campestris* (SM). a. The general view of white colored testis (T) in the immature male reproductive system. b. The general view of yellow colored testis (T) in the mature male reproductive system. c. The testis follicles under the sheath covering the testicle in an immature male. d. The testicular follicles under the sheath covering the testicle in the adult insect.

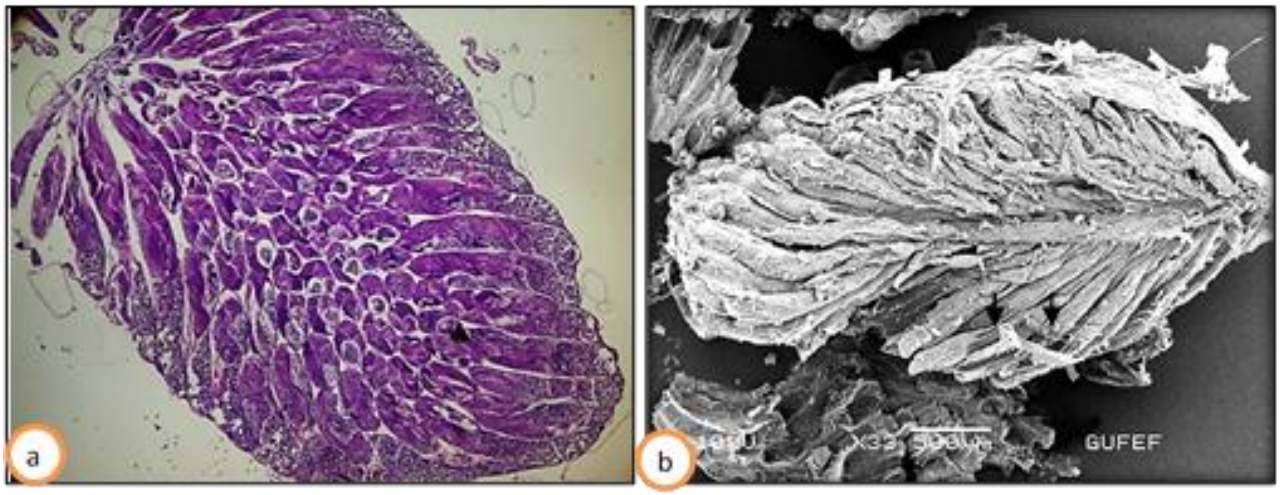

Figure 2. The general view of testis follicles in *G. campestris*. a. Stages of spermatogenesis seen in testicular follicles in longitudinal section of mature testis (x100) (H&E) (LM). b. SEM photograph of the longitudinal section of testis follicles (arrow ⟶)

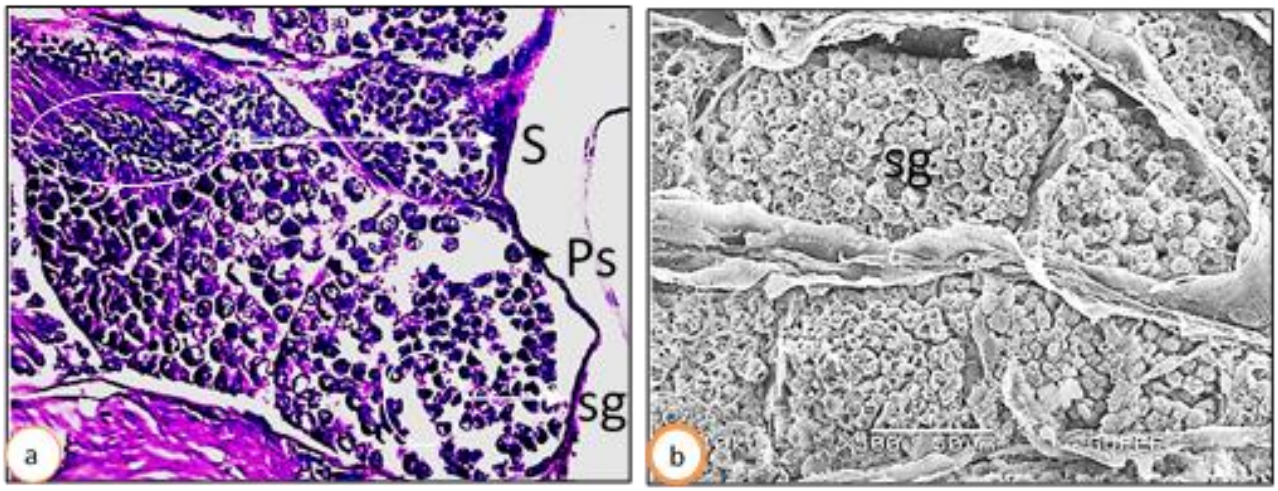

Figure 3. a. Spermatogonia (Sg) and spermatocytes (Sp) in the growth zone (×400, H&E) (LM). b. Peritoneal sheath (Ps) surrounding the testis and spermatogonia in the testis (SEM).

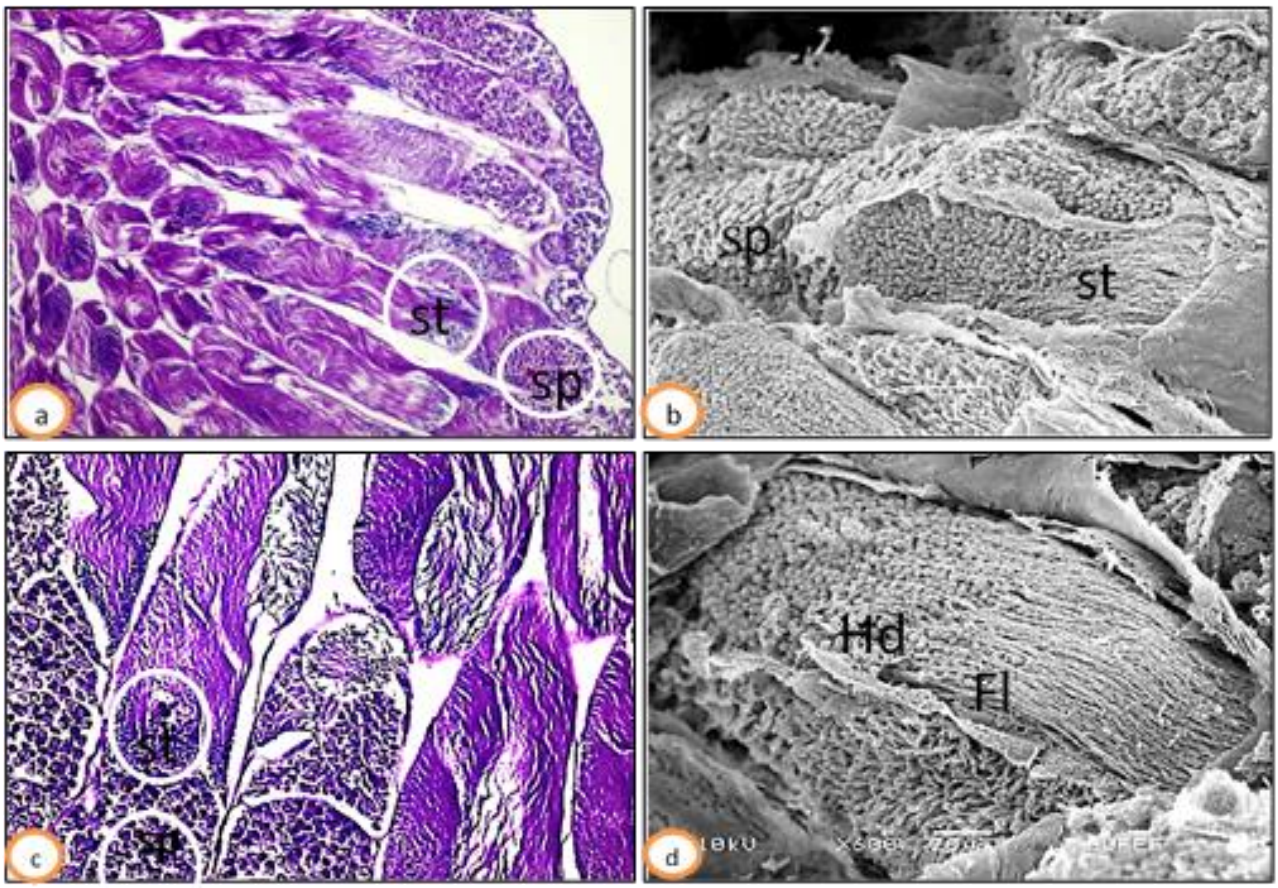

Figure 4. Spermatocytes and spermatids found in the maturation zone of testicular follicles. a, b. The transformation of spermatocyte into spermatids in the testicular follicle (x200) (H&E) (LM and SEM). c. The histological cross-section of spermatocytes (Sp) and spermatids (St) at the maturation zone (x200) (H&E) (LM). d. The flagellum (Fl) and round-shaped head regions (Hd) in spermatids (SEM).

The male reproductive system of *Orphulella punctata* (Orthoptera: Acrididae) consists of two testes and testicles surrounded by an orange-colored sheath; however in *Poecilimon cervus* (Tettigoniidae) testes are yellow. (Silva et al., 2018; Polat, 2016).

The male genital system of *Tetrix arenosa angusta* (Hancock) (Orthoptera: Tetrigidae) is consist of the testes, vasa efferentia, vasa deferentia, accessory glands, ejaculatory duct and intromittent organ and a pair of testes of a mature grouse locust. The testicles extend in the hemocoel between the dorsal diaphragm and the alimentary canal (Widdows &Wick, 1959).

The male reproductive system of *Tylopsis liliifolia* (Orthoptera: Tettigonidae) composed of a couple of testicles disposed laterally along the alimantary canal. However, male reproductive system of Acrididae testes structure is different. Male reproductive system of Grassopher (Orthoptera: Acrididae) consists of two testes and each testis consists of a series of slender follicles (Viscuso & Vitale, 2015).

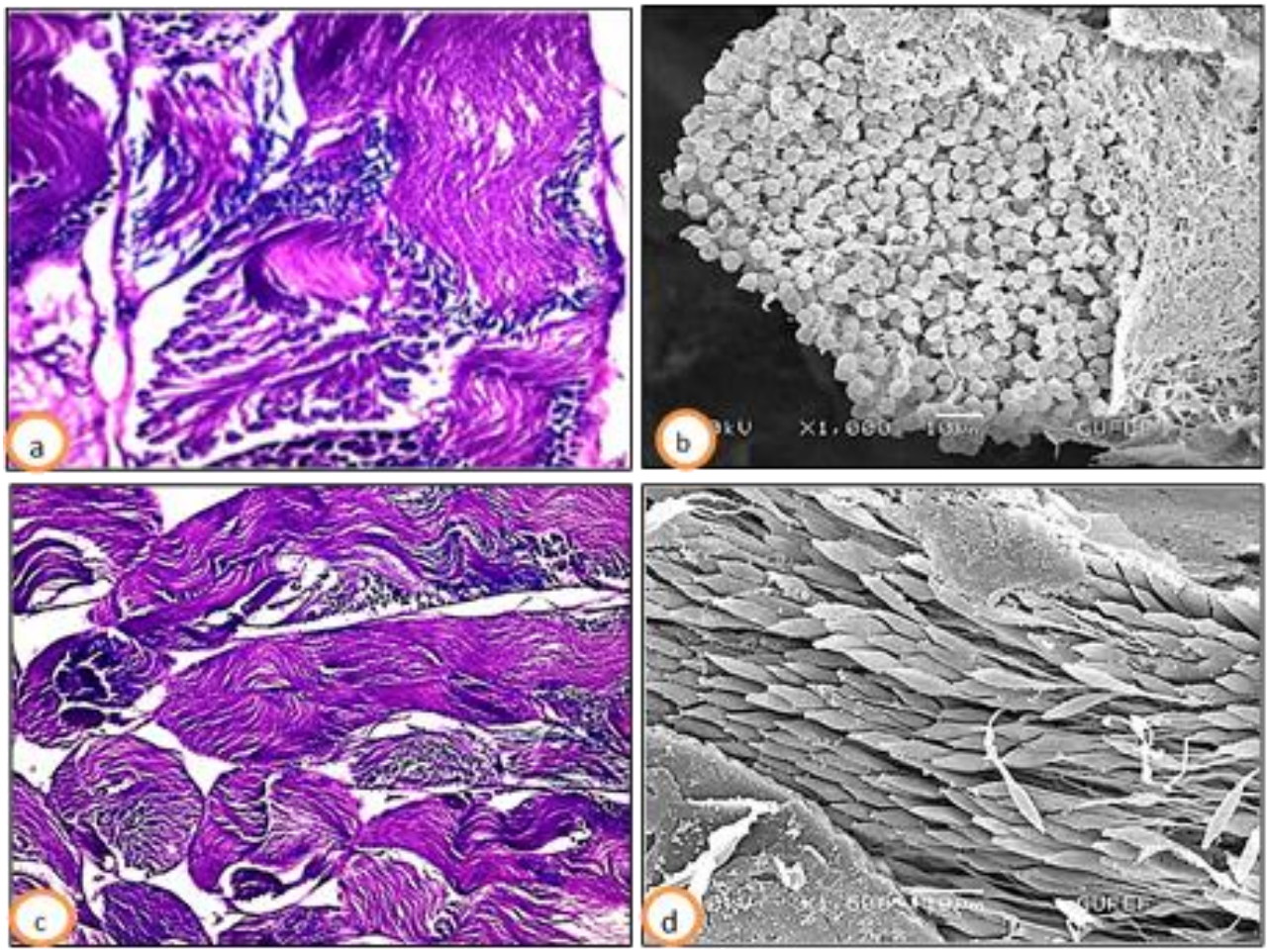

Figure 5. The spermatids and spermatozoa in differentiation zone. a. Differantation of spermatids to spermatozoa in testicular folicle (x400) (H&E) (LM). b. The round head regions in the spermatids (SEM). c. The histological section of spermatozoa in regular bundles shaped (x400) (H&E) (LM). d.The spermatozoon head and flagellum parts (SEM).

Male reproductive system of *G. campestris* consist of a couple of testes and every testis is comprised of 140 testicular tubules. In *G. assimilis* (Orthotera: Gryllidae) each testis is comprised approximalety 280 follicles. The follicles are slightly spindle- shaped. In *Gryllodes sigillatus* (Walker) (Orthoptera: Gryllidae) every testis is occurig approximalety 154 follicles (Nandchahal, 1972). In *Tylopsis liliifolia* (Orthoptera: Tettigoniidae) either testis is formed of 30 to 39 separate testicular follicles.

Additionally, every testicular follicle has a characteristic teardrop shape. There is little change in the shape between the follicles (Viscuso & Vitale, 2015). However, *Parrettix toltecus* (Orthoptera: Tetrigidae) consists of 9 follicles and *Tetrix vittatum* Zett. Consists of 30 follicles each testes (Widdows & Wick, 1959). Male reproductive system of *Baeacris punctulatus* (Orthoptera: Acrididae) consists of two testes and each testis has 30 follicles (Michel & Teran, 2005). In *Orphulella punctata* (Orthoptera: Acrididae) each testis consists of 4 follicles and their structure is filiform. (Silva et al., 2018). The number of testicular follicles varies by species. e.g. *Chortophaga viridifasciata* (Orthoptera, Acrididae) 26-28, *Melanoplus differentialis* (Orthoptera, Acrididae) 188 and *Romalea microptera* (Orthoptera, Romaleidae) (Silva et al., 2018).

In the male reproductive system of *G. campestris* every testis is consist of many testis follicles in which varied stage of spermatogenesis (spermatocytes, spermatids, and spermatozoa). The structure and arrangement of the sperm is generally the sperm of other locusts. It is similar to its structure. Initially, the germ cells at the proximal end of the testicular follicle undergo mitosis to form spermatocytes. Later, spermatids are formed from the spermatocytes in the middle region of the follicles through meiosis. At last, spermatids, in the proximal region of the follicle differentiation into spermatozoa. Similar developments have been seen in *O. punctata* (Orthoptera, Acrididae), *Pseudochorthippus parallelus parallelus* (Orthoptera, Acrididae), *P. cervus* (Orthoptera, Tettigoniidae) (Polat, 2016; Silva et al., 2018; Polat et al., 2019). At the end of the differentiation stage, to form bundles of sperm, a large number of sperms comes together. *Orphulella punctata* showed 450 spermatozoa per bundle and for Orthoptera, the presence of sperm in bundles occurs in Eumastacidae and Acrididae with number of sperm per bundles range from 256 to 2048 (White, 1954; Silva et al., 2018).

Vas deferens of *G. campestris* is quite long and thin. Vas deferens connects the testicles to the ejaculatory canal. Vas deferens connects testes to the ejaculatory duct in the male reproductive system of *P. parallelus parallelus* too. It is a long thin canal originating from the middle part of the testis in vas deferens, *P. Parallelus parallelus, P. cervus,* and *G. sigillatus.* (Nandchahal, 1972; Polat, 2016; Vitale et al.,

2011; Viscuso & Vitale, 2015). Accessory glands are a part of the male reproductive system, differ in shape and number. Although, they are quite similar histologically. In *G. campestris*, the accessory glands are on both sides of the ejaculatory duct and they seem to be many of white tubules. In *Locusta migratoria migratorioides*, the accessory glands are on either sides of the ejaculatory duct (Gallois & Cassier, 1991, Polat et al., 2020). Accessory glands in *G. sigillatus*, consists of six groups of tubules of various colors and sizes. (Nandchahal, 1972).

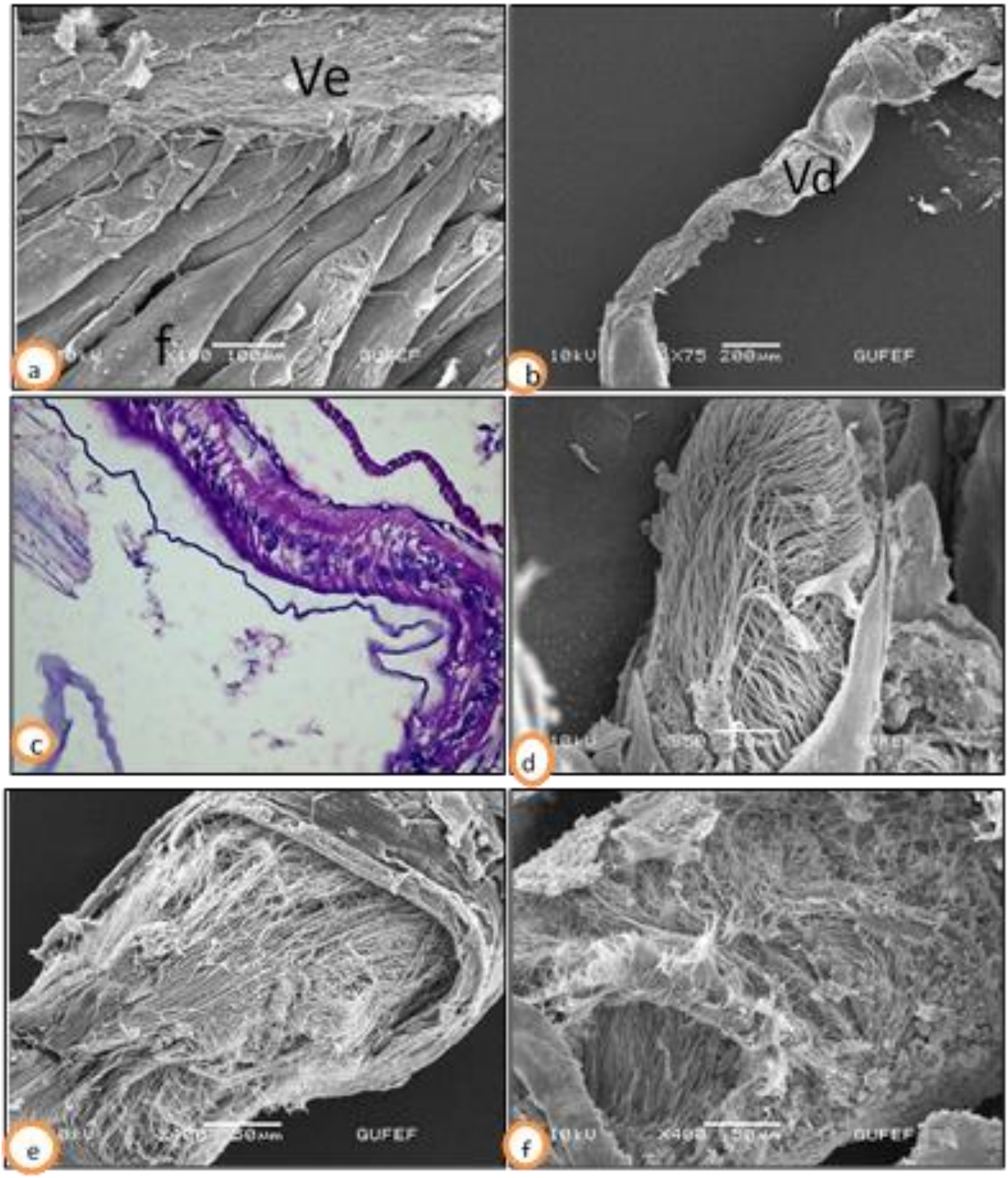

Figure 6. The vas deferens in *G. campestris*. a. The connection of testicular follicles to the vas deferens (Vd) by the vas efferentia (Ve) (SEM). b. The sperm tails in stack in the vas deferens lumen (SEM). c. The muscle layer and mono layered epithelium with oval nucleus surrounding the vas deferens (x1000) (H&E) (LM). d-f. The mature sperm bundles in the lumen of vas deferens (SEM).

In *Locusta migratoria, Gomphocerus rufus, S. gregaria* and *Camnula pellucida* consist of 16 tubules but *P. parallelus parallelus* consists of 10 tubles (Kaulenas, 2012; Chapman, 2013; Polat et al., 2020). Even so *Gryllus sp*. (Orthoptera, Gryllidae) consists of approximately 600 tubles (Chapman, 2013). Accessory glands of *G. campestris* have no cuticle layer on the wall. Accessory glands produced secretions formed plays a role in the production of spermatophore and transfer of sperm to female individual (Chapman, 2013).

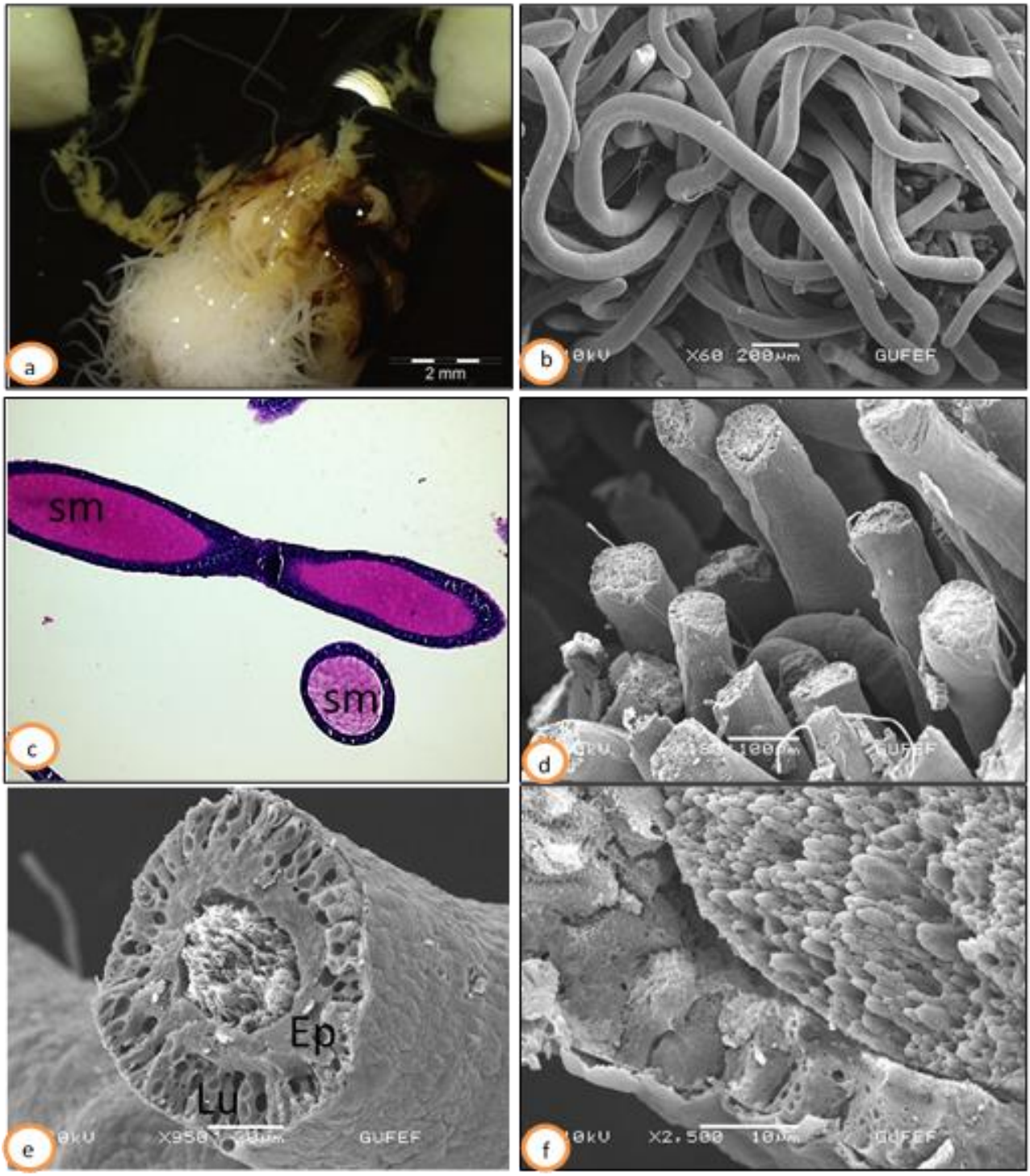

Figure 7. The accessory glands in *G. campestris*. a. The general view of white, thin, long and complex accessory glands (SM). b. The accessory glands with smooth surface (SEM). c. The longitudinal and cross section of accessory gland (x200) (H&E) (LM). d-f. A single layer of epithelium surrounding the accessory glands and lumen filled with secretory material (Sm) (SEM). Ep-epithelium, Lu-lumen, Sm-secretion material.

Male reproductive system of *G. campestris* has of a spermatophore which was fixed at the level of the female gonophore. The spermatophore is flask-shaped with a wider part called ampulla with a neck and attachment plate and with a long sperm tube. The sperm sac nearly similies a pear which is white and contained the mixed up spermatodesms and a dense yellowish secretion.

In *Decticus verrucivorus* (Orthoptera: Tettigoniidae), spermatophore is connected externally to the female's genitalia and occurs of two parts: a large, gelatinous, sperm-free portion, the spermatophylax, used as food by the female after mating; and a sperm-containing ampulla, eaten after the spermatophylax has been eaten (Wedell & Arak, 1989). In the species examined (Orthoptera: Tettigoniidae), the spermathopore have a similar morphology. They were flask-shaped, with a wider part called ampulla, with a long peduncule or neck. Spermatophore of *T. Liliifolia*, the sac is opalescent white

and contained the mixed up spermatodesm and a dense, colourless secretion but *Bolivarius siculus* has yellowish secretion (Viscuso et al., 2015) Spermatophore of *Gryllus bimaculatus* (Orthoptera: Gryllidae) occurs of an ampulla (containing the spermatozoa), a long slender sperm tube and an attachment plate (Hall et al., 2000).

Consequently, the purpose of this article is to contribute to explaining the male reproductive system of *G. campestris*, providing biological control against this species and revealing properties that may be useful for future studies on the taxonomy and phylogeny of other Orthoptera species.

Ethics committee approval: Ethics committee approval is not required for this study.

Conflict of interest: The authors declares that there is no conflict of interest.

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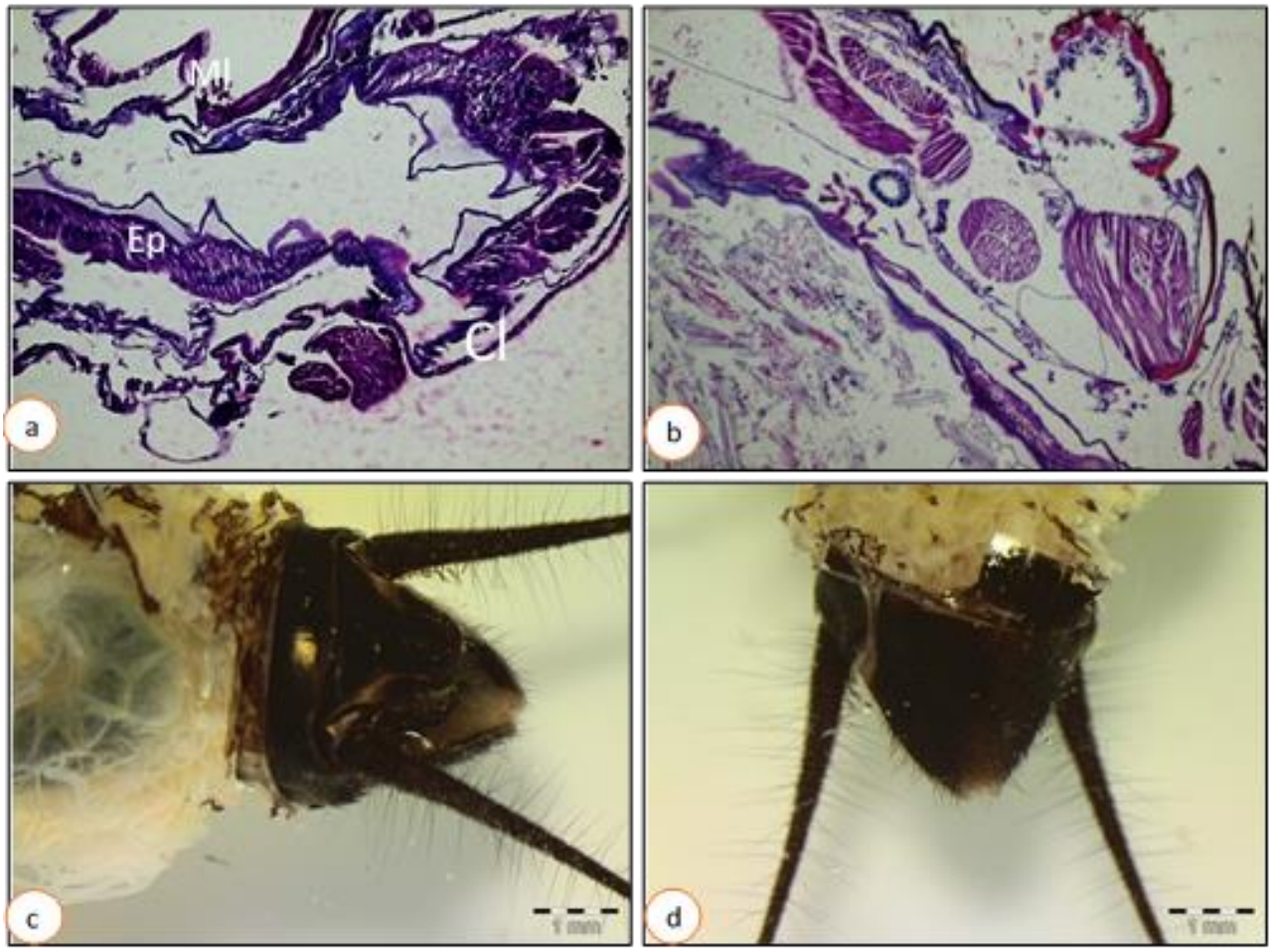

Figure 8. The ejaculator canal and genital chamber of *G. campestris*. a. The cuticular layer (Cl), a single layer cylindrical epithelial (Ep) and muscle layer (Ml) surrounding ejaculatory duct (x400) (H&E) (LM). b. The histological section of muscle bundles between ejaculatory duct and genital chamber (x400) (H&E) (LM). c, d. The general view of male genital of *G. campestris* (SM).

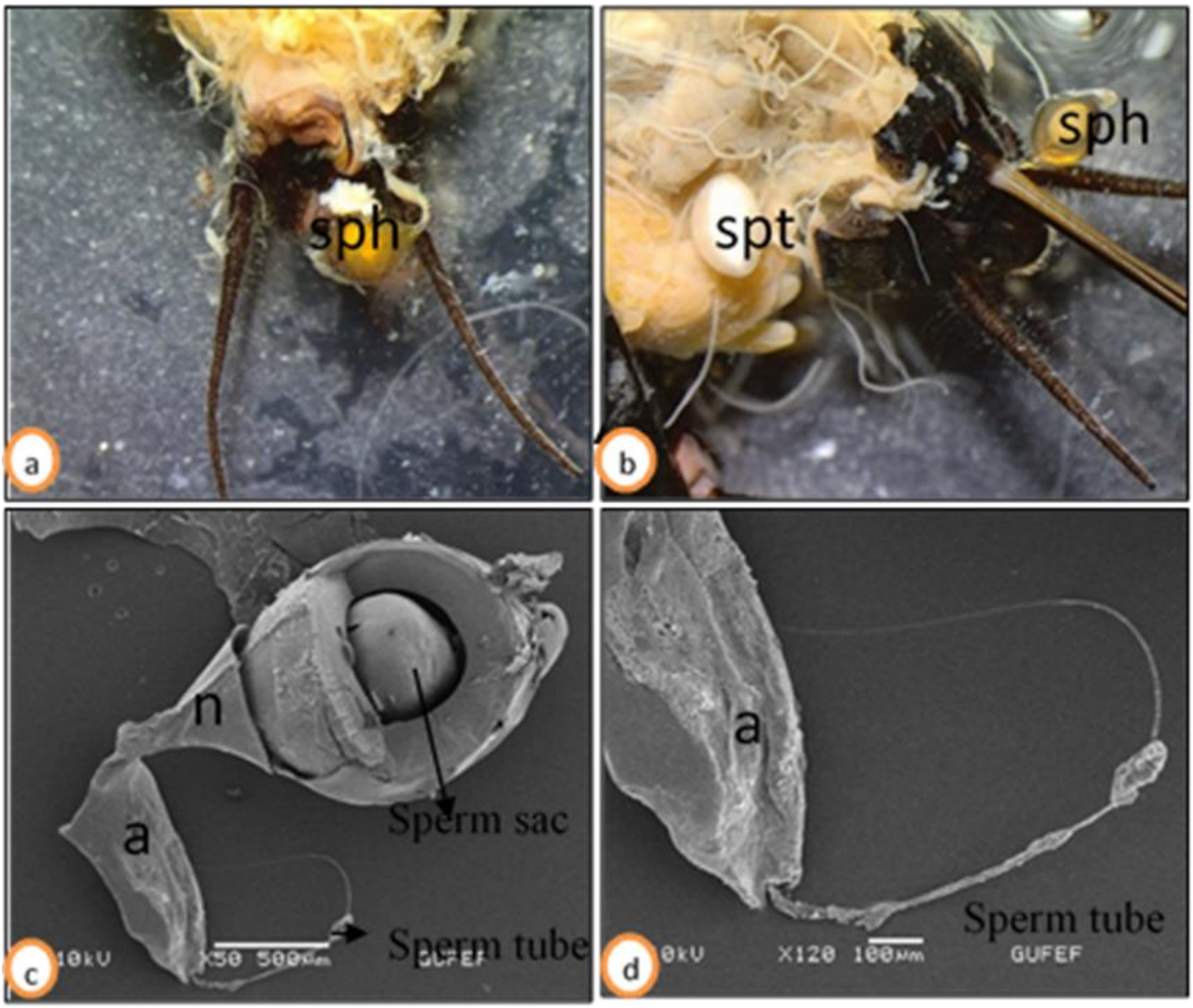

Figure 9. The spermatheca (spt) and spermatophore (sph) in *G. campestris*. a. The general view of spermatophore in male. b. The general view of spermatophore and ovipositor in female (SM). c-d. The sperm sac. d The neck (n), attachment plate (a) and spermtube (SEM).

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Anti-Colorectal Cancer Effects of Medicinal Plants: *Euphorbia helioscopia*, *Ferula elaeochytris*, and *Sideritis albiflora*

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Abstract: Phytochemicals, extracts, and mixtures obtained from plants have been offered as an option for cancer treatment and prevention for modern drug discovery in recent years. For this purpose, in this study, anti-colorectal cancer effects of the hexane, acetone, methanol, and water extracts obtained by sequential extraction from *Euphorbia helioscopia* L., *Ferula elaeochytris* Korovin, and *Sideritis albiflora* Hub.-Mor. on DLD-1 cell line were investigated *in vitro* by using Alamar blue assay. Dose-dependent inhibition was detected in the viability of DLD-1 cell line. In all three plants species, *E. helioscopia* (IC_{50}: 140.83±0.31 µg/mL), *F. elaeochytris* (IC_{50}: 67.93±0.12 µg/mL), and *S. albiflora* (IC_{50}: 85.12±0.10 µg/mL) methanol extracts showed higher anti-colorectal effects on DLD-1 cell line compared to other extracts tested for the same species. In addition, the IC_{50} value of doxorubicin used as a standard was found as 6.10±0.55 µg/mL. With the results obtained, as the first report highlighting *in vitro* anti-colorectal cancer effects of the studied plant species on DLD-1 cell line, promising marks were obtained from the analysis of the extracts as anti-cancer sources for plant-derived drug applications.

Keywords: Plant species, extracts, cytotoxic activity, DLD-1 cell line, Alamar blue assay.

Tıbbi Bitkilerin Anti-Kolorektal Kanser Etkileri: *Euphorbia helioscopia*, *Ferula elaeochytris* ve *Sideritis albiflora*

Öz: Bitkilerden elde edilen fitokimyasallar, ekstreler ve karışımlar, son yıllarda modern ilaç keşfi için kanser tedavisinde ve önlenmesinde bir seçenek olarak sunulmuştur. Bu amaçla, bu çalışmada sıralı ekstraksiyon kullanılarak *Euphorbia helioscopia* L., *Ferula elaeochytris* Korovin ve *Sideritis albiflora* Hub.-Mor. bitkilerinden elde edilen hekzan, aseton ve metanol ekstrelerinin DLD-1 hücre hattı üzerindeki anti-kolorektal kanser etkileri Alamar mavisi testi kullanılarak *in vitro* olarak incelendi. DLD-1 hücre hattının canlılığında doza bağlı inhibisyon tespit edildi. Üç bitki türünün hepsinde, *E. helioscopia* (IC_{50}: 140.83±0.31 µg/mL), *F. elaeochytris* (IC_{50}: 67.93±0.12 µg/mL) ve *S. albiflora* (IC_{50}: 85.12±0.10 µg/mL) metanol ekstreleri aynı türler için test edilen diğer ekstrelere kıyasla DLD-1 hücre hattı üzerine daha yüksek anti-kolorektal kanser etkisi gösterdi. İlaveten, standart olarak kullanılan doksorubisinin IC_{50} değeri 6.10±0.55 µg/mL olarak belirlendi. İncelenen bitki türlerinin DLD-1 hücre hattı üzerindeki *in vitro* anti-kolorektal kanser etkilerini vurgulayan bu ilk çalışma ile bitki kaynaklı ilaç uygulamaları için anti-kanser kaynakları olarak ekstraktların analizinden umut verici sonuçlar ortaya çıkarıldı.

Anahtar kelimeler: Bitki türleri, ekstre, sitotoksik aktivite, DLD-1 hücre hattı, Alamar mavisi testi.

1. Introduction

Throughout history, people have preferred and used plants in nature for the treatment of many diseases (Aras et al., 2021). Although developing technology and newly produced treatment techniques have brought new drugs, the dangerous side effects of these drugs have reached levels that cannot be ignored and the interest in scientific studies on plants and their bioactive properties has deepened (Yuan et al., 2016). It has been reported that the population benefiting from plants containing phytochemical compounds with bioactive properties in the treatment/prevention of many health problems constitutes 80% of the whole world population and this rate is even higher in developing countries (Ozkan et al., 2016). The concept of treatment named 'Complementary Medicine' has gained more importance from the past to the present, and in some countries, government incentives are also given for research on the discovery of endemic plant species and their medical properties (Tekeli et al., 2019; Vickers & Zollman, 1999). The World Health Organization (WHO), in a report prepared based on scientific studies on medical plants by researchers from 91 countries, reported that there are approximately 20,000 species of plants used in the treatment of diseases (Patra et al., 2018). So far, it is known that around 10,000 phytochemical compounds have been identified in the plant kingdom, including tannins, flavones, triterpenoids, phenolics, steroids, saponins, polysaccharides, and alkaloids and still a large percentage has not been identified (Barbosa et al., 2013). In recent studies, natural phytochemicals with antioxidant, antimicrobial, antifungal, antidiabetic, anti-inflammatory, anticancer, and antihypertensive properties have been suggested as valuable for human health (Demir & Akpınar, 2020). The use of plants for medicinal purposes has increased with the discovery that chemical compounds

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used in drug production show similarities with plant active substances. In the past, the use of plant origin drugs was more common; this rate has gradually decreased as a result of the development of chemical applications. However, in recent years, with the research and development of new therapeutic uses of plants, the demand for natural herbal products has increased (Ekor, 2013). Due to the rich chemical structures of plants, it has been one of the research areas of pharmacology to use it for the development of new and highly effective drug formulations. In developed and developing countries, 25% of prescription medicines consist of active ingredients obtained from plants (Demir & Akpınar, 2020).

Cancer is defined as an abnormality in the structure of the cell as a result of genetic or epigenetic changes. This negative change occurs in cancer cells uncontrolled and rapidly. In addition, cancer cells have the ability to escape from physiological suppressors and spread (metastasize) with the mutations they undergo. These rapid and active growth processes of tumor cells cause cancer (with 277 different types) to be the second major disease-causing death worldwide (Hassanpour & Dehghani, 2017). It has been stated that colorectal cancer is in the third place after lung and breast cancer deaths in females, and lung and prostate cancers in cancer deaths in males. Approximately one million people get colorectal cancer every year and around five hundred thousand people die from this disease (Bar-Shalom et al., 2019). At the same time advanced age, adenomas, genetic factors, family history, obesity, lifestyle, unbalanced eating habits, radiation, and some chronic diseases such as chronic inflammatory and diabetes mellitus are effective in the etiology of the disease (Aiello et al., 2019).

Nowadays, the low selectivity of drugs used in routine cancer therapies and the side effects they cause in multiple drug use increase the importance of drug researches on this subject. In this direction, the plant kingdom is seen as the most valuable source of new generation treatment agents in terms of drug discovery. In this study, anti-colorectal cancer effects of *Euphorbia helioscopia* L., *Ferula elaeochytris* Korovin, and *Sideritis albiflora* Hub.-Mor. extracts on the DLD-1 cell line were investigated.

2. Material and Methods

2.1. Plant materials

The herbarium numbers, collection areas, and information about the other characteristics of the studied plant species were given in Table 1. The voucher specimens have been deposited at the herbarium of Natural Products Laboratory of Muğla Sıtkı Koçman University, Muğla, Turkey.

Table 1. Herbarium numbers, collection areas, and information about other characteristics of the studied plant species

	Species		
	Euphorbia helioscopia L.	*Ferula elaeochytris* Korovin	*Sideritis albiflora* Hub.-Mor.
Herbarium number	MUMED1235	MUMED1051	EGE42372
Common name	Spurge-(Sütleğen)	Rod-(Çakşır)	Mountain tea-(Dağ çayı)
Genus	*Euphorbia*	*Ferula*	*Sideritis*
Family	Euphorbiaceae	Apiaceae	Lamiaceae
Collection area	Artvin-Turkey	Bayburt-Turkey	Muğla-Turkey
Bioactive properties of species	Antioxidant, anti-proliferative, anti-inflammatory, anti-bacterial, anti-fungal, anthelmintic, wound healing, anticholinesterase, and anti-urease activities (Deveci et al., 2018a; Yang et al., 2021)	Antioxidant, anti-diabetic, anti-tyrosinase, anticholinesterase, and cytotoxic activities (Baykan et al., 2020; Deveci et al., 2018b; Deveci et al., 2020)	Antioxidant, anti-ulcer, anti-diabetic, anti-tyrosinase, and anticholinesterase activities (Askun et al., 2009; Deveci et al., 2019; Deveci et al., 2020)

2.2. Extraction

Powdered *E. helioscopia, F. elaeochytris,* and *S. albiflora* samples were separately extracted with *n*-hexane, acetone, and methanol (24 h and 4 times at room temperature) to produce the extracts, respectively. After filtration, the remaining solvents were removed by evaporation at 40°C under vacuum to give the extracts. The remaining plant parts were extracted with water at 80°C for one day and lyophilized to obtain the water extracts. All extracts were stored at +4°C until analysis.

2.3. Cell viability

RPMI-1640 growth medium (ATCC, Virginia, USA) and incubation conditions of 10% fetal bovine serum (FBS), 1% penicillin/streptomycin, 2 mM L-glutamine (Sigma, St. Louis, Missouri, USA) in 5% CO_2 at 37°C and 90-95% humidity were used for cultivation of DLD-1 (colorectal cancer) cell line.

2.4. Cell viability assay

$1x10^4$ cells with growth medium were placed into 96-well plate and left to incubate in 5% CO_2 at 37°C for 24 h until attachment occurred to the bottom. After addition of different concentrations of the plant extracts in the range of 1 µg/mL and 1000 µg/mL to the each well, viability and proliferation of the cells were determined in refer to Alamar Blue assay as defined previously (Karakurt & Adali, 2016; Deveci et al., 2021). The results were measured at 570 nm and 610 nm by using a 96-well microplate reader (MultiskanGo, Thermo Scientific Co., MA, USA). Doxorubicin was used as a standard. IC_{50} values of the plant extracts were estimated as µg/mL through the sigmoidal curve plotted between the inhibition rate (%) and the concentration (µg/mL). Anti-colorectal cancer effect results were measured and calculated by using GraphPad Prism (GraphPad Software v5.0, USA).

2.5. Statistical analysis

All data on anti-colorectal cancer effect tests were the average of three parallel sample measurements. Data were recorded as mean ± S.E.M. Significant differences between means were determined by *t*-test, p values <0.05 were regarded as significant.

3. Results

Anti-colorectal cancer effects of the hexane, acetone, methanol, and water extracts of *E. helioscopia* and *F. elaeochytris,* the hexane and methanol extracts of *S. albiflora* on DLD-1 cell line were screened by Alamar blue assay. The results in Table 2 showed the IC_{50} values of the extracts. The cell viability (%) values of *E. helioscopia* etxtracts were presented in Fig. 1a, *F. elaeochytris* extracts in Fig. 1b, *S. albiflora* extracts in Fig. 1c. Heat Map analysis of the dose-dependent inhibition of the extracts on DLD-1 cell line were given in Fig. 2. Dose-dependent inhibition was detected in the viability of DLD-1 cell line. In all plant species, *F. elaeochytris* (IC_{50}: 67.93±0.12 µg/mL), *S. albiflora* (IC_{50}: 85.12±0.10 µg/mL), and *E. helioscopia* (IC_{50}: 140.83±0.31 µg/mL) methanol extracts showed higher anti-colorectal cancer effects on DLD-1 cell line compared to other extracts tested for the same species.

Table 2. IC_{50} values (µg/mL) of the extracts

Plant species	Extracts	Code	DLD-1 cell line
E. helioscopia	Hexane	EHH	142.01±0.06
	Acetone	EHA	245.38±0.92
	Methanol	EHM	140.83±0.31
	Water	EHW	> 250
F. elaeochytris	Hexane	FHH	> 250
	Acetone	FHA	102.95±0.41
	Methanol	FHM	67.93±0.12
	Water	FHW	187.44±0.24
S. albiflora	Hexane	SAH	> 250
	Acetone	SAA	NT[c]
	Methanol	SAM	85.12±0.10
	Water	SAW	NT[c]
Standard	Doxorubicin[b]		6.10±0.55

[a] IC_{50} values represent the means ± SEM of three parallel measurements (p<0.05). [b] Positive control. [c] Not tested.

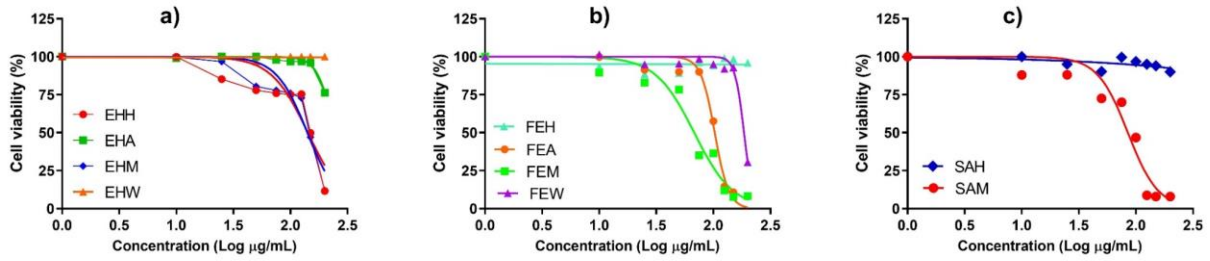

Figure 1. Cell viability (%) values of a) *E. helioscopia* extracts b) *F. elaeochytris* extracts c) *S. albiflora* extracts. Coded as: hexane (EHH), acetone (EHA), methanol (EHM), water (EHW) extracts of *E. helioscopia;* hexane (FEH), acetone (FEA), methanol (FEM), water (FEW) extracts of *F. elaeochytris;* hexane (SAH), methanol (SAM) extracts of *S. albiflora*

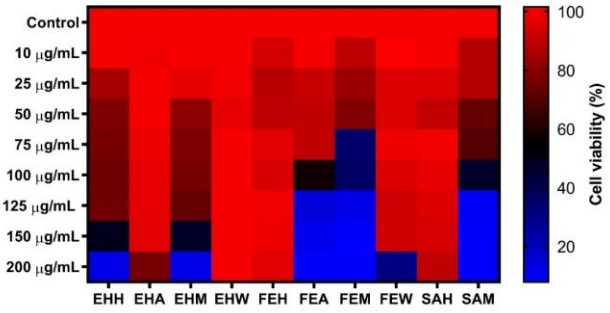

Figure 2. Heat Map analysis of the dose-dependent inhibition of the extracts on DLD-1 cell line. Cell viability decreased from red to blue color. Coded as: hexane (EHH), acetone (EHA), methanol (EHM), water (EHW) extracts of *E. helioscopia*; hexane (FEH), acetone (FEA), methanol (FEM), water (FEW) extracts of *F. elaeochytris*; hexane (SAH), methanol (SAM) extracts of *S. albiflora.*

4. Discussion

In recent years, phytochemicals in plants have been presented as an option for the treatment and prevention of cancer in terms of complementary medicine as they have fewer side effects. (Chromanska et al., 2017). It is known that natural products of plant origin are better tolerated and these properties attract the attention of modern drug discovery. Estimated statistics revealed that only 10% of the plant population was studied for pharmacological applications (Singh et al., 2016a; Wu et al., 2017). Approximately 60% of anti-cancer drugs are of natural origins such as microorganisms, vertebrates, plants, and invertebrates. Many studies have confirmed that most chemotherapeutic drugs target proliferative cells unspecifically and are cytotoxic. This situation later leads to the poor prognosis of cancer patients. Therefore, it is aimed to research and develop new anti-cancer agents that selectively affect cancer cells (Singh et al., 2016a; Singh et al., 2016b). The development of plant ingredients forms the beginning of traditional medicine and today plants still remain an important source of pharmaceutical agents. Particularly, the basis of cancer chemotherapy has been established especially by natural products for the last few years. Although combinatorial chemistry affords a wide diversity of new and synthetic drugs, natural products can offer effective compounds for the development of new agents with improved biological properties (Lahlou, 2013). This success in drug discovery is due to the high chemical diversity of natural resources; however, the chemical variability occurring in the plant population complicates the characterization processes of a large number of metabolites. The conventional methods used to discover the most active molecules have different disadvantages, consisting of a complex structure and long periods of time

such as separation, isolation, and identification studies from a crude extract. The screening of plant-derived mixtures is becoming an effective way to quickly select metabolites with biological properties (Koçyigit et al., 2016).

Herein, *F. elaeochytris* methanol extract showed the highest anti-colorectal cancer effect on DLD-1 cell line. Also, all methanol extracts revealed the highest anti-colorectal cancer effect on DLD-1 cell line when compared to other extracts. In our previous study, catechin hydrate (105.5±2.3 µg/g) was reported as a major phenolic compound in *F. elaeochytris* methanol extract (Deveci et al., 2018b). It is elucidated that catechin derivatives act by suppressing the levels of phospho-AKT and nuclear *β*-catenin in colorectal cancer models and induction of apoptosis in different animal models (Yang et al., 2014). *S. albiflora* methanol extract was found as the second anti-colorectal cancer active extract and we identified caffeic acid (8.915±0.005 µg/g) as the major phenolic compound in our previous research (Deveci et al., 2019). Chiang et al. (2014) documented that caffeic acid derivatives suppress the PI3K/Akt and integrin-mediated signaling pathways and the proliferation of colorectal cancer cells through induction of cell cycle arrest in the G0/G1 phase. It has also been proven in previous studies that methanol is a more selective solvent for the extraction of bioactive compounds. The higher effects of the methanol extracts may be due to the synergistic effects of other secondary compounds in addition to the reported phenolic compounds (Altemimi et al., 2017; Truong et al., 2019).

There is a limited number of studies in the literature on anti-colorectal cancer effects of plant species belonging to *Euphorbia*, *Ferula*, and *Sideritis* genus. This is the first report highlighting anti-colorectal cancer effects of *E. helioscopia*, *F. elaeochytris*, and *S. albiflora* extracts on the DLD-1 cell line. Previously, the infusion of *Sideritis syriaca* was reported to inhibit HT-29 cell growth with value of ~ 60% at 1.00 µg/µL concentration after 48 h by Kogiannou et al. (2013). The IC_{50} value of siderol, a well-known major constituent of the genus *Sideritis*, was calculated as 26.4±3.7 µM in DLD-1 cell line (Tomou et al., 2020). The hexane extract of *Ferula hermonis* root (IC_{50}: 25 µg/mL) was found to have moderate sensitivity on the LoVo cell line (Abutaha et al., 2019). In a different study, cytotoxic activities of the methanol 80% extracts and the hexane, chloroform, and methanol fractions of *Ferula szowitsiana*, and *Ferula hirtella* were tested on the HT-29 line. The IC_{50} values were in the methanol 80% extracts and hexane, chloroform and methanol fractions were found as 36, 33, 102, > 300 µg/mL for *Ferula szowitsiana*; 89, 26, 110, > 300 µg/mL for *Ferula hirtella* (Hamzeloomoghadam et al., 2013). Growth inhibition rates of *Euphorbia helioscopia* petroleum ether, chloroform, ethyl acetate, and *n*-butanol extracts on SW-480 cell line were reported as ~ 80, 65, 45, 80% at 150 µg/mL concentration and ~ 75, 60, 40, 75% at 200 µg/mL concentration (Wang et al., 2012). Cytotoxicity of euphodendrophanes A, B, C, D, E F, and tigliane euphodendriane A isolated from *Euphorbia dendroides* on DLD-1 line was reported with IC_{50} values of 59.3, 22.1, 42.7, 37.9, 75.3, and 27.4 µM, respectively (Aljancic et al., 2011). Superior cytotoxic effect was observed in the methanol extract of *Euphorbia hierosolymitana* on HCT-116 cell line with IC_{50} value of 4.22 µg/mL (El-Hallouty et al., 2020). Mesas et al. (2021) reported the cytotoxic effect of *Euphorbia lathyris* ethanol extract (IC_{50}: 72.9±1.27 µg/mL) against HCT-115 cell line. In a study on *Euphorbia macrorrhiza*, inhibition ratios of Caco-2 cell line were recorded in the essential oil, hexane, chloroform, and ethyl acetate fractions as 96.32, 72.52, 8.52, 2.32% for root parts and 96.12, 83.57, 68.22, 4.61% for aerial parts at 250 µg/mL concentration. In addition, *n*-butanol and residual methanol fractions of both parts of *Euphorbia macrorrhiza* were found as inactive (Lin et al., 2012). There are similarities and differences between our results and the literature. These differences may be due to effects such as discrepancy of plant species, cell line, extraction solvent, and methods.

In conclusion, this study is the first to examine anti-colorectal cancer effects of *E. helioscopia*, *F. elaeochytris*, and *S. albiflora* against DLD-1 cell line. The results support that the studied plant species will be an important step in the discovery of effective natural agents in the treatment of colorectal cancer and will illuminate further studies.

Ethics committee approval: Ethics committee approval is not required for this study.

Conflict of interest: The authors declares that there is no conflict of interest.

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Research Article / Araştırma Makalesi

Comparison of Different Life Stages of Total, Phospholipid and Triacylglycerol Fatty Acids of *Lucilia sericata*

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Abstract: *Lucilia sericata*, which belongs to the Calliphoridae family (Diptera), is used as a debridement tool in open necrotic wounds that do not respond to conventional treatments. Knowing the total, phospholipid (PL), and triacylglycerol (TAG) fatty acid content of *L. sericata* fly, which is important for health, in its different stages is important both in terms of taxonomy and physiology. After *L. sericata* samples used in the study were obtained commercially, they were bred under laboratory conditions and then the fatty acids in different stages were analyzed by gas chromatography. Sixteen fatty acids are determined as a result of the analysis. When individual fatty acids are considered, Palmitic acid (16:0), Palmitoleic Acid (16:1n-7), oleic acid (18:1n-9) and Linoleic Acid (18:2n-6) were found to be major ones, while the others are detected in trace amounts. It is determined that, out of total, PL and TAG, saturated fatty acids (SFA) are found at a high percentage in the eggs, monounsaturated fatty acids (MUFA) are the highest in the larvae, and polyunsaturated fatty acids (PUFA) are the highest in fly and pupa. Furthermore, out of total, PL and TAG, ∑PUFA is at a low percentage in the egg and the larval stages. Different results are found at different stages in this study. This may be because fatty acid percentages that change during metamorphosis meet different physiological needs at different phases.

Keywords: Maggot, fly, Calliphoridae, gas chromatography.

Lucilia sericata'nın Total, Fosfolipid ve Triaçilgliserol Yağ Asitlerinin Farklı Yaşam Evrelerinin Karşılaştırılması

Öz: Calliphoridae ailesindeki (Diptera) olan *Lucilia sericata*, geleneksel tedavilere yanıt veremeyen açık nekrotik yaralarda debridman aracı olarak kullanılmaktadır. Sağlık açısından önemli olan *L. sericata* sineğine ait farklı evrelerindeki total, fosfolipit (PL) ve triaçilgliserol (TAG) yağ asit içeriğinin bilinmesi hem taksonomik hem de fizyolojisi açılardan önem taşımaktadır. Çalışmada kullanılan *L. sericata* örnekleri ticari olarak temin edildikten ve laboratuvar koşullarında üremesi sağlandıktan sonra farklı evrelerindeki yağ asitleri gaz kromatografisi ile analizleri yapılmıştır. Yapılan analiz sonucunda 16 yağ asidi belirlenmiştir. Bireysel yağ asitleri incelendiğinde Palmitik asit (16:0), Palmitoleik Asit (16:1n-7), oleik asit (18:1n-9) ve Linoleik Asit (18:2n-6) majör yağ asitleri olarak belirlenmiştir. Diğerleri eser miktarda tespit edilmiştir. Total, PL ve TAG'de doymuş yağ asitleri (SFA) yumurtada; Tekli Doymamış Yağ Asitleri (MUFA) larvada, Çoklu doymamış yağ asitleri (PUFA) sinek ve pupada yüksek yüzdede olduğu belirlenmiştir. Ayrıca total, PL ve TAG'de yumurta ve larva evresinde ∑PUFA'nın düşük yüzdede olduğu görülmüştür. Çalışmamızda farklı evrelerde farklı sonuçlar tespit edilmiştir. Bunun nedeni metamorfoz süresince değişen yağ asidi yüzdelerinin farklı evrelerindeki farklı fizyolojik ihtiyaçlarının karşılanması şeklinde açıklanabilir.

Anahtar kelimeler: Maggot, sinek, Calliphoridae, gaz kromotografisi.

1. Introduction

Lipids play a key role in insect biochemistry as energy sources, hormones, and structural compounds. It has also been found that lipids are the main energy source in insect embryogenesis (Gilbert, 1967). Dietary lipids as an energy source are more important than dietary proteins. Furthermore, fatty acids such as linoleic acid (18:2n-6) and linolenic acid (18:3n-3) play an important role in the transition from pupal to adolescent. In addition, fatty acids play an important role as precursors in the biosynthesis of waxes, pheromones, and eicosanoids. They are also known to be involved in preventive secretions (Wakayama et al., 1980; Stanley-Samuelson et al., 1988; Başhan, 1996). Eicosanoids are an important intermediate in many fields of invertebrate biology, such as reproduction (Stanley & Miller, 1998), ion transport (Nor Aliza et al., 2001), hormone signal transfer system (Keeley et al., 1996; Ali & Steele, 1997) and immune system (Nor Aliza et al., 2001). Eicosanoids also occupy a place between the animal population and the host-parasite relationship in which the prey-predator relationship is involved (Nor Aliza et al., 2001). It has been reported that fatty acids stored in the form of triacylglycerol (TAG) serve as the main energy source in insects when they are not receiving food and during long flights (Downer & Matthews, 1976; Beenakkers et al., 1985).

It has been claimed that the fatty acid composition of many insect species changes depending on the developmental stages of the insects (Stanley-Samuelson et al., 1988). Madariaga et al. (1974) found that the fatty acid distribution of *Dacus oleae* species, Pagani et al. (1980) gave that of *Ceratitis capitata* species, and Janda (1975) determined that of *Galeria mellonella* differs depending on the developmental stages. In their study, Hodges and

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Barras (1974) found that the fatty acid distribution in the Phospholipid (PL) fraction of the egg, the larva, the pupa, and the adult individuals of *Dentroctonus frontalis* was different.

In this study, *Necrophage* larvae of the *Calliphoridae* family, the most commonly *Lucilia sericata*, is a debridement tool used in wound treatment for biotherapy purposes in open necrotic wounds that do not respond to traditional treatments (Horobin et al., 2003; Britland et al., 2011). The average size of the adult *L. sericata* flies, which are bluish-green or metallic green, is 2-15 mm, their eggs are small, pale yellowish-white and have cylindrical oval structure, and the larvae are headless and footless and consist of 12 segments. Peritreme are narrow and closed in posterior stigmas. Their front ends are thin, while their backsides are larger in diameter, cylindrical and there is a pair of mouth hooks or lip scleritis in the head part (Bolaban, 2009). In the present paper, it was aimed to compare the total, PL and TAG fatty acid content in different stages of *L. sericata* fly that is important for health.

2. Material and Methods

Commercially supplied *L. sericata* flies were produced in a laboratory environment at 25 ºC for 16 hours a day and 8 hours at night in 60*120 cm cages in the air conditioning room. Eggs were obtained by placing livers of chicken, turkey and beef in the environment where these flies lived. The eggs were collected and placed in 50 ml test tubes and 35 ml of 0.05% sodium hypochlorite was added to separate the eggs. At the end of this process, sodium hypochlorite was decanted, 35 ml of 5% formaldehyde was placed on the eggs to sterilize them. The sterilized eggs were filtered in a Buchner funnel, washed with sterile isotonic 3-4 times to remove the effect of formaldehyde and the sterile eggs were transferred to a sterile liver agar medium in a 90 mm petri dish. After the media containing the eggs were incubated overnight at 25-30 °C in an incubator, the larvae were obtained from the eggs. Eggs obtained from adult flies, hatching larvae and pupa samples occurring from larvae were collected and stored at -20 ºC until the samples were analyzed.

2.1. Fatty acid analysis of total, phospholipids, and triacylglycerols

Insects were homogenized in a chloroform-methanol (2:1 v/v) mixture in a high-speed homogenizer (Folch et al., 1957). For fractionation of TAG and PL in samples, thin layer chromatography (TLC) plates (Silica gel 60G (Merck)) were placed into the driving tank containing the mixture of petroleum ether-diethyl ether-acetic acid (80:20:1) to separate PL and TAG from each other.

2.2. Gas Chromatography Conditions

Fatty acid analysis of fat samples converted to methyl esters was carried out by a SHIMADZU GC 2010 PLUS model Gas Chromatography device using a flame ionization detector (FID) and DB-23 (Bonded 50 % cyanopropyl) (J & W Scientific, Folsom, CA, USA) capillary column (30m x 0.25mm inner diameter x 0.25µm film thickness). Detector temperature: 250 °C; injector temperature: 250 °C; injection: Split-model 1/20. Gas flow rates: Carrier gas: Helium 0.5 ml/min for 30 m column; hydrogen: 30 ml/min; dry air: 400 ml/min. Column (oven) temperature: waiting time, 2 min at 170 °C; 2 °C/min until 210 °C, waiting time 20 min; total analysis time: 42 minutes. A mixture of methyl esters of fatty acids (Sigma-Aldrich Chemicals) was used as a standard for the identification of fatty acids. Chromatograms for methyl esters of fatty acid and amounts of total fatty acid were obtained on a computer with the computer program GC Solution (Version 2.4). Peaks in the chromatograms of the analyzed samples were identified by comparing the retention times of the methyl esters of all fatty acids in the standard. The results are given as a qualitative value in % fatty acid. Comparison of fatty acid percentages was performed with SPSS 16 computer program by one-way analysis of variance (ANOVA). Differences were determined by the TUKEY HSD test. As a result of the statistics, the differences were considered to be significant when the data were at the $P < 0.05$ level.

3. Results

A total of 16 fatty acids were determined as a result of the analysis. The main fatty acids were determined as Palmitic Acid, Palmitoleic Acid, Stearic acid, Oleic Acid and Arachidonic Acid. Other fatty acids such as myristic and pentadecanoic were present only in trace proportions (Table 1).

The total, PL and TAG fatty acid contents (in %) of *L. sericata* adult, pupa, larvae and eggs are given in Table. When individual fatty acids were considered, it was observed that 16:0, 16:1n-7, 18:1n-9 and 18:2n-6 were major ones, while others were detected in trace amounts. Out of total, PL and TAG, percentage of 16:0 was the highest in the egg, while it was the lowest in pupa out of total and TAG; the percentage of 16:1n-7 was similar in all phases; 18:1n-9 was the highest in the larva out of total and PL, in the pupa out of TAG, while it was the lowest in the egg out of total, in the pupa out of PL, and in the fly out of TAG. On the other hand, the percentage of 18:2n-6 was the lowest in the egg and larva, while it was at a similar percentage in the other stages (Table 1).

Total fatty acid distribution was in the order of MUFA>SFA>PUFA in all except eggs, while this order was SFA>MUFA>PUFA in the egg. Out of PL, there was an order of MUFA>PUFA>SFA in the fly, SFA>MUFA>PUFA in the egg, MUFA>SFA>PUFA in the larva, and SFA=MUFA>PUFA in the pupa. Out of TAG, the order was SFA>MUFA>PUFA in the fly and in the egg, MUFA>SFA>PUFA in the larva, MUFA>SFA=PUFA in the pupa. Out of total, PL and TAG, the percentage of ∑SFA was the highest in the egg. In total MUFA, total and PL were the highest in the larva, TAG was the highest in the pupa, while PUFA was at the lowest percentage in the egg and larva (Table 1).

When the total fatty acid distribution was considered, it was found that 16:0 and consequently total SFA increased, while 18:1n-9, ∑MUFA, 18:2n-6 and ∑PUFA decreased in the egg. In the case of the larva, 18:1n-9 and ∑MUFA increased, whereas 18:2n-6 and ∑PUFA decreased. Out of PL, the percentage of 16:0 and consequently total SFA was high in the egg, while that of 18:2 and total PUFA was low. In the case of the larva, the percentage of 18:1n-9 and total MUFA increased, whereas that of 18:2n-6 and total PUFA decreased. Out of TAG, the percentage of 16:0 and consequently total SFA was high in the egg, while that of 18:2n-6 and total PUFA was low.

Percentage of 18:1n-9 and total MUFA increased, while that of 18:2n-6 and total PUFA decreased in the larva. Out of total, PL and TAG fatty acid, percentage of SFA was high in the egg, percentage of MUFA was high in the larva, whereas that of PUFA was high in the fly and pupa. Furthermore, out of total, PL and TAG, the percentage of ∑PUFA was low in the egg and larval stages (Table).

Table 1. Distribution of fatty acid Total. Phospholipids (PL), and Triaçilgliserol (TAG) in *Lucilia sericata*

	Total				**PL**				**TAG**			
Fatty Acid	**Adults**	**Eggs**	**Larvae**	**Pupae**	**Adults**	**Eggs**	**Larvae**	**Pupae**	**Adults**	**Eggs**	**Larvae**	**Pupae**
12:0	0.08 ±0.01	0.24 ±0.02	0.59 ±0.05	0.09 ±0.01	0.18 ±0.01	0.17 ±0.01	0.08 ±0.01	0.10 ±0.01	0.11 ±0.01	0.18 ±0.01	0.25 ±0.02	0.16 ±0.01
14:0	2.02 ±0.16	3.62 ±0.29	3.07 ±0.24	1.90 ±0.15	0.98 ±0.08	2.92 ±0.23	2.28 ±0.18	1.69 ±0.13	2.91 ±0.23	3.53 ±0.28	3.19 ±0.25	1.04 ±0.08
15:0	0.29 ±0.02	0.68 ±0.05	0.40 ±0.03	0.19 ±0.02	0.20 ±0.02	0.39 ±0.03	0.49 ±0.04	0.41 ±0.03	0.16 ±0.01	0.70 ±0.06	0.39 ±0.03	0.09 ±0.01
16:0	24.56 ±1.96	42.18 ±3.37	26.22 ±2.09	22.81 ±1.82	20.47 ±1.63	36.07 ±2.88	27.00 ±2.15	31.96 ±2.55	30.73 ±2.45	35.27 ±2.81	27.73 ±2.21	12.81 ±1.02
17:0	0.17 ±0.01	0.06 ±0.01	0.27 ±0.02	0.14 ±0.01	0.33 ±0.03	0.28 ±0.02	0.30 ±0.02	0.14 ±0.01	0.20 ±0.02	0.26 ±0.02	0.26 ±0.02	0.08 ±0.01
18:0	5.52 ±0.44	8.26 ±0.66	2.08 ±0.17	5.56 ±0.44	4.87 ±0.39	12.09 ±0.96	1.20 ±0.10	3.00 ±0.24	8.19 ±0.65	7.44 ±0.59	2.39 ±0.19	3.88 ±0.31
∑S.F.A.	**32.64 ±2.60**	**55.05 ±4.39**	**32.63 ±2.60**	**30.69 ±2.45**	**27.03 ±2.16**	**51.92 ±4.14**	**31.35 ±2.50**	**37.29 ±2.98**	**42.29 ±3.37**	**47.37 ±3.78**	**34.21 ±2.73**	**18.07 ±1.44**
16:1n-7	14.19 ±1.13	13.51 ±1.08	13.37 ±1.07	13.50 ±1.08	14.13 ±1.13	5.82 ±0.46	19.06 ±1.52	12.37 ±0.99	12.27 ±0.98	11.83 ±0.94	11.89 ±0.95	5.05 ±0.40
18:1n-9	25.64 ±2.05	16.82 ±1.34	41.00 ±3.27	26.73 ±2.13	26.41 ±2.11	31.14 ±2.48	35.45 ±2.83	22.75 ±1.82	27.60 ±2.20	29.11 ±2.32	43.34 ±3.46	58.40 ±4.66
20:1n-9	0.33 ±0.03	0.54 ±0.04	0.30 ±0.02	0.15 ±0.01	0.84 ±0.07	0.77 ±0.06	0.31 ±0.02	0.47 ±0.04	0.41 ±0.03	0.38 ±0.03	0.35 ±0.03	0.49 ±0.04
∑M.U.F.A.	**40.16 ±3.20**	**30.87 ±2.46**	**54.67 ±4.36**	**40.39 ±3.22**	**41.37 ±3.30**	**37.74 ±3.01**	**54.81 ±4.37**	**35.59 ±2.84**	**40.28 ±3.21**	**41.32 ±3.30**	**55.58 ±4.43**	**63.94 ±5.10**
18:2n-6	20.54 ±1.64	10.74 ±0.86	9.86 ±0.79	21.44 ±1.71	23.23 ±1.85	6.69 ±0.53	11.70 ±0.93	19.96 ±1.59	13.71 ±1.09	8.80 ±0.70	8.23 ±0.66	14.57 ±1.16
18:3n-6	0.49 ±0.04	0.59 ±0.05	0.14 ±0.01	0.71 ±0.06	0.46 ±0.04	0.35 ±0.03	0.15 ±0.01	0.49 ±0.04	0.40 ±0.03	0.36 ±0.03	0.09 ±0.01	0.22 ±0.02
18:3n-3	0.49 ±0.04	0.21 ±0.02	0.32 ±0.03	0.44 ±0.03	0.88 ±0.07	0.30 ±0.02	0.22 ±0.02	0.36 ±0.03	0.29 ±0.02	0.45 ±0.04	0.17 ±0.01	1.29 ±0.10
20:2n-6	0.06 ±0.00	0.03 ±0.00	0.18 ±0.01	0.16 ±0.01	0.08 ±0.01	0.35 ±0.03	0.06 ±0.00	0.07 ±0.01	0.08 ±0.01	0.15 ±0.01	0.23 ±0.02	0.08 ±0.01
20:3n-6	0.11 ±0.01	0.08 ±0.01	0.31 ±0.02	0.20 ±0.02	0.07 ±0.01	0.11 ±0.01	0.22 ±0.02	0.10 ±0.01	0.13 ±0.01	0.09 ±0.01	0.29 ±0.02	0.05 ±0.00
20:4n-6	4.39 ±0.35	2.07 ±0.17	1.52 ±0.12	5.43 ±0.43	4.27 ±0.34	1.53 ±0.12	1.35 ±0.11	5.13 ±0.41	2.34 ±0.19	1.03 ±0.08	1.11 ±0.09	1.45 ±0.12
20:5n-3	1.12 ±0.09	0.36 ±0.03	0.38 ±0.03	0.54 ±0.04	2.61 ±0.21	1.01 ±0.08	0.15 ±0.01	1.01 ±0.08	0.48 ±0.04	0.43 ±0.03	0.10 ±0.01	0.33 ±0.03
∑P.U.F.A.	**27.20 ±2.17**	**14.08 ±1.12**	**12.70 ±1.01**	**28.92 ±2.31**	**31.60 ±2.52**	**10.35 ±0.83**	**13.84 ±1.10**	**27.12 ±2.16**	**17.43 ±1.39**	**11.31 ±0.90**	**10.21 ±0.81**	**17.99 ±1.44**
n3	1.61 ±0.13	0.57 ±0.05	0.69 ±0.06	0.98 ±0.08	3.49 ±0.28	1.32 ±0.10	0.37 ±0.03	1.37 ±0.11	0.77 ±0.06	0.88 ±0.07	0.27 ±0.02	1.62 ±0.13
n6	25.59 ±2.04	13.51 ±1.08	12.00 ±0.96	27.94 ±2.23	28.11 ±2.24	9.03 ±0.72	13.47 ±1.07	25.75 ±2.05	16.66 ±1.33	10.43 ±0.83	9.94 ±0.79	16.37 ±1.31
n3/n6	0.06	0.04	0.06	0.04	0.12	0.15	0.03	0.05	0.05	0.08	0.03	0.10

Values are provided as mean ± SE

SFA saturated fatty acid, MUFA monounsaturated fatty acid, PUFA polyunsaturated fatty acid

4. Discussion

A total of 13 fatty acids, 3 monounsaturated and 8 polyunsaturated, were detected in the analysis performed on the larva and the adults of *Ochlerotatus euedus*, *O. subdiversus*, *O. flavescens*, *O. caspius*, *Aedes cinereus* and *Anopheles messeae* (Sushchik et al., 2013). In their work Gołębiowski et al. (2014) identified a total of 26 fatty acids, 6 monounsaturated and 3 polyunsaturated, between C6 and C24 in *L. sericata* larva, and a total of 21 fatty acids in pupa, 4 monounsaturated and 3 polyunsaturated. It was found that 16:0 (20.5%), 16:1n-9 (15.4%), and 18:1n-9 (56.9%) as the major fatty acid in the larva; 16:0 (29.5%), 16:1n-9 (16.0%), and 18:1n-9 (45.6%) as the major fatty acid in the pupa. Zhang et al. (2010) determined a total of 10 fatty acids in their total fatty acid analysis on *L. sericata larvae*. They determined SFA as 20.57%, MUFA 60.32% and PUFA 19.11%. In our study, out of total fatty acid, the highest one was MUFA (54.67%), then SFA (32.63%) and the lowest one was PUFA (12.70%), showing a similar distribution with that study. However, unlike that study, 16 fatty acids were found in our study.

Palmitic and oleic acids were at the highest level in the samples of all species in the analysis carried out on the egg samples of four fly species. Percentage of palmitic acid was 30.40% in *L. excision*, 30.68% in *C. megacephaly*, 31.08% in *L. cuprina*, 30.29% in *C. albiceps*, whereas that of oleic acid was found to be 41.03% in *L. excision*, 40.81% in *C. megacephaly*, 44.9% in *L. cuprina*, and 41.03% in *C. albiceps*.

L. cuprin had the highest oleic acid concentration. It has been noted that the fatty acid composition of different samples varies quantitatively between genera and even species (Lunas et al., 2019). In our study, it was similarly high in terms of total fatty acid. However, the percentage of palmitic acid was the highest in the egg (42.18%), while that of oleic acid was higher in the larva (41.00%). Hence, the values in different life stages of the same sample were different. These results are similar to the findings of Jacob and Hanssen (1979) who described the quantitative differences between fatty acid profiles of four adult dragonfly species. Likewise, Thompson (1973) and Paul et al. (2017) stated that the fatty acid compositions of different insect species showed significant quantitative differences. These data in our study are very important for determining the quantitative differences in taxonomic terms.

Gołębiowski et al. (2012) found that female and male *L. sericata* fatty acid profiles were from C6 to C20, a total of 15 fatty acids in males and 16 in females. They found that 16:0 (29.4% in females, 19.5% in males), 16:1n-9 (10.3% in females, 14.7% in males) and 18:1n-9 (40.6% in females and 57.4% in males) were major fatty acids in both sexes. In our study, 16:0 (25.56%), 16:1n-9 (14.19%), 18:1n-9 (25.64%) and 18:2n-6 (20.54%) were determined to be major ones. Monounsaturated and polyunsaturated fatty acids are generally found in many insect species. Although these fatty acids are detected in aquatic insect larvae *Stictochironomus pictulus* lipids, the presence of polyunsaturated fatty acids at 20:4n-3 and 20:5n-3 is rather unusual. In their study, they found that the ratio of 20:4n-3 and 20:5n-3 were 1.6 and 1.0% in females. In our study, the percentage of 20:4n-3 was between 1.03% and 5.43%, while that of 20:5n-3 was between 0.33% and 2.61% out of total, PL and TAG.

Cakmak et al. (2004) studied different developmental stages and different lipid fractions of *Myrmeleon inconspicuus* species. In this study, they observed that there was a decrease in 16:1n-7, while an increase was observed in 18:2n-6 in the adult stage in both fatty acid distributions in the PL and TAG fractions *of M. inconspicuus* larva and adult individuals. In our study, 16:1n-7 PL was low in the egg (5.82%), TAG was low in the pupa (5.05%), and the percentage of PL was highest in Larva (19.06%), whereas it was similar in pupa (12.37%) and fly (14.13%). TAG showed a similar distribution in the fly (12.27%), the egg (11.83%) and the larva (11.89%). In the case of 18:2n-6, PL was three times lower in egg and two times lower in larva than that in fly and pupa. TAG decreased 56% in eggs and larvae compared to flies and pupae. Ogg and Stanley-Samuelson (1992) suggested that insects undergoing different metamorphoses have different fatty acid content. In their study, they determined that the differences observed in the fatty acid distribution in the egg, larva, pupa, and adult stages of insects undergoing holometabol metamorphosis were more pronounced than those observed in insects with hemimetabol. This is because the extent of tissue organization in insects undergoing complete metamorphosis (holometabol) is more complex than insects that undergo semi-metamorphosis (hemimetabol) (Crippsc & De Renobales, 1988).

As a result of the investigation of the fatty acid composition of the late stage larva and pupa of *Achroia grisella*; there was no statistical difference between the two groups in terms of SFA percentages, while the percentage of unsaturated fatty acids was higher in the larval stage than that in the pupal stage, and the percentage of supersaturated fatty acids was higher in the pupal stage compared to that in the larval stage. The reason for these differences was explained as the different physiological needs that occur during the metamorphosis of insects (Nurullahoglu, 2003). In our study, out of SFA, total, and PL was statistically high in the eggs; out of TAG, they were low in the larva and the pupa. In the case of MUFA, total, PL and TAG were 1.7 times higher in the larva than fly, egg and pupae. The percentage of supersaturated fatty acids was approximately two times lower in the eggs and larvae compared to other stages.

In a study conducted on *G. mellonella*, the percentages of 18:1n-9 and 16:0 were very high, while the percentage of 18:2n-6 was the third largest one in the fatty acid profile of the fifth, sixth and seventh stage larvae and pupae of the species (Aktümsek et al., 2000). Similar results were found in our study. The percentage of 16:0 and 18:1n-9 increased significantly, while the percentage of 18:2n-6 decreased from the 5th larval stage to the pupal stage of *G. mellonella*, and the percentage of 18:3n-3 was lower in the 6th and 7th stage larva than that in the 5th stage, whereas it was higher in the pupal stage. This is because varying fatty acid percentages during metamorphosis meet different physiological needs in different stages of the insect (Aktümsek et al., 2000). In our research, it was found that 16:0 is higher out of total, PL, and TAG in the larva and 18:1n-9 is higher in pupa out of TAG; 18:2n-6 and 18:3n-3 were found to be similarly lower. This is due to the difference between species during metamorphosis. In another study, the percentage of 16:1n-7, 16:0, and 18:1n-9 was high and similar in the fatty acid composition of *A. grisella* larva and pupa (Nurullahoglu, 2003). Another study showed that the highest percentages in the fatty acid composition of end stage larva and pupa of *P. interpunctella* correspond to 18:1n-9 and 16:0 (Seven, 2004). In the analysis of fatty acid composition of the late stage larva and pupa of *Tenebrio molitor*, Taskin and Aksoylar (2010) found that 12:0-18:2n-6 fatty acids are responsible for the total fatty acid composition in both stages. They found that oleic acid had the highest percentage and 16:0 and 18:2n-6's also had high percentages. In our research data, we found that 16:0, 18:1n-9 and 18:2n-6 were high out of total, PL, and TAG. Oleic acid is a fatty acid used for growth and as an energy source (Dadd, 1973). They attributed the reason for the high oleic acid in insects to this, which was found to be similarly high in our study as well.

Kalyoncu and Ozge (2014) investigated the fatty acid composition of *P. interpunctella* larvae, pupae, and adults. They identified 20 fatty acids ranging from 12-22 carbon fatty acids as a result of the analysis and determined that the largest percentage in the larval developmental stage corresponded to 16:0 while 18:1n-9 had the highest percentage in the pupal and adult stages. According to our research data, out of total fatty acids, 16:0 had the highest percentage out of saturated fatty acids; 18:1n-9 was high in the other stages except egg (16.82%), out of the monounsaturated fatty acids. This fatty acid was found to be 1.58 times more in the larva. They found that the percentage of SFA was the highest in the larval stage,

while the percentage of PUFA was the highest in the pupal stage, and they stated that there were differences in fatty acid percentages depending on the developmental stages (Kalyoncu & Ozge, 2014).

Insects meet their energy needs from stored TAGs during their pupal stages. During the pupa phase, TAG is consumed slowly, accelerating towards the end of the metamorphosis (Ogg & Stanley-Samuelson, 1992). In their study on the fatty acid composition in PL and TAG fractions of *M. inconspicuus* larva and adult individuals, Cakmak et al. (2004) found that 16:1n-7 decreased, whereas 18:2n-6 increased in both fractions in the adult stage. In our study, similar decreases and increases were observed in both PL and TAG.

18:2n-6, 20:3n-6 and 20:4n-6 in the PL fraction of thorax of individuals belonging to the *P. americana* species, and 18:1n-9 and eicosadienoic acid (20:2n-6) in the abdomen part were higher than those in other body parts (Jurenka et al., 1988). The ratio of 18:2n-6 was high in the PL fraction of the head and thorax of female and male individuals of the *Magicicada septendecim*, and that of 18:1n-9 was high in the abdomen (Hoback et al., 1999). In our investigation, 16:1n-7, 18:1n-9 and 20:3n-6 were higher in the larva; 18:2n-6, 20:4n-6 and 20:5n-3 in the pupa, 20:2n-6 and 20:5n-3 in the eggs. Phospholipid fatty acid content was different in eggs, larvae, pupae, and adults of *D. frontalis* (Hodges & Barras, 1974). Ogg and Stanley-Samuelson (1992) obtained similar findings from *M. sexta*. Bozkus (2003) noted increases and decreases in fatty acids in PL fractions at different developmental stages such as nymphs and adults. Similarly, in our study, it was observed that different fatty acids were present at different stages.

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Conflict of interest: The authors declares that there is no conflict of interest.

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Research Article / Araştırma Makalesi

The Role of Citrus Nobiletin on Oxidative Stress Levels and Superoxide Dismutase Activities in Metastatic Castration-Resistant Prostate Cancer

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Abstract: Nobiletin (NOB) is a polymethoxylated flavone. It has multiple biologic activities that can modulate oxidative stress in many cancer types. However, there is no study in the literature that has examined the effects of NOB on oxidative stress levels in Metastatic Castration-Resistant Prostate Cancer (MCRPC) yet. Motivated from this gap, we investigated the impact of NOB on oxidative stress and superoxide dismutase (SOD) enzyme activities in MCRPC as a preliminary study. For this purpose, PC-3 and HUVEC cells were used to determine the effects of NOB on the amount of Malondialdehyde (MDA), hydrogen peroxide (H_2O_2), and proline as well as SOD enzyme activities. NOB potentially induced SOD enzyme activities but the level of MDA, H_2O_2, and proline decreased after incubation with NOB in PC-3 cells ($p<.05$ and $p<.001$ were considered statistically significant). Our results confirmed that NOB acted as a protective agent for cancer cells and could selectively regulate oxidant status in MCRPC cells. Consequently, these preliminary findings provide better insight into the role of citrus NOB on oxidative stress levels and antioxidant enzyme activities in MCRPC. Additionally, there is a need to elucidate the molecular mechanisms of this cytoprotective effect of NOB as a potential chemotherapeutic agent.

Keywords: Antioxidant effect, cancer, flavanoid, malondialdehyde, proline.

Metastatik Kastrasyona Dirençli Prostat Kanserinde Narenciye Nobiletin'in Oksidatif Stres Düzeyleri ve Süperoksit Dismutaz Aktiviteleri Üzerindeki Rolü

Öz: Nobiletin (NOB) polimetoksile bir flavondur ve birçok kanser türünde oksidatif stresi modüle edebilen çok sayıda biyolojik aktiviteye sahiptir. Bununla birlikte, literatürde, MCRPC'de Metastatik Kastrasyona Dirençli Prostat Kanserinde (MCRPC) NOB'nin oksidatif stres seviyeleri üzerindeki etkilerine dair henüz bir çalışma bulunmamaktadır. Bu nedenle, ön çalışma olarak MCRPC'de NOB'nin oksidatif stres ve süperoksit dismutaz (SOD) enzim aktiviteleri üzerindeki etkilerini belirlemeyi amaçladık. Bu amaçla, çalışmada NOB'nin Malondialdehit (MDA), hidrojen peroksit (H_2O_2) ve prolin miktarı ile SOD enzim aktiviteleri üzerindeki etkilerini belirlemek için PC-3 ve HUVEC hücreleri kullanıldı. NOB'un potansiyel olarak SOD enzim aktivitelerini indüklediği, ancak PC-3 hücrelerinde NOB ile inkübasyondan sonra MDA, H_2O_2 ve prolin seviyesinin azaldığı tespit edildi ($p<.05$ ve $p<.001$ istatistiksel olarak anlamlı kabul edildi). Elde edilen veriler, NOB'nin kanser hücreleri için koruyucu bir ajan olarak hareket ettiğini ve MCRPC hücrelerinde oksidan durumunu seçici olarak düzenleyebildiğini doğruladı. Sonuç olarak, bu ön bulgular, MCRPC'de turunçgil NOB'nin oksidatif stres seviyeleri ve antioksidan enzim aktiviteleri üzerindeki rolü hakkında daha iyi fikir vermektedir. Ek olarak, potansiyel bir kemoterapötik ajan olarak NOB'nin bu sitoprotektif etkisinin moleküler mekanizmalarının aydınlatılmasına ihtiyaç vardır.

Anahtar kelimeler: Antioksidan etki, flavonoid, kanser, malondialdehyde, proline.

1. Introduction

In metastatic castration-resistant prostate cancer (MCRPC), therapies such as androgen ablation cannot be successful and oxidative stress has been regarded as one of the qualities of the aggressive disease phenotype (Shen & Abate-Shen, 2010). In particular, oxidative stress is related to MCRPC development, advancement, and response to therapy. Reactive oxygen species (ROS) are produced directly in damaged tissue in response to pro-inflammatory cytokines, growth factors, exposure to chemicals, and other stressors. Oxidative signals can regulate the expression of various cytokines and chemokines (TNFα, IL-1β, IL-6, IL-8). In inflammatory microenvironment conditions, the subsequent stimulation of ROS production causes DNA damage and increases mutation rate when it continues for a long time. This may induce oncogenic transformation in cancer-related genes and result in DNA damage, apoptosis, and genetic alterations of essential cellular proteins that regulate the cell cycle (Ma, 2010). To balance ROS-induced oxidative damage, cells have developed various antioxidant processes to sustain their genomic constancy such as enzymatic scavengers including superoxide dismutase (SOD), catalase as well as glutathione peroxidase, antioxidant enzymes involved in cell defence, DNA repair enzymes, and cellular mechanisms of genomic control. While SOD superoxide anions catalyze H_2O_2, catalase and glutathione peroxidase convert H_2O_2 into the water and the accumulation of peroxides is prevented (Acharya et al., 2010). In this context, current treatment options in MCRPC are low and oxidative stress plays a vital role in MCRPC development (Amaral et al., 2012). Therefore, it is essential to investigate the effects of alternative treatment strategies on oxidative stress markers in MCRPC.

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Nobiletin (NOB) is a bioactive polymethoxylated flavone (5,6,7,8,3',4'-hexamethoxyflavone) which was found in citrus peels and had multiple biologic activities (Bernini et al., 2011; Zheng et al., 2019; Dusabimana et al., 2019). Flavonoids exhibit anti-carcinogenic characteristics in vitro and reduce cancer risk by protecting cells from oxidation or inflammation by changing the level of sex hormones, reducing angiogenesis or cell proliferation, and regulating apoptosis (Gates et al., 2009). NOB is anti-apoptotic, anti-inflammatory, anti-tumor, and antioxidant and an effective inhibitor against human prostate cancer cells and melanoma cells (Kunimasa et al., 2010; Lee et al., 2013; Xiao et al., 2016; Huang et al., 2016; Surichana et al., 2018). Additionally, studies have shown that NOB has an anti-inflammatory effect and exhibit an inhibiting effect on tumor invasion, proliferation, and metastasis and inhibit the creation of reactive oxygen species (ROS) by increasing the superoxide dismutase (SOD) and glutathione (GSH) activity and reduction in the making of malondialdehyde (MDA) and H2O2-induced PC12 cells (Luo et al., 2008; Lu et al., 2010; Güney Eskiler et al.; 2018; Liu et al., 2019; Deveci Ozkan et al., 2020). In addition, researchers reported that nobiletin impeded cell proliferation both depending on the dose and duration and prevented cell cycle progression in G1 (Morley et al., 2007). Nobiletin can induce DNA damage that contributes to apoptosis by regulating polymerase activity in cancer cells (Zhang et al., 2020). It has been suggested that NOB may be a pathway that triggers pyroptosis associated with apoptosis by inducing IL-1β expression (He et al., 2015). NOB is hypothesized to inhibit metastasis by impeding the activity of activator protein-1, a dimeric protein, preventing DNA binding (Kawabata et al., 2005; Goh et al., 2019). Another suggestion proposes that NOB functions through the Nuclear Factor-kappa B route and differentiates gene expression via regulating its promoter regions (Xiong et al., 2015; Park et al., 2016). As a food ingredient, nobiletin may be a favorable new anti-prostate cancer functional food component that can generate ROS-mediated apoptosis in prostate cancer cells.

No study has been conducted in the literature that shows the effects of NOB on oxidative stress levels and antioxidant enzyme activities in MCRPC. Therefore, in this study, we aimed to determine the effects of NOB on oxidative stress in MCRPC (PC-3) and control cells (HUVEC). To clarify if NOB affects oxidative stress, we measured the amount of Malondialdehyde (MDA), hydrogen peroxide (H2O2), and proline as well as SOD (superoxide dismutase) enzyme activities. Thus, it is aimed to obtain information that will contribute to the potential of NOB as a functionally unique and possible chemopreventive agent in inflammation-related tumor formation such as MCRPC.

2. Material and Methods

2.1. Cell culture and NOB treatment

In this study, the PC-3 (prostate cancer) cell line was used as MCRPC cells and human umbilical vein endothelial cells (HUVEC) were evaluated as a control cell line. All cells were bought from American Type Culture Collection (ATCC). PC-3 was cultured in RPMI-1640 (Thermo Fisher, USA) and HUVEC cells were grown in DMEM medium (Thermo Fisher, USA) supplemented with 0.1% penicillin and streptomycin, 10% FBS, and incubated at 37°C with 5% CO_2. The optimal treatment concentration and times for NOB in PC-3 and HUVEC cell lines were determined in our previous studies (Deveci Ozkan et al., 2020). Therefore, all cells were treated with NOB (80 µM for PC-3 and HUVEC, respectively) for 48 h in all experiments. Additionally, cells were grown in a culture medium without NOB used as a negative control.

2.2. Antioxidant enzyme activity and oxidative stress assays

2.2.1. Preparation of cell lysate

To determine the effects of NOB on antioxidant enzyme activity and oxidative stress level, the cells ($5x10^5$) were seeded six-well plates and treated with indicated concentrations (80 µM for PC-3 and HUVEC, respectively) of NOB for 48h. After incubation, the cells were treated with RIPA lysis and extraction buffer (Sigma Aldrich, USA), centrifuged for 10 min at 10.000 rpm at 4°C. After centrifugation, obtained supernatant was used for the analysis of antioxidant enzyme activity and oxidative stress.

2.2.2. Determination of Superoxide Dismutase (SOD) activity

SOD activity was established phytochemically according to the proposed method of Beauchamp and Fridovich (1971). SOD accelerates superoxide radicals' degradation (O^{2-}), H_2O_2, and molecular oxygen formed during oxidative energy production. SOD activity is determined by the inhibition of NBT (nitrobluetetrazolium) and formazan (blue crystals) formation. The blue-purple color formation from NBT with the effect of light is inversely proportional to the activity of the SOD enzyme. One unit of SOD activity was determined as the quantity of enzyme needed to reduce 50% of the NBT kept under light at 560 nm. Therefore, SOD enzyme activity was calculated according to the formula below.

%Inhibition = [(Blank OD – Sample OD)/Blank OD] x 100

2.2.3. Determination of Malondialdehyde (MDA) level

MDA level was verified by the proposed method of Ohkawa et al. (1979). According to this method, the thiocarboxylic acid (TBA) test, which accepts MDA as the final product of lipid peroxidation, was used. The formation of MDA content resulting from the TBA reaction is accepted as a lipid peroxidation measure. The amount of MDA in 1 ml of solution was calculated according to the formula below and the results were given as MDA (nmol/gram tissue) (Ananieva et al., 2002).

MDA (nmol/g): [(A532-A600)/155000] x 106

2.2.4. Determination of Proline Level

Proline level was determined by the proposed method of Myara et al. (1982). According to this method, the proline level was determined by measuring absorbance in a spectrophotometer. Absorbance values were obtained from an ultraviolet spectrophotometer (Shimadzu UV mini-1240 spectrophotometer) at 520 nm wavelength. Proline concentration was calculated as µmol/g according to the proline standard curve.

2.2.5. Determination of Hydrogen Peroxide (H_2O_2) level

H_2O_2 level was determined by the proposed method of

Jana and Choudhuri (1981). According to this method, the H_2O_2 level was determined by measuring absorbance in a spectrophotometer. Absorbance values were obtained from an ultraviolet spectrophotometer (Shimadzu UV mini-1240 spectrophotometer) at 410 nm wavelength. H_2O_2 concentration was calculated according to the proline standard curve.

2.3. Statistical analysis

Statistical analyses were carried out via Graph pad Prism v9.0 (Software, CA) and showed the mean ± standard deviation of three independent experiments. One-way analysis of variance (ANOVA) and Tukey's test was utilized to obtain multiple comparisons ($p<.05$ and $p<.001$ were taken as statistically significant).

3. Results

Our results showed that the SOD activities of the cells incubated with NOB for 24 and 48 h showed a significant increase in PC-3 cells (9.71 ± 2.04 and 9.36 ± 2.32, respectively) compared to the control cells not treated with NOB (7.98 ± 0.41 and 6.87 ± 0.13, respectively). Moreover, a similar significant increase was observed in HUVEC cells treated with NOB compared to the control group level in both treatment times (*$p<.05$, **$p<.001$, Table 1 and Fig. 1). As we expect that a significant decrease was observed in MDA levels of the PC-3 cells treated with NOB (0.19 ± 0.03) compared to the control (0.37 ± 0.06) for 48 h but a significant increase was determined in HUVEC cells treated with NOB (0.69 ± 0.28) compared to the control (0.60 ± 0.47) for 48h (*$p<.05$, **$p<.001$, Table 1 and Fig. 2). Additionally, a similar significant decrease was observed for in H_2O_2 concentration of the PC-3 cells treated with NOB for 24 and 48h (0.21 ± 0.08 and 0.34 ± 0.01, respectively) compared to the control (1.49 ± 0.05 and 0.12 ± 0.05, respectively) but a significant increase was determined in HUVEC cells treated with NOB compared to the control for both 24 and 48h (*$p<.05$, **$p<.001$, Table 1 and Fig. 3). Besides, the proline level of the cells incubated with NOB showed a statistically non-significant decrease in PC-3 and HUVEC cells compared to the control cells for 48h (Table 1 and Fig. 4). Therefore, according to our results, MDA and H_2O_2 levels were significantly lower whereas SOD activity was significantly higher in the MCRPC cells treated with NOB.

Table 1. Comparison of the oxidative stress levels and antioxidant enzyme activities according to the control group and NOB treated group in PC-3 and HUVEC for 24 and 48h.

Parameters		PC-3-Control Mean ± SD *(n=3)*	PC-3-NOB Mean ± SD *(n=3)*	HUVEC-Control Mean ± SD *(n=3)*	HUVEC-NOB Mean ± SD *(n=3)*
SOD (unit/mg)	24h	7.98 ± 0.41	9.71 ± 2.04*	8.36 ± 0.38	14.21 ± 1.29**
	48h	6.87 ± 0.13	9.36 ± 2.32*	3.38 ± 0.78	11.06 ± 0.86**
MDA (µM)	24h	0.53 ± 0.21	0.56 ± 0.15	0.42 ± 0.21	0.47 ± 0.08*
	48h	0.37 ± 0.06	0.19 ± 0.03*	0.60 ± 0.47	0.69 ± 0.28**
H_2O_2 (µM)	24h	1.49 ± 0.05	0.21 ± 0.08**	0.16 ± 0.05	0.21 ± 0.01**
	48h	0.12 ± 0.05	0.34 ± 0.01**	0.06 ± 0.01	0.10 ± 0.07**
Prolin (µM)	24h	9.46 ± 1.83	6.68 ± 0.83	4.27 ± 0.57	6.85 ± 1.18
	48h	11.22 ± 1.22	7.63 ± 1.46	7.98 ± 0.08	7.27 ±1.07**

The results are expressed as mean ± SD of eight experiments. *$p< .05$ and **$p< .001$ significantly different compared to the controls.

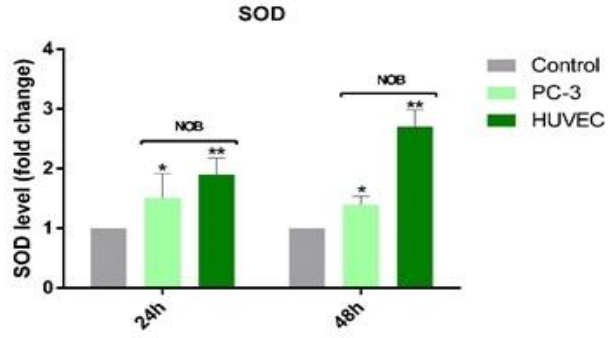

Figure 1. Relative enzyme activity level of SOD in PC-3 and HUVEC cells. To determine the enzyme activity level of SOD in PC-3 and HUVEC cells were treated with 80 mM NOB, for 24 and 48 h. *(*$p<.05$, **$p<.001$*, NOB: Nobiletin).

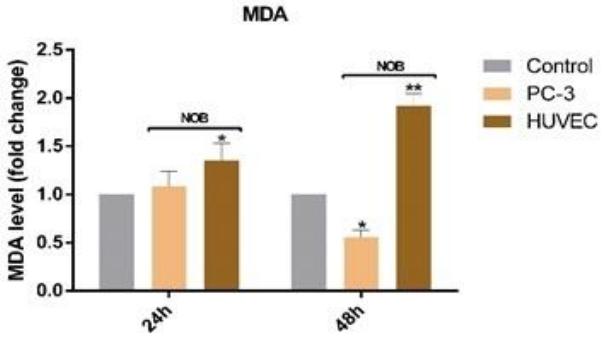

Figure 2. Relative level of MDA in PC-3 and HUVEC cells. To determine the level of MDA in PC-3 and HUVEC cells were treated with 80 mM NOB, for 24 and 48 h. *(*$p<.05$, **$p<.001$*, NOB: Nobiletin).

4. Discussion

In this study, the role of citrus nobiletin on oxidative stress levels and antioxidant enzyme activities was investigated in metastatic castration-resistant prostate cancer cells for the first time. Our preliminary findings demonstrated that NOB could modulate oxidative stress in PC-3 cells.

Oxidative stress is one of the inevitable consequences of aerobic life and increasing evidence indicates that the accumulation and formation of ROS and reactive nitrogen species play a significant role in various age-related diseases such as prostate cancer. Besides, if this oxidative stress that can cause damage to the cells is not repaired, the development of carcinogenesis will be inevitable over the years (Cooke et al., 2000; DeWeese et al., 2001; Cooke et al., 2003). Moreover, antioxidant enzymes' activities decrease when the balance of ROS-antioxidant is disturbed in prostate cancer cells (Zhou et al., 2006). Our study found significantly higher SOD (antioxidant enzyme) activity levels in NOB-treated PC-3 cells. This finding has supported that NOB has a potential protective role on the cells from oxidative stress by increasing the level of antioxidant enzymes.

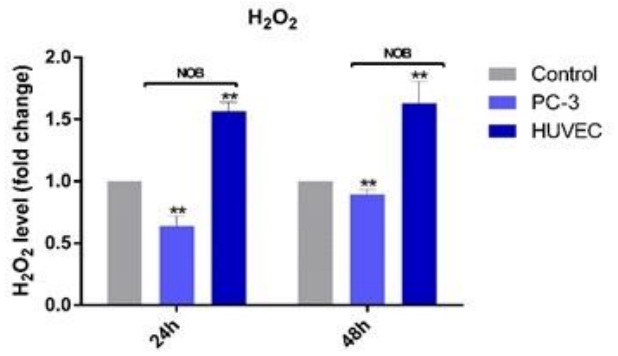

Figure 3. Relative concentration of H_2O_2 in PC-3 and HUVEC cells. To determine the concentration of H_2O_2 in PC-3 and HUVEC cells were treated with 80 mM NOB, for 24 and 48 h. *(*p<.05, **p<.001*, NOB: Nobiletin).

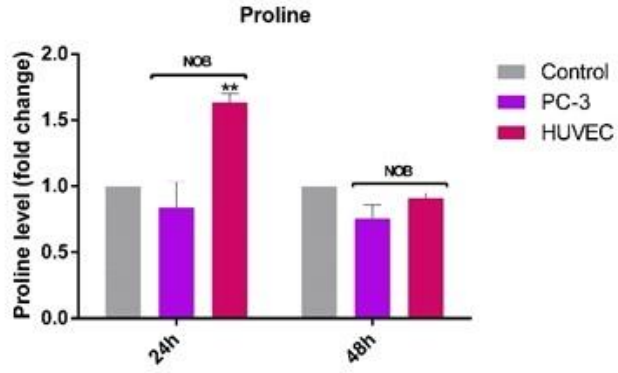

Figure 4. Relative level of proline in PC-3 and HUVEC cells. To determine the level of proline in PC-3 and HUVEC cells were treated with 80 mM NOB, for 24 and 48 h *(*p<.05, **p<.001*, NOB: Nobiletin).

Oxidation of lipid peroxidation is one of the most widely reported markers of oxidative stress as a contributor to cancer and MDA is the most frequently studied of these markers. In one study, Meng et al. (2017) showed that Cu^{2+} -induced intracellular ROS accumulation and increased MDA levels with apoptosis reduced by astaxanthin treatment and this might have a protective effect against Cu^{2+} -induced oxidative damage by reducing ROS and MDA levels. Similarly, our results revealed that NOB treatment decreased the MDA level in PC-3 cells compared to the control cells not treated with NOB.

Acute and chronic inflammatory cells such as prostate cancer produce superoxide, H_2O_2, and other ROS and recently it has been shown that oxidative stress is higher in prostate cancer cells compared to normal cells. In a study, the effect of doxorubicin, a chemotherapeutic drug, on H_2O_2 production in PC-3 cells was examined and an increase in intracellular H_2O_2 was observed as doxorubicin concentration increased (Wagner+ et al., 2005). However, according to the results of our study, as a substance with the potential of being a chemotherapeutic agent, NOB reduced the hydrogen peroxide level in PC-3 cells compared to the control cells not treated with NOB. On the contrary, our findings demonstrated that NOB increased the H_2O_2 level in HUVEC cells. Our results confirmed that NOB acted as a protective agent for cancer cells and could selectively regulate oxidant status in MCRPC cells. H_2O_2 activates the cellular or mitochondrial apoptotic pathway (Cho et al., 2015). Considering all these data, nobiletin reduced the cytotoxicity induced by H_2O_2, scavenging ROS, reducing MDA, especially in PC-3 cells, and restoring the activities of antioxidants (Lu et al., 2010; Malik et al., 2015). In this study, it can be assumed that the protection provided by nobiletin may be due to its antioxidant effect.

Extracellular matrix (ECM) proteins are a large source of amino acids that are likely to be discharged into the tumor microenvironment through the activity of matrix metalloproteinases and/or collagenases emitted by cancer cells, thereby affecting the metabolism of cancer cells. There are specifically Glycine and Proline, as a non-essential amino acid, among the ECM proteins. Additionally, many studies have demonstrated that proline metabolism impacted many pathways in the control of cancer cell plasticity and had the potential to be a prognostic marker and potential therapeutic target (Phang & Liu, 2012; Phang et al., 2015). Many cancer cells can use proline to produce ATP and ROS and proline oxidation had an essential role in cancer cells' survival (Phang, 2019; Huynh et al., 2020). Thus, our results showed that NOB treatment decreased the proline level in PC-3 cells but increased in HUVEC cells compared to those not treated with NOB. These findings suggested that NOB can modulate oxidant status through ECM and selectively regulate oxidant status in MCRPC cells, consistent with the literature. Nobiletin may be an efficient cytostatic anti-cancer agent. Inhibition of cell spread without stimulating cell mortality might be valuable in the treatment of tumors in a way that is less likely to generate cytotoxicity and mortality in non-tumor tissues.

5. Conclusion

The imbalance between antioxidants and oxidants occurs by decreasing the number of antioxidants or increasing the number of oxidants in the cell and can induce positive responses, including cellular proliferation or activation and negative responses as growth suppression and cell mortality. Thus, stress development studies have proven to be instructive for the development of cancer treatments and are useful in developing anti-cancer strategies. Therefore, these preliminary findings provide better insight into citrus nobiletin's role, as a cytoprotective and potential chemotherapeutic agent, on oxidative stress levels and antioxidant enzyme activities in MCRPC through by antioxidant properties of NOB.

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Conflict of interest: The authors declare that there is no conflict of interest.

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Research Article / Araştırma Makalesi

An Evaluation on the Last Ecdysis in Galeodids (Galeodidae, Solifugae, Arachnida)

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Abstract: In this study, the observations on pre-ecdysis and post-ecdysis during the last ecdysis of *Galeodes* sp. were recorded. During the ecdysis, a strand-like structure, intracuticular fibre, occurs between the old cuticle and the new one. This intracuticular fibre consists of the tracheal tubes as well as the old cuticle pieces. Thanks to this structure, the solifuge continues its biological activities such as breathing, even at a minimum level, during the quiescence process of the ecdysis. Forming male cheliceral flagellum during the last ecdysis leads to accurate sex determination.

Keywords: Solifuge, moulting, exuvia, trachea.

Galeodidlerde Son Deri Değişimi Üzerine Bir Değerlendirme (Galeodidae, Solifugae, Arachnida)

Öz: Bu çalışmada *Galeodes* sp. türüne ait son deri değişimi öncesinde ve sonrasındaki gözlemler kaydedilmiştir. Deri değişimi sırasında eski deri ile yeni deri arasında kütikulalar arası iplik benzeri doku oluşmaktadır. Bu doku kütikula parçalarının yanısıra içerisinde trake borularını da içermektedir. Böğü bu yapı sayesinde deri değişimi sırasındaki durgunluk sürecinde, solunum gibi hayati faaliyetlerini minimum düzeyde de olsa sürdürmektedir. Son deri atımından sonra oluşan bireyin yetişkin erkek ya da dişi olduğu kesinleşmektedir.

Anahtar kelimeler: Böğü, deri değiştirme, eksuvia, trake.

1. Introduction

The arachnids of the order Solifugae are fast-moving predaceous animals, comprising more than 1100 species belonging to 138 genera and 12 extant families (Harvey, 2003; Erdek, 2019). Many solifuges are predominantly univoltine and active for only short periods of their life cycle, not living for more than one year (Punzo, 1998). Muma (1966) recorded his observations on post-embryonal ecdysis of *Eremobates durangonus* Roewer, 1934. Haupt (1982) questioned that the shedding of cuticle causing problems for the functional permanence of cuticular sense structures during ecdysis and studied the hair regeneration of a chemotactile sensillum in *Gluvia dorsalis* (Latreille, 1817). Wharton (1981) pointed out that moulting caused a dental modification in *Biton (Biton) striatus* (Lawrence, 1928). It was previously suggested that the flagellum is formed just before males attain sexual maturity during the last ecdysis (Punzo, 1998).

The purpose of this study was to observe and record the events that occur during the pre-ecdysial and post-ecdysial development of the last ecdysis in a species of *Galeodes* and evaluate its exuvial morphology.

2. Material and Methods

The pre-ecdysial specimen of *Galeodes* sp. was collected in southeast Turkey (Hakkari Province, Demirtaş Village, Yağmurlu Hamlet, 37°43'58.14" N, 43°59'35.22"E, 2229m a.s.l., 29.06.2019) (Fig. 1A-B) and kept under observation in the laboratory (Fig. 1C-F). The species could not be fully identified because of the insufficient number of specimens and also its main characteristics were not fully formed. Specimens were maintained in the laboratory in a glass bell jar (50 cm diameter and 20 cm height) containing a layer of soil about 4-5 cm in height at room temperature. A small stone was put to provide a hiding place during the daylight hours and wet cotton wool for providing humidity. For feeding, larvae of *Tenebrio molitor* Linnaeus, 1758 (Coleoptera) were provided daily. Digital images were taken using a digital camera attached to Leica DFC295 stereomicroscope. For scanning electron microscopy (SEM) analysis, the exuvial junction part was cleaned and air-dried. After gold-coated in a Quorum SC7620 sputter coater, it was studied and photographed at an accelerating voltage of 10kV in a ZEISS Sigma 300 scanning electron microscope at the Science Application and Research Center in Van Yüzüncü Yıl University.

3. Results

The preecdysial galeodid was found in a burrow under a stone in the habitat (Fig. 1A-B). The chelicerae, pedipalps, four pairs of legs, and opistosoma of galeodid were bent up dorsally in the 17-day period between the date taken from the habitat to moulting (Fig. 1C-D). The chelicerae and propeltidium were swollen and the ocular tubercle became transparent in 3-4 days (Fig. 1D). The galeodid squirmed its opisthosoma at irregular intervals.

The first exuvial rupture occurred in propeltidial surface behind the ocular tubercle (Fig. 2B-C). There was

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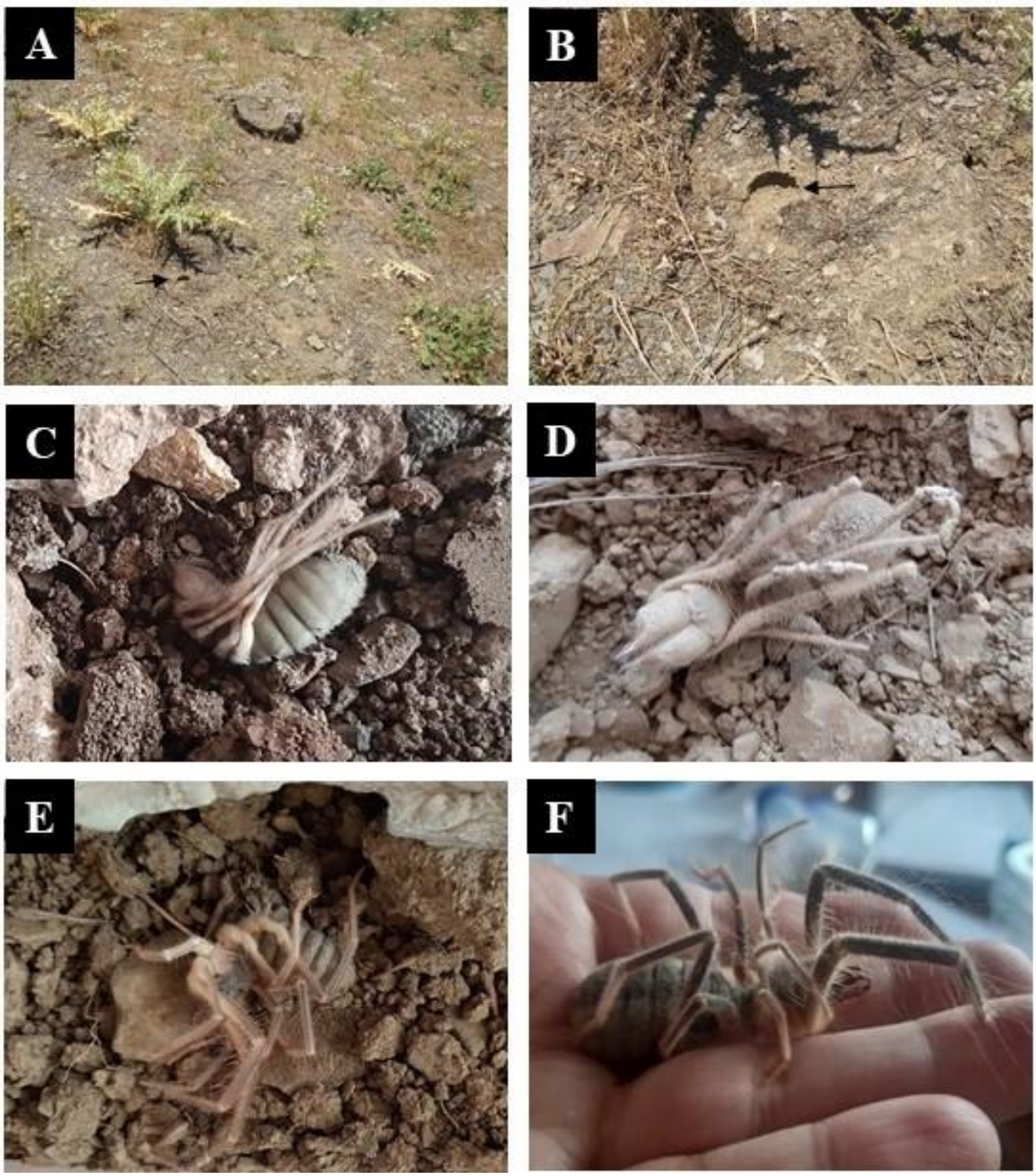

Figure 1. The burrow of *Galeodes* sp. in habitat (A). Close-up view of the burrow (B). Dormancy position in preecdysial galeodid, laterally (C), dorsally (D). The general overview of galeodid just after ecdysis (E), a few days after ecdysis (F).

an intracuticular fibre, whitish and 2.8 cm in length, on the propeltidial exuvium behind the transversal rupture (Fig. 2A-C). The muscle-like structure was also observed on coxal junction of pedipalp as well as the intracuticular fibre (Fig. 2D). There are local moulting areas on the ventral surface of pedipalpal coxa, whose socket structures were clearly visible (Fig. 2D, 3D). This intracuticular fibre consists of folded exuvial skin and tube-like tracheae under this exuvial skin (Fig. 3A, C). The tracheal surface is thrown into longitudinal folds equally (Fig. 4A-B) and the surface of tracheae reveals tiny transversal anastomosing folds (Fig. 4B).

The chelicerae tip and the tarsal claws of the legs have not shed their skin and have fallen off as a whole because they are completely renewed. (Fig. 2A-B). Just after ecdysis, the male cheliceral flagellum was visible and the whole body surface seemed glossy, soft, and fragile (Fig. 1E). The chelicerae, pedipalps, four pairs of legs, and opisthosoma of galeodid were bent up dorsally. Within a few days, the surface became dull and sensilla on the body surface became more visible (Fig. 1F). The last ecdysis process was completed in about 16 hours and it received its first food about 68 hours after ecdysis. In this process, the galeodid did not move actively on the ground, just only small twitches occurred in its place (Fig. 1E).

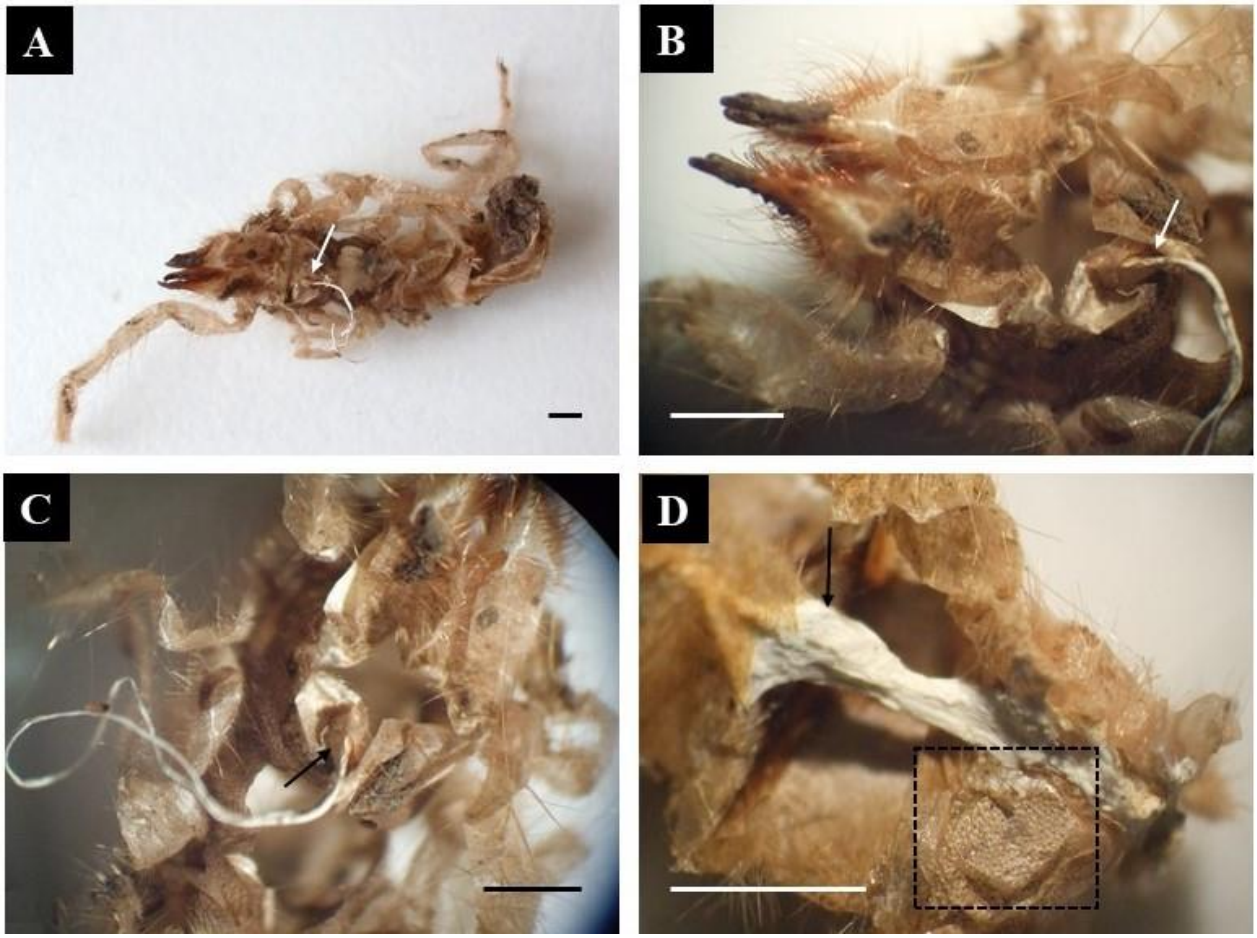

Figure 2. The exuviae of *Galeodes* sp., dorsally (A-D), Exuvial sheath of whole body (A), Exuvial sheath of chelicerae and propeltidium (B-C). ventral surface of pedipalpal coxa (D). (Arrow show intracuticular fibre (C) and muscle-like structure (D), square shows setal moulted area of pedipalpal coxa) (Scale bars: 2mm).

4. Discussion

The behaviors and events before and after moulting during the last ecdysis were observed and reported in the present study. The reason it is called "the last ecdysis" because the male flagellum is formed at the end of this ecdysis process. Partial moulting was observed especially for proximal segments of extremities. This moulting was like breakages of sensilla. Most of the sensilla on the body surface especially on chelicerae were not moulted. The moult cycle culminates in ecdysis. Haupt (1982) indicated that the old sensilla in the old cuticle remain connected to the epidermis during apolysis by dendrites, though the exuvial space widening. In this study, it was observed that the intracuticular fibre consists of exuvial sheath and tracheal trunks. Until the solifuge comes out of the exuvial sheath, this intracuticular fibre remains as a connective tissue that provides its connection with the external environment. During the ecdysis, exuvial space widens, apolysis is highly likely to occur in this process with freeing of the epidermis from the old exoskeleton. It is also thought that the intracuticular fibre located near the rupture point is contributed to the opening of the first rupture on the propeltidium. Altner & Prillinger (1980) indicated for hemimetabolous insect that the development starts with the outgrowth of the dendritic outer segment together with the cuticular sheath and in the moulting process elongation of both structures brought about a strand that bridges the ecdysial space between the sensillum of old cuticle and the anlage of the new sensillum within epidermis. This strand is most probably same as the structure defined as intracuticular fibre in this study. They also emphasized that the cuticular sheath might be protecting the elongated dendrites from the enzymes in the exuvial space. The hard structures like tarsal claws on legs, cheliceral tip, and teeth not moulted literally but left incomplete pieces. Wharton (1981) also mentioned this process as changing of teeth size and shape with each moulting. In this case, teeth and tarsal claws will be small in size and this will cause systematic questioning because of dentition.

Punzo (1998) said that burrows are utilized for the digestion of food, oviposition, aestivation, ecdysis, and as places for protection during periods of inactivity. In this study, the burrows observed in the habitat and under laboratory conditions were circular and lateral lying position of preecdysial galeodid at equal height with of the burrows. During this quiescence stage, solifuge only twisted its opisthosoma in the burrow. It is thought that these twistings were effective in the formation of the circular burrow.

Ethics committee approval: Ethics committee approval is not required for this study.

Conflict of interest: The author declares that there is no conflict of interest.

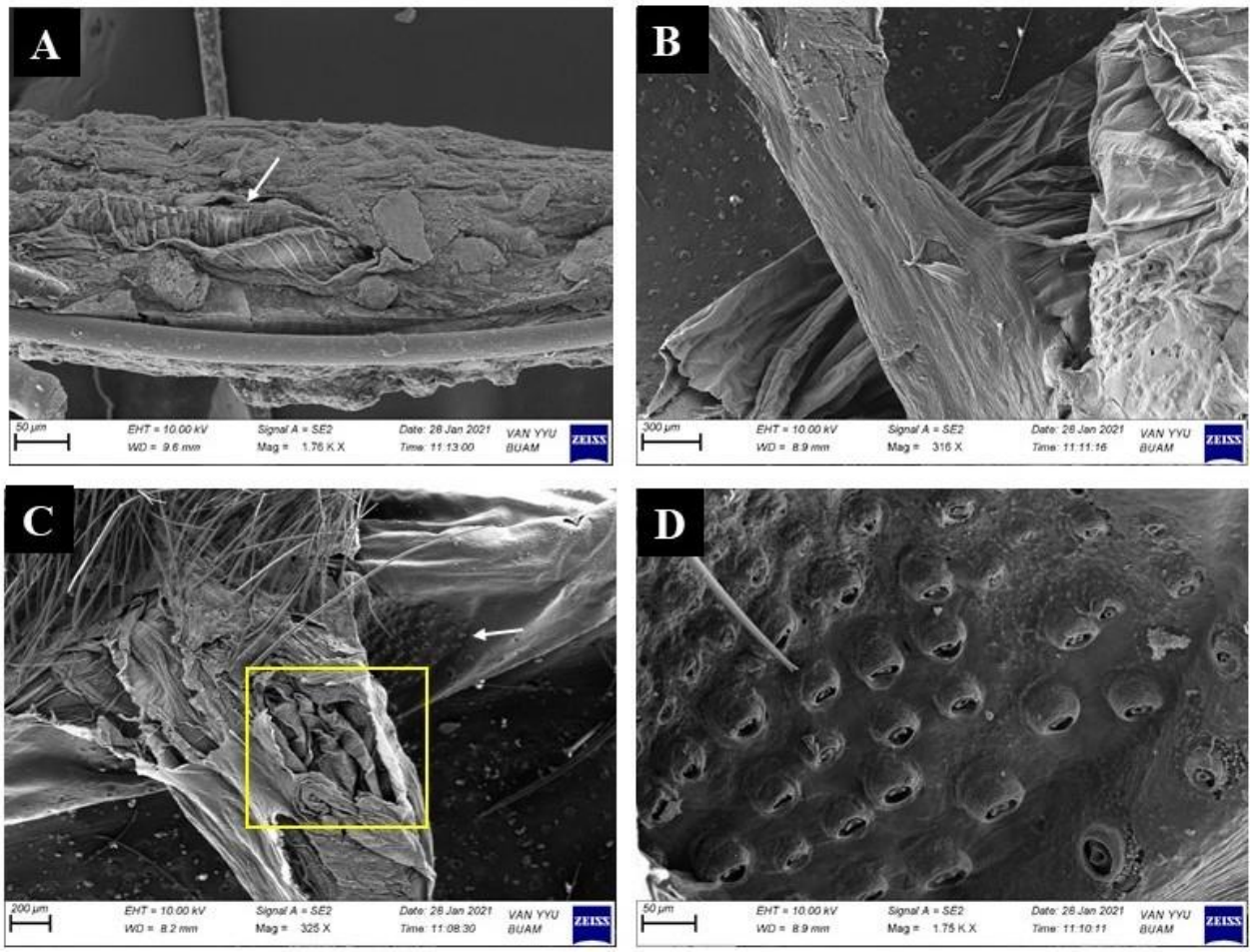

Figure 3. The intracuticular fibre connected to the propeltidial exuvium of *Galeodes* sp., dorsally (A-C), (Arrows show trachea in Fig. A and setal moulting area in Fig. C, and the square shows tracheae in Fig. C). Sockets of the moulting setae (D).

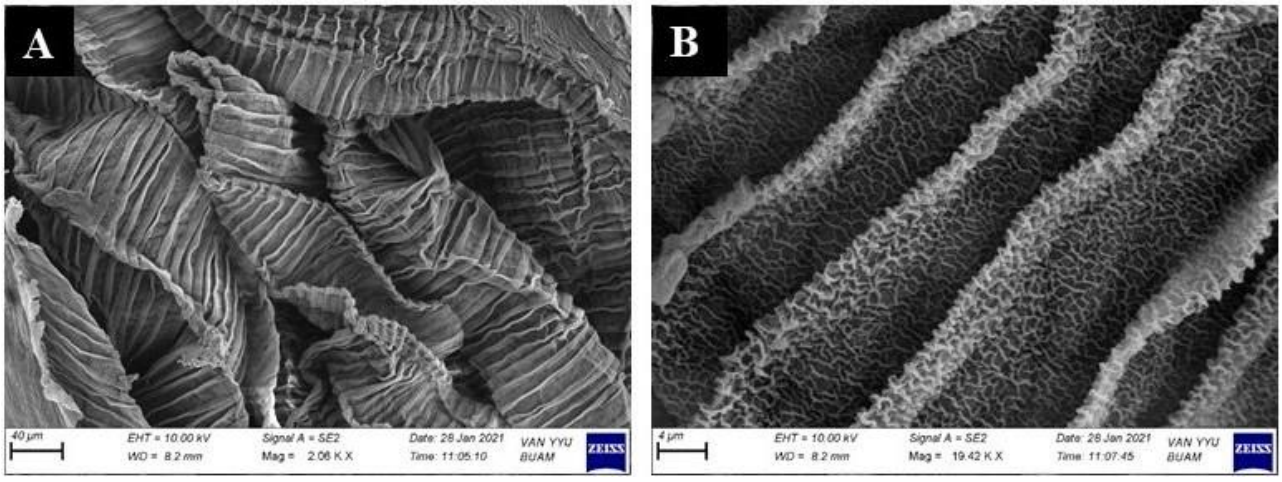

Figure 4. The transversal folds of tracheae in intracuticular fibres connected to exuviae of *Galeodes* sp. (A), Close-up to the transversal folds of tracheae (B).

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Note / Not

Note on the Endemic Status of Harran Fringe-fingered Lizard, *Acanthodactylus harranensis* Baran, Kumlutaş, Lanza, Sindaco, Ilgaz, Avcı, & Crucitti, 2005

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Abstract: Harran Fringe-fingered Lizard, *Acanthodactylus harranensis* has been assumed to be endemic to Turkey so far. Here an ancient personal finding from Syria is presented that was recorded ten years before the classification of the species. The newly acquired distributional data of this endangered species is discussed, including the consequences about the survival of the species.

Keywords: Turkey, Syria, Mesopotamian Plain, Harran Fringe-Fingered Lizards, endemism.

Harran Tarak Parmaklı Kertenkelesi, *Acanthodactylus harranensis*'in Baran, Kumlutaş, Lanza, Sindaco, Ilgaz, Avcı, & Crucitti, 2005 Endemik Statüsü Hakkında Bir Not

Öz: Harran Kertenkelesi, *Acanthodactylus harranensis*'in şimdiye kadar sadece Türkiye'ye endemik olduğu varsayılmaktaydı. Bu çalışmayla, türün tasnifinden on yıl önce Suriye'den eski bir kişisel bulgu sunulmuştur. Nesli tükenmekte olan türün yeni elde edilen dağılış verileri ve türün devamlılığı da dahil olmak üzere tartışılmıştır.

Anahtar kelimeler: Türkiye, Suriye, Mezopotamya ovası, Harran Kertenkelesi, endemizm.

Mayıs 1995'te Suriye'nin kuzey kesimlerinde, Türkiye sınırına yakın bir bölgede, yapılan arazi çalışması sırasında tahıl tarlasının kenarında bulunan sığ bir hendekte *Acanthodactylus* Fitzinger, 1834 cinsine ait bir örneğe rastlanmıştır (Şekil 1). Erkek numunenin yakalandığı lokalite haritada tespit edilememiş olup, yerel tabelada Bi'r Abou olarak adlandırılan yerleşim biriminin 9 km batısında (36.666ºN 39.679ºE) bulunmaktadır. Kertenkele örneği elle yakalandıktan sonra renk-desen özelliklerinin tespiti için fotoğrafı çekilmiştir. Örnek eterle bayıltıldıktan sonra önceden hazırlanmış uygun tespit karışımı (%70'lik etanol) vücut boşluğuna enjekte edilmiştir. Örnek hâlihazırda Rotterdam Doğa Tarihi Müzesi'nde muhafaza edilmektedir (Müze numarası 95-215). Örneğe ait bazı morfolojik karakteristikleri (pholidosis, vücut ölçümü ve oranlar) o zaman belirlenmiştir.

Toplanan örneğin bazı morfolojik özellikleri *A. grandis* Boulenger, 1909 türüne yakın olmakla birlikte (parmaklarda dört sıra pul, ventral plakların eğik dizilmesi gibi), ergin erkek örnek bazı farklı özellikler göstermekteydi ve o yıllarda mevcut olan literatürler ile (Arnold, 1983; Salvador, 1982) numunenin taksonomik durumu net olarak belirlenememiştir.

Ergin erkek numuneye ait bazı morfolojik özellikler şu şekilde sıralanabilir: Baş+gövde uzunluğu 97 mm; kuyruk rejenere olmuş; ventralia enine 12-14 sıra; ventralianın konfigürasyonu komplike, yani merkez kısmında uzunlamasına sıra halinde, öncesi ve arkasında eğik sıralar halinde; parmaklarda dört sıra pul; kulak açıklığının önündeki pulların pektinatlı; subocularis dördüncü ve beşinci supralabial arasına sıkışmış, ama dudak kenarına kadar ulaşmıyor; gularia 30; dorsalia 52; collaria 11; femoral porlar (sol) 25; kuyruğun proksimal pulları neredeyse düz, sonrasında karinalı; başın yan tarafında 5 koyu dikey bant mevcut; sırt tarafı ağ şeklinde desenli.

Şekil 1. Suriye'nin kuzeyinde (36.666ºN 39.679ºE) gözlenen *Acanthodactylus harranensis* örneği

Figure 1. *Acanthodactylus harranensis* specimen from Northern Syria (36.666ºN 39.679ºE)

Baran et al. (2005) on yıl sonra Harran'dan (Şanlıurfa, Türkiye) yeni bir Lacertid türü olarak *Acanthodactylus harranensis* türünü tanımlamıştır. Yeni takson sadece tip lokalitesi olan Harran harabelerinden rapor edildiğinden

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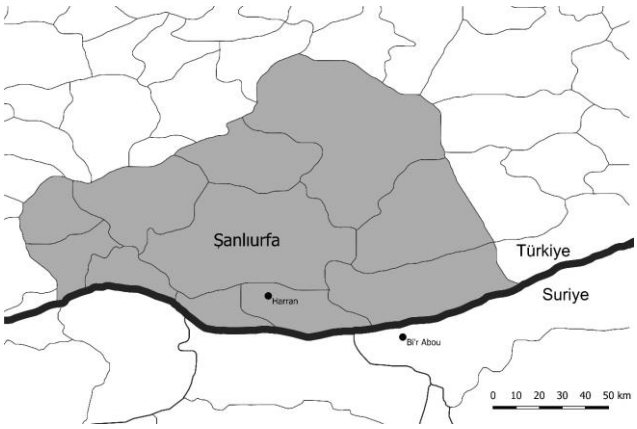
Türkiye'ye endemik olarak kabul edilmektedir (Baran et al., 2021).

Suriye'de *Acanthodactylus* cinsine dahil 5 türün [*A. boskianus asper* (Audouin, 1829), *A. grandis* Boulenger, 1909, *A. orientalis* Angel, 1936, *A. robustus* Werner, 1929 ve *A. tristrami* (Günther, 1864).] var olduğu bilinmektedir (lacerta.de). Söz konusu türler göz önünde bulundurulduğunda, *A. orientalis*, *A. robustus*, *A. tristrami* ve *A. boskianus* subocular plağın dudak kenarına ulaşması, parmaklarında üç sıra pulun varlığı ve ventral plaklarının tam uzunlamasına sıra teşkil etmeleri ile *A. harranensis* türünden ayrışmaktadırlar (Baran et al., 2005).

Suriye'de bulunan tek bir örneğin morfolojik özellikleri *A. harranensis* türüne birebir uygunluk göstermektedir. Mevcut örnek, ventral plakların sayısı ve konfigürasyonunun yanında, kuyruğun proksimal pullarının neredeyse düz olması ve tipik sırt deseninden (ağsı), *A. grandis* türünden farklılık göstermektedir. Ayrıca sırt ortasındaki enine bir sıradaki dorsal pulların sayısının (52) az olmasıyla da Suriye'de bulunan *A. grandis* türünden (56-63, Salvador, 1982) farklılık göstermektedir.

Numunenin bulunduğu lokalite ile Türkiye sınırı arasında 7 km, Harran İlçesi (Şanlıurfa) (Tip lokalite) ile ise kuş bakışı 61 km mesafe bulunmaktadır. Türün tip lokalitesinin yanında ve mevcut çalışmada incelenen örneğin yakalandığı lokalite, aşağıda Şekil 2'de verilmiştir. Söz konusu iki lokalite Mezopotamya Ovası'nın aynı kuzey bölümünde yer almaktadır.



Şekil 2. *Acanthodactylus harranensis*'in tip lokalitesini ve Suriye'de bulunan yeni lokaliteyi gösteren harita.

Figure 2. The map showing the type locality of *Acanthodactylus harranensis* and the new locality found in Syria.

Bu çalışmayla Harran Tarak Parmaklı Kertenkelesi'nin Türkiye ile birlikte Suriye'de de yayılış gösterdiği ortaya konulmuş ve tür endemik olma statüsünden çıkmıştır. *Acanthodactylus harranensis* muhtemelen Mezopotamya'nın kuzey parçasına endemik bir kertenkele türüdür. Daha önce kurak bir yapıda olan Harran Ovası, 1995 yılından itibaren Atatürk Barajı suları ile sulanmaya başlanmış ve Suriye sınırına kadar olan türün yaşamasına uygun alanlar tarlalara dönüştürülmüştür. Söz konusu durum Güneydoğu Anadolu Bölgesi'nde Harran Tarak Parmaklı Kertenkelesi'nin yarı kurak olan orijinal habitatının yok olması sonucunu beraberinde getirmiştir. Nesli tehlike altında olan türün (Kaska et al., 2009), özellikle Suriye'nin kuzeyinde yer alan sulanmayan sahalarda varlığını sürdürme şansı mevcuttur. Suriye içerisinde kalan bölge yeterince araştırılmamıştır. Bilinen populasyonu oldukça yaşlı bireylerden oluşan türün (Beşer et al., 2019), dağılış sahasının tam olarak belirlenmesi için özellikle Suriye'nin kuzeyi başta olmak üzere Türkiye-Suriye sınırı boyunca daha kapsamlı arazi çalışması yapılması gerekmektedir. Bölgenin güvenlik durumu şu aşamada buna imkan tanımamaktadır.

Etik kurul onayı: Bu çalışma için etik kurul onayı alınmasına gerek yoktur.

Çıkar çatışması: Yazar, çıkar çatışması olmadığını beyan etmiştir.

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Note / Not

A Stowaway from Cyprus; *Heteropoda venatoria* Linnaeus, 1767 (Araneae, Sparassidae)

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Abstract: It is a known phenomenon that the circulation of alien species increases due to many different variables such as the increase in human commercial activities, the ease of travel between continents, and the decrease in their duration. Pantropical origin *Heteropoda venatoria*, due to its synanthropic tendencies, is a spider species that is inclined to travel frequently and to disperse in the geographies it reaches. In this brief paper, a female *Heteropoda venatoria* that arrived from Japan to Cyprus via a merchant ship is presented.

Keywords: Giant crab spider, exotic, spider, alien species.

Kıbrıs'tan Kaçak Bir Yolcu; *Heteropoda venatoria* Linnaeus, 1767 (Araneae, Sparassidae)

Öz: Dünya üzerinde insana ait ticarî faaliyetlerin artması, kıtalar arası seyahatlerin kolaylaşıp, sürelerinin azalması gibi birçok farklı değişkene bağlı olarak yabancı türlerin dolaşımlarının arttığı bilinen bir olgudur. Pantropical kökenli *Heteropoda venatoria*, sinantropik eğilimleri dolayısıyla sık sık seyahate ve ulaştığı coğrafyalarda dağılım göstermeye yatkın bir örümcek türüdür. Bu kısa makalede, Japonya'dan Kıbrıs'a ticarî bir gemi aracılığıyla ulaşan dişi bir *Heteropoda venatoria*'dan bahsedilmektedir.

Anahtar kelimeler: Dev yengeç örümceği, egzotik, örümcek, yabancı tür.

"Dev Yengeç Örümceği" ya da "Muz Örümceği" olarak da isimlendirilen *Heteropoda venatoria* Linnaeus, 1767, avcı örümcekleri bünyesinde barındıran Sparassidae familyasına ait bir türdür. Sparassidae üyeleri, Dünya genelinde 87 cinse ait 1262 türle temsil edilmekte olup (WSC, 2021), orta-büyük boylu araneomorf örümceklerdir. Ekribellat, entelijin, sekiz gözlü, laterigrad bacaklı ve çift tarsal tırnaklıdırlar. Ayrıca metatarsuslarının dorsal ucunda üç loblu yumuşak bir zarın varlığı familyanın tanılayıcı özelliklerindendir (Jocqué & Dippenaar-Schoeman, 2007).

Sparassid Örümcekler Kıbrıs'ta beş tür ile temsil edilmektedirler. Bunlar: *Eusparassus walckenaeri* (Audouin, 1826); *Micrommata formosa* Pavesi, 1878; *M. ligurina* (C. L. Koch, 1845); *M. virescens* (Clerck, 1757) ve *Olios suavis* (O. Pickard-Cambridge, 1876)'dır (Bosmans et al., 2019). Bunların tamamı Akdeniz havzası ve çevresinde görülmesi olanaklı, yerli türlerdir.

Doğal dağılım alanı Tropikal Asya olan *Heteropoda venatoria* zaman içinde insan faaliyetlerine bağlı olarak, özellikle ticarî muz taşımacılığı sebebiyle, Pasifik Adaları, Yeni Dünya (Kuzey, Orta ve Güney Amerika), Makaronezya, Avrupa ve Afrika'ya da giriş yapmıştır (WSC, 2021).

Heteropoda venatoria, saldırgan değil tam tersine ürkek bir örümcek türüdür. Rahatsız edilmesi halinde son derece hızlı hareket eder. İri cüssesine rağmen yassı vücudu ve esnek bacakları sayesinde küçük çatlaklara giriş çıkış yapabilme kabiliyetine sahiptir. Günümüzde muz tarımında, muzu zararlı böceklere karşı korumak için biyolojik mücadele ajanı olarak kullanılmaktadır. İnsanla teması durumunda nadiren ısırılma vakası görülse de, *H. venatoria*'nın zehrinin tıbbî önemi yoktur. Isırılan bölgede geçici ağrı ve şişlik bilinen semptomlardandır (Ewunkem et al., 2016).

Olay: 04.12.2020 günü Kıbrıs, Girne, Taşkent'te faaliyet gösteren kâr amacı gütmeyen özel bir kuruluş olan "Kıbrıs Yaban Hayat Araştırma Enstitüsü" arandı. Telefondaki kişi "yaklaşık 45 gün evvel Japonya'nın Osaka Limanından ayrılan bir yük gemisinden indirilen konteynerde büyük bir örümceğin varlığından" bahsediyordu. Bunun üzerine kişi ile temasa geçen Kıbrıs Yaban Hayat Araştırma Enstitüsü görevlisi, örümceği enstitüye bağlı Yaban Hayat Kurtarma ve Rehabilitasyon Merkezine iletmek üzere tutanak karşılığında teslim aldı. Tutanakta örümceğin durumu kritik (24 saat içerisinde ölebilir) olarak işaretlenmiştir. Nitekim örümcek merkeze naklinin hemen ardından yüksek olasılıkla susuzluk, ağır stres ve üşüme gibi nedenlerden dolayı ölmüştür. Bunun ardından etil alkole konulan örnek, enstitü uzmanlarınca, Jäger (2000)'e göre, *Heteropoda venatoria* (dişi birey) olarak teşhis edilip koruma altına alınmıştır (Şekil 1).

Heteropoda venatoria'nın ticarî gemiler aracılığıyla seyahat edip, ulaştıkları coğrafyalarda yaşama imkânı bulmaları daha önceleri çeşitli yazarlar tarafından bildirilen bir olgudur. Örneğin, Taucare-Ríos & Brescovit

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(2011)'in Şili kayıtları bunlardan sadece biridir. Bizim kaydımızın ada faunası açısından sevindirici tarafı; örneğin tek, sağlıksız ve doğaya kavuşamadan alıkonulmuş olmasıdır.

Şekil 1. *Heteropoda venatoria*, ölüm sonrası fotoğraf. Ölçek çizgisi: 10 mm

Kıbrıs'ın yoğun ticarî ve turistik trafiği adaya yabancı türlerin girişini kolaylaştırmaktadır. Örneğin bir yaprak zararlısı olan *Chrysolina* (*Chrysolinopsis*) *americana* (Linnaeus, 1758) (Coleoptera, Chrysomelidae) türü bu makalenin değerlendirilme sürecinde Kıbrıs'tan rapor edilmiştir (Hadjiconstantis & Zoumides, 2021). Yine bir başka Örümceğimsi türü olan *Euscorpius italicus* (Herbst, 1800) (Scorpiones: Euscorpiidae)'nin de adadaki varlığı muhtemelen taşınmaya bağlıdır (Yağmur, 2012). Bununla birlikte, mevcut literatüre göre Kıbrıs'ta dağılım gösteren örümceklerin tamamı yerli türler olup içlerinde yabancı tür bulunmamaktadır (Bosmans et al., 2019). Ancak ada olması sebebi, ulaşım ve taşıma yoğunluğu dolayısıyla yabancı türlerin Kıbrıs'a giriş olasılığı her zaman mümkün olup doğası açısından potansiyel bir tehlike arz etmektedir. Bunun önüne geçebilmek adına adaya giren ticarî mallara yönelik kontrollerin yoğunlaştırılması, gümrük memurlarına ve özellikle giriş bölgelerinin yakınlarında yaşayan halka yönelik eğitim çalışmaları akla ilk gelen önlemler arasında sıralanabilir.

Teşekkür: Örümcek örneğinin enstitüye intikalini sağlayan, fotoğraflarını çeken Mustafa Güray Bukan'a teşekkür ederiz.

Etik kurul onayı: Bu çalışma için etik kurul onayı alınmasına gerek yoktur.

Çıkar çatışması: Yazarlar, çıkar çatışması olmadığını beyan etmiştir.

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Erratum / Düzeltme

Erratum to: Searching of the Genetically Modified Organisms and Their Products' Status and Evaluation of Food Safety and Regulations in Turkey in terms of the Forensic Sciences

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