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(1977-2023)

Sevgili meslektaşımızı 6 Şubat 2023 tarihinde Kahramanmaraş'ta meydana gelen depremde kaybetmiş olmanın derin üzüntüsünü yaşıyoruz. Merhumeye Allah'tan rahmet, ailesine ve yakınlarına başsağlığı dileriz.

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Saccharomyces cerevisiae L. Hücre Kültürü Oksidatif Stres Modelinde Bazı Fosfazen Türevlerinin Biyokimyasal Aktiviteleri

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ÖZET

Bu çalışmada, *Saccharomyces cerevisiae* L. kültür ortamında bazı fosfazenlerin biyokimyasal aktiviteleri belirlendi. Deneysel uygulamada farklı fosfazen molekülleri kullanıldı. Çalışma kapsamındaki deney grupları; kontrol grubu, H_2O_2 (Hidrojen peroksit) ve fosfazen molekülleri grupları şeklinde düzenlendi. Gruplar hazırlandıktan sonra, kontrol grubu dışında diğer kültürlere 30 µg fosfazen ve 100 µl H_2O_2 ilave edildi. 30 °C de 72 saat inkübasyona bırakıldı. İnkübasyon sonunda hücre peletleri ayrıldı. Elde edilen süpernatantdan Glutatyon S-Transferaz (GST) ile total protein düzeyleri belirlendi. Hekzan/izopropanol alkol karışımı ile elde edilen homojenattan da yağ asidi ve lipofilik moleküllerin analizi yapıldı. Deney sonuçlarına göre, fosfazen molekülü ve H_2O_2 ilave edilen maya hücrelerinde total protein değerleri ile GST değerlerinde paralel bir artış gözlenirken, bazı gruplarda protein miktarında artış saptandığı halde GST düzeyinde azalma olduğu belirlendi. *S. cerevisiae'* nin membran yapısında önemli bir yer kaplayan ergosterolün, T3 ve T3B kodlu fosfazenler ile H_2O_2 gruplarında kontrol grubuna göre yüksek, T4 kodlu fosfazenler ile H_2O_2 gruplarında ise düşük olduğu belirlendi. Sonuç olarak; *S. cerevisiae* kültür ortamına fosfazen ve H_2O_2 moleküllerin eklenmesinin, ergosterol ve yağ asidi sentezi ile yağ asitlerinin hidrokarbon zincirine çift bağ girişi yapan enzimlerin son ürünlerinde artışlara ya da azalışlara neden olduğu ortaya konulmuştur.

Biyokimya

Araştırma Makalesi

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Anahtar Kelimeler
Fosfazen
H_2O_2 (Hidrojen peroksit)
Lipofilik moleküller
Saccharomyces cerevisiae L.
Yağ asiti

Antioxidant Effects of Some Phosphazene Derivatives in *Saccharomyces cerevisiae* L. Cell Culture Oxidative Stress Model

ABSTRACT

In this study, biochemical activities of some phosphazenes were determined in *Saccharomyces cerevisiae* L. culture medium. Different phosphazene molecules were used in experimental practice. The experimental groups within the scope of the study were organized as control group, H_2O_2 (Hydrogen peroxide) and groups of phosphazen molecules. After the groups were prepared, 30 µg of phosphazen and 100 µl of H_2O_2 were added to other cultures, except for the control group of *S. cerevisiae* culture. It was incubated for 72 hours at 30 °C. At the end of incubation, cell pellets were separated. Glutathione S-Transferase (GST) and Total protein levels were determined from supernatant. The fatty acid and lipophilic molecules were analyzed from the homogenate obtained with the hexane / isopropanol alcohol mixture. According to our experimental results, while total protein values and GST values increased in parallel with the phosphazen molecule and H_2O_2 added yeast cells, GST levels were decreased in some groups, although an increase in the amount of protein was observed. Ergosterol, which has an important place in the membrane structure of *S. cerevisiae*, was found to be higher in T3 and T3B coded phosphazenes and H_2O_2 groups

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Fatty acid

compared to the control group, and low in T4 coded phosphazenes and H_2O_2 groups. Our study results revealed that as a result of the addition of phosphazen and H_2O_2 molecules to the culture medium of *S. cerevisiae,* ergosterol and fatty acid synthesis, fatty acids cause increases or decreases in the end products of enzymes that double-enter the hydrocarbon chain.

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GİRİŞ

Fosfazen; aynı grup azot ve fosfor atomlarının oluşturduğu $(R)_3$ P=NR (R:halojen, alkoksi, amino, alkil ve aril) yapısındaki bileşiklere denir (Civan, 2014). Fosforun azot ile yaptığı bileşikler üç ana grupta incelenebilir. P ile N arasındaki bağ sayısı tek olduğu zaman fosfazan H_2N-PH_4, çift olduğu zaman fosfazen HN=PH_3, üç olduğu durumda fosfazin N≡PH_2 olarak adlandırılmaktadır. Yapılarında yer alan fonksiyonel gruplarının bağlanma durumlarına göre isimler alarak sınıflandırılırlar. Fonksiyonel grup, fosfazen halkasında yer alan bir fosfora bağlanmışsa spiro adını alır, aynı halkada yer alan farklı iki fosfora bağlanmışsa ansa ve iki tane fosfazen halkasını birleştirecek şekilde bağlanmışsa bino fosfazen adını almaktadır (Civan, 2014). Fosfazenler; monofosfazenler, siklofosfazenler ve polifosfazenler olmak üzere üç grupta incelenebilir. Siklo ve polifosfazenler; en iyi bilinen ve üzerinde en çok çalışılan P-N bileşikleridir (Kılıç ve ark., 1996; Carriedo ve ark., 1996; Allock ve ark., 1996; Laguna ve ark., 2002).

Siklofosfazenler inorganik yapıya sahiptirler. Asit içeren alkoller ve aromatik halka yapısına sahip fenollerin tepkimesi sonucunda sentezlenebilmektedirler (Uslu & Yesilot, 2015; Sazhin ve ark., 2011). Klor atomlarının fosfora bağlanma şekillerinden kaynaklı olarak meydana gelen fiziksel ve kimyasal özellikler bulunmaktadır (Okutan ve ark., 2011). Farklı özelliklere sahip olan fosfazenler yapılarındaki bağlanmalara göre değişkenlik göstermektedir. Bu değişkenlikler fosfazenler için farklı kullanım alanı oluşturmaktadır. Polifosfazenler; organik yapıda olan sıvıları ayırma, buharlaştırma ve iyonlara ayırma gibi durumlarda membran filtreleri olarak ve bazı siklofosfazenler de kanser ilaçlarında kullanılmaktadır (Kılıç ve ark., 1996; Xia ve ark., 2017: Onder & Ozay 2021; Şenkuytu ve ark., 2022).

1900'lü yılların sonlarına doğru fosfazen bileşikleri ile ilgili yapılan çalışmalar hız kazanmıştır. Halkalı fosfazenlerle başlayan bu çalışmalar günümüzde geliştirilen tekniklerle artış göstermiştir (Dumanoğulları, 2006).

Monofosfazenlerle (basit fosfazenler) yapılan çalışmalar, bu moleküllerin hücrelerin büyümesi üzerine etkileri olduğunu ortaya koymuştur. Ayrıca yüksek oranda uygulanan fosfazen moleküllerinin bakteri ve maya hücreleri üzerinde antimikrobiyal etkiye sahip olduğu, bazı fosfazen moleküllerinin ise maya hücreleri üzerinde herhangi bir etkiye sahip olmadığı belirlenmiştir. İn vitro ortama verilen bazı fosfazenlerin de hücre büyümesini olumlu yönde etkilediği tespit edilmiştir (Öztürk ve ark. 2000).

Son zamanlarda fosfazen türevleri yüksek seçici antikanser madde (Siwy ve ark., 2006), anitmikrobiyal ajan (Işıklan ve ark., 2010; Çıralı ve ark., 2015; Binici ve ark., 2022) ve biyomedikal malzemelerin (Greish ve ark., 2005) tasarımında bir hayli dikkat çekmektedir. Ayrıca; canlılarda ilaç etkileşimlerini tespit etmek amacıyla da fosfazenler kullanılmaktadır (Koçak ve ark., 2013; Ozay & Ozay 2014; Sun ve ark., 2015). Tüm bu bilgiler ışığında bu çalışmada; biyoteknolojik önemleri oldukça geniş olan fosfazenlerden yeni sentezlenmiş olan bazı fosfazen gruplarının *S. cerevisiae* hücre kültürü oksidatif stres modelinde biyokimyasal parametreler üzerine etkilerinin belirlenmesi amaçlanmıştır.

Fosfazen Molekülleri

Bu moleküllerin sentezi TÜBİTAK tarafından desteklenen TBAG-107T407 nolu proje ile yapılmıştır.

MATERYAL ve METOD

T3= 22 mg**; T3A=** 27mg; **T3B=** 28 mg; **T3C=** 27 mg; **BrFOS=** 20 mg; **T4A=** 20 mg; **T4B=** 20 mg; **T4C=** 20 mg tartılarak 5 ml DMSO (dimethyl sülfoxide)'da çözdürüldü. Kültür ortamına ekim yapılana kadar + 4 °C de bekletildi.

Maya Kültür Ortamının Hazırlanması

Deneyde kullanılacak olan *S. cerevisiae* gelişimi ve çoğalması için YEPD (200 mL için; 2 g maya ekstrak, 4 g baktopepton, 4 g glukoz) besiyeri ortamı her grup için tekrar sayısı (n) = 5 olacak şekilde hazırlandı.

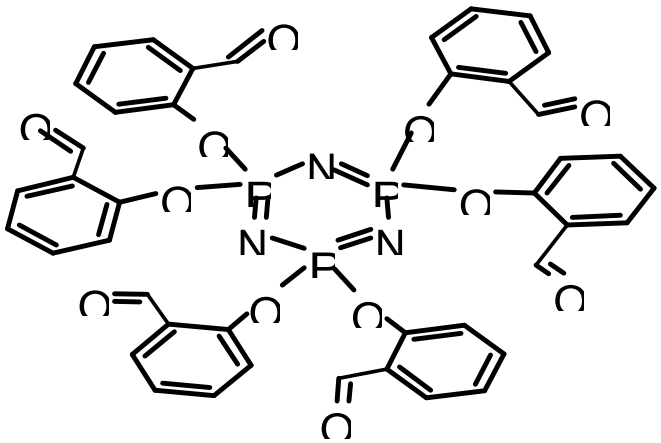
Besiyeri ortamı hazırlandıktan sonra aşağıdaki gruplara ayrıldı:

Kontrol grubu: Bu gruptaki *S. cerevisiae* hücreleri

için, YEPD besiyeri ortamı hazırlandı.

Fosfazen Uygulama Grupları: Bu gruptaki *S. cerevisiae* hücreleri için, YEPD besiyeri ortamı içerisine maya hücresi inoküle edildikten sonra OD600 değerleri 0.4-0.6 civarına [yaklaşık olarak 1-3 107 hücre ml (Bergman, 2001)] ulaşınca fosfazen maddelerinin her birinden 30 µg konsantrasyon olacak şekilde gruplar hazırlandı. Ayrıca aynı şartlarda H_2O_2 grubu da hazırlanarak % 35'lik H_2O_2 karışımından 100 µL her bir H_2O_2 grubuna eklendi ve aynı şartlarda inkübasyona bırakıldı.

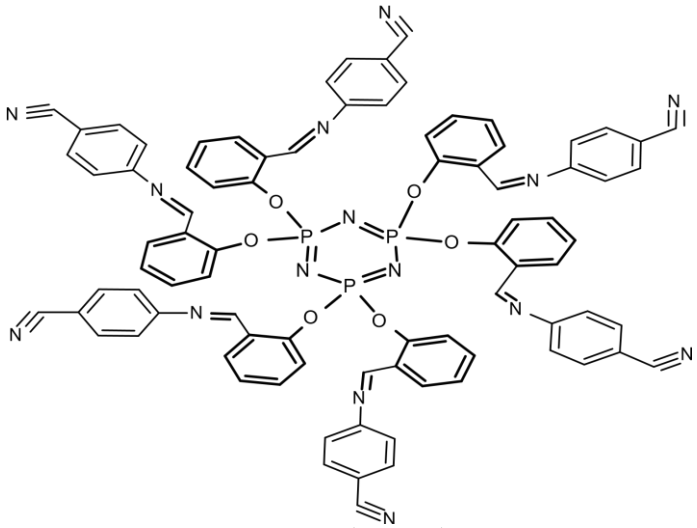
Her bir fosfazen madde ayrı bir grup olarak belirlenip deneysel çalışma işlemi yürütüldü ve aşılama işleminden sonra kültürler 30 °C'de 72 saat inkübasyona bırakıldı. Bu sürenin sonunda laboratuvar şartlarında kültürlerin 517 nm'deki hücre yoğunlukları ölçüldükten sonra, 6000 rpm'de 5 dakika süreyle +4 °C'de santrifüj edilerek hücreler



T3= Hexa (phenoxy) cyclotriphosphazene, Molekül ağırlığı: 861.621786

Şekil 1. T3= Hexa (phenoxy) cyclotriphosphazene, Molekül ağırlığı: 861.621786

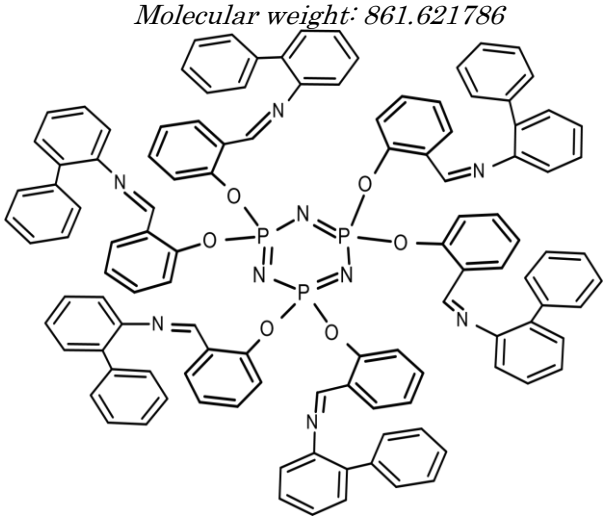
Figure 1. T3= Hexa (phenoxy) cyclotriphosphazene, Molecular weight: 861.621786



T3A= 4-aminobenzonitril (SALF1), Molekül ağırlığı: 1462.345746

Şekil 2. T3A= 4-aminobenzonitril (SALF1), Molekül ağırlığı: 1462.345746

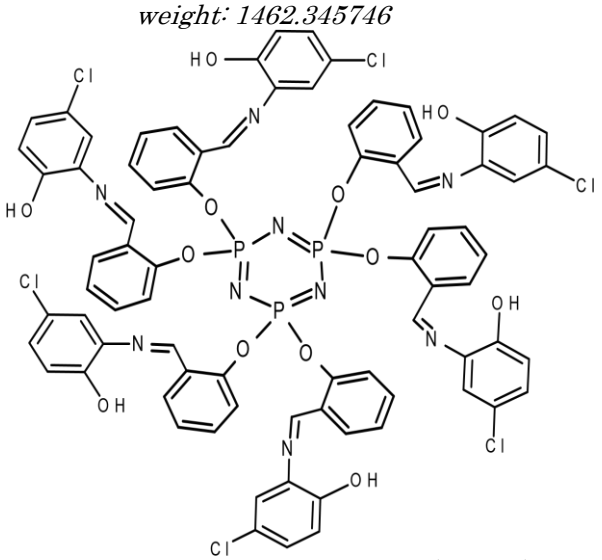
Figure 2. T3A= 4-aminobenzonitril (SALF1), Molecular weight: 1462.345746



T3B= 2-aminobifenil (SALF2), Molekül ağırlığı:1768.864746

Şekil3. T3B= 2-aminobifenil (SALF2), Molekül ağırlığı:1768.864746

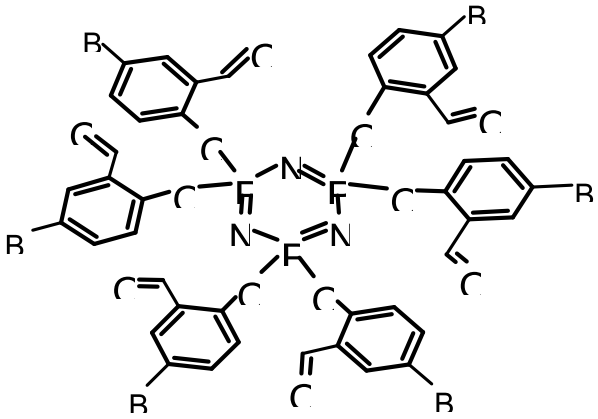
Figure 3. T3B= 2-aminobifenil (SALF2), Molecular weight:1768.864746



T3C= 2-amino-4-klorofenol (SALF3), Molekül ağırlığı:1614.955746

Şekil 4. T3C= 2-amino-4-klorofenol (SALF3), Molekül ağırlığı:1614.955746

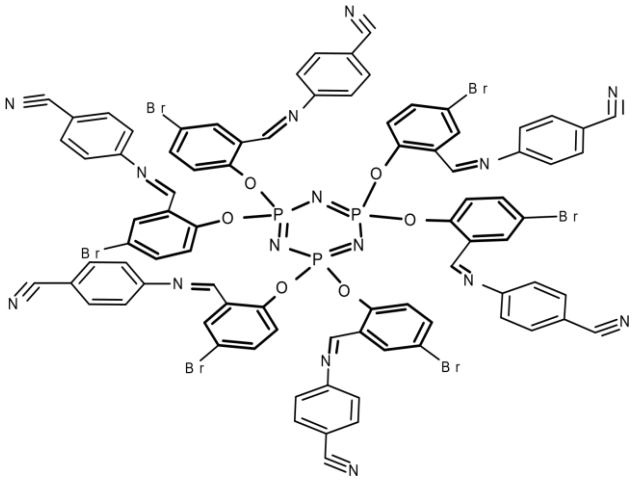
Figure 4. T3C= 2-amino-4-klorofenol (SALF3), Molecular weight:1614.955746



BrFOS= Hekza (4-Bromo-2-Formil-Fenoksi) Siklotrifosfazen, Molekül ağırlığı: 1334.998)

Şekil 5. BrFOS= Hekza (4-Bromo-2-Formil-Fenoksi) Siklotrifosfazen, Molekül ağırlığı: 1334.998)

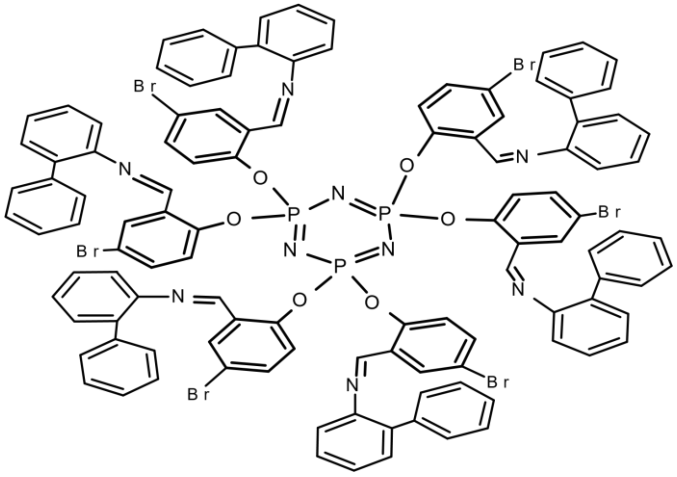
Figure 5. BrFOS= Hekza (4-Bromo-2-Formil-Fenoksi) Siklotrifosfazen, Molecular weight: 1334.998



T4A= 1.4-aminobenzonitril, Molekül ağırlığı: 1935.722

Şekil 6. T4A= 1.4-aminobenzonitril, Molekül ağırlığı: 1935.722

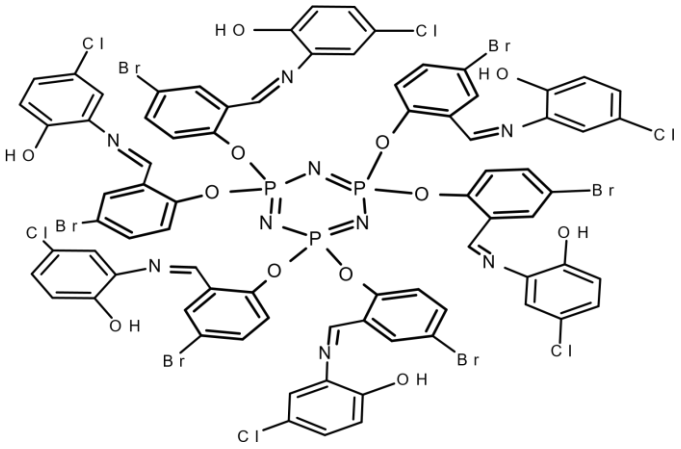
Figure 6. T4A= 1.4-aminobenzonitril, Molecular weight: 1935.722



T4B= 2-aminobifenil, Molekül ağırlığı: 2242.241

Şekil 7. T4B= 2-aminobifenil, Molekül ağırlığı: 2242.241

Figure 7. T4B= 2-aminobifenil, Molecular weight: 2242.241



T4C= 2-amino-4-klorofenol, Molekül ağırlığı: 2088.332

Şekil 8. T4C= 2-amino-4-klorofenol, Molekül ağırlığı: 2088.332

Figure 8. 2-amino-4-klorofenol, Molecular weight: 2088.332

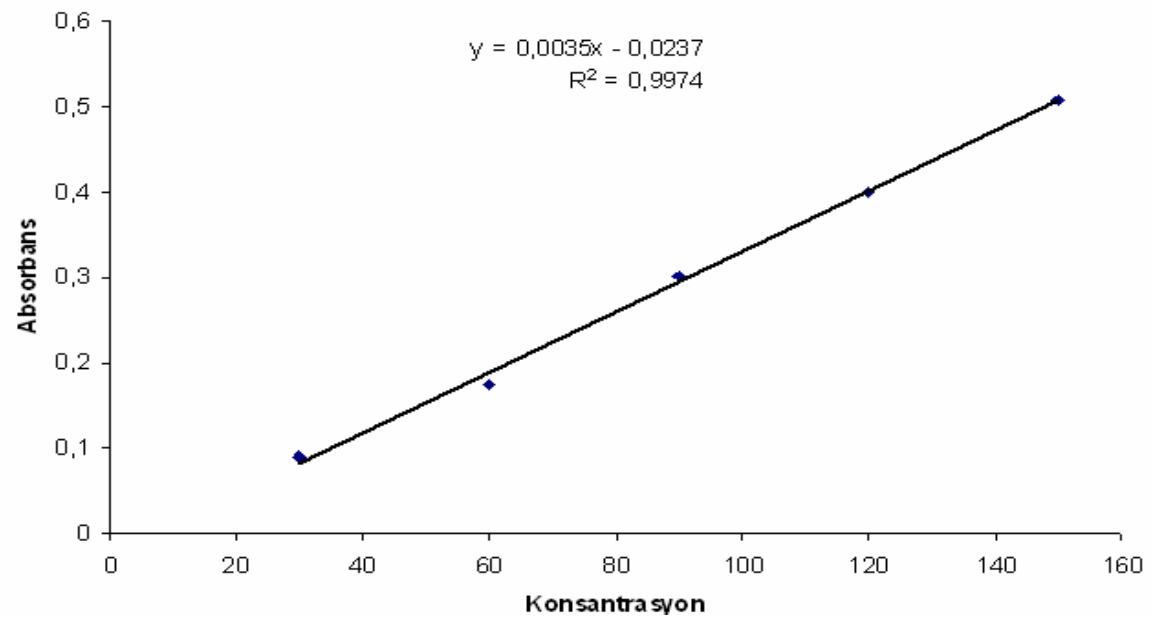
toplandı. Hücreler pellet olarak toplandıktan sonra yaş ağırlıkları belirlendi. Hücre pelletleri, 20 mM Tris HCl-baz (pH= 7.4) ve 20 mM EDTA karışımı ile homojenize edilip santrifüj edildikten sonra supernatant kısmı ile GST ve total protein, pelet kısmı ileyağ asidi ölçümleri yapıldı.

Maya Hücresinde Total Protein Miktarının Ölçülmesi

Örneklerin total protein miktarlarının ölçümü Lowry (1950) yöntemine göre yapıldı. Gruplar 750 nm'de blank'a karşı okundu ve okunan değerlere göre Şekil 9'daki kalibrasyon eğrisi oluşturuldu. Örneklerin protein miktarları elde edilen bu kalibrasyon eğrisindeki denklem vasıtasıyla hesaplandı, sonuçlar (mg/g) şeklinde verildi.

Glutatyon S-Transferaz (GST) Enzim Aktivitesi Tayini

Glutatyon S-transferaz tayini Habig (1974) tarafından geliştirilen metoda göre yapılmıştır. GST'nin bütün izozimleri için 1-kloro-2,4-dinitrobenzen (CdNB) substrat olarak kullanılır. GST enzimi tarafından CdNB, indirgenmiş glutatyon (GSH) ile konjuge edilerek glutatyonun oksidasyonuna bağlı olarak 340 nm'de absorbans yükselmektedir. Enzim aktivitesinin tayini için 3 dakika boyunca 340 nm'de yükselen absorbanslar okunarak 340 nm'de (ε=340: 9,6 mM cm^{-1}) 1 dakikada süpernatantta bulunan 1 mg toplam protein başına oluşturulan tioeter miktarı belirlenmiştir.



Şekil 9. Protein kalibrasyon eğrisi
Figure 9. Protein calibration curve

Yağ Asidi Metil Esterlerinin Hazırlanması

Hücre peleti örneklerinde yağ asidi ekstraksiyonu Hara ve Radin tarafından tanımlanan yönteme göre yapıldı (Hara & Radin, 1978). Doku örnekleri 3:2 (v/v) oranında hekzan-isopropanol karışımı ile homojenize edildi. Homojenizasyon sonrası bu homojenat +4 °C'de 9050 × g'de 10 dk. santrifüj edilerek elde edilen üst kısımdan yağ asidi analizi yapıldı.

Yağ asidi bileşimini belirlemek için ayrılan örneklerin üzerine %2'lik metanolik sülfürik asitten ilave edildi, iyice karıştırılarak 55 °C'de 15 saat etüvde metilleşmeye bırakıldı (Christie, 1999). Süre sonunda tüpler etüvden çıkarıldı, soğuduktan sonra %5'lik NaCl ilave edilerek iyice karıştırıldı. Tüpler içinde oluşan yağ asidi metil esterleri hekzan ile ekstrakte edildi ve hekzan fazı üsten pipetle alınarak %2'lik potasyum bikarbonat ($KHCO_3$) ile muamele edildi ve fazların ayrılması için 10 saat beklendi. Süre sonunda metil esterlerini içeren karışımların, 45 ºC'de ve azot gaz akımı altında çözücüleri uçuruldu, 1 mL n-heptan ile çözüldü ve yağ asidi metil esterleri gaz kromatografisinde analiz edildi. Bu analiz için SP™-2380 kapiler GC kolon (L×ID. 30 m × 0,25 mm, df 0,20 µm) (Sigma) kullanıldı.

Yağ Asidi Metil Esterlerinin Gaz Kromatogafik Analizi

Lipit ekstraktı içindeki yağ asitleri metil esterlerine dönüştürüldükten sonra Shimadzu marka GC 2001 Plus gaz kromatografisi ile analiz edildi. Analiz sırasında kolonun sıcaklığı 148–218 ºC, enjeksiyon sıcaklığı 245 ºC ve dedektör sıcaklığı 290 ºC olarak tutuldu. Kolon sıcaklık programı 148 ºC'den 218 ºC'ye kadar programlandı. Sıcaklık artışı 180 ºC'ye kadar 5 ºC dk^{-1} ve 200 ºC'den 218 ºC' ye kadar 4 ºC dk^{-1} olarak ayarlandı. 218 ºC'de 4 dakika tutulacak ve toplam süre 28 dakika olarak belirlendi. Taşıyıcı gaz olarak helyum gazı kullanıldı. Analiz sırasında örneklere ait yağ asidi metil esterlerinin analizinden önce, standart yağ asidi metil esterlerine ait karışımlar enjekte edilerek, her bir yağ asidinin alıkonma süreleri belirlendi. Bu işlemden sonra gerekli programlamalar yapılarak örneklere ait yağ asidi metil esterlerinin analizleri yapıldı (Tvrzicka ve ark., 2002).

A, D, E ve K Vitamin ve Ergosterol Miktarlarının HPLC Cihazı ile Analizi

A, D, E ve K vitaminleri ve steroller için alınan örneklerin üzerine % 5'lik metanolik potasyum hidroksit (KOH) çözeltisi ilave edildi. Örnekler vortekslendikten sonra 85 ° C'de 30 dk etüvde bekletildi. Sabunlaşmayan lipofilik kısma 5 mL HİP karışımı ilave edildi ve 24 saat oda sıcaklığında bekletildi. Süre sonunda üst faz alınıp 37 °C'de azot ortamında buharlaşmaya bırakıldı. Kalan kısım 1 mL (% 60+% 40,v/v) asetonitril/ metanol karışımında çözülerek otosampler viallerine alındı. Analiz, Shimadzu marka HPLC cihazı ile yapıldı. (Sánchez-Machado vd., 2002; Lopez-Hernandez vd., 2006).

İstatiksel Analizler

İstatistik analizleri için, SPSS 15.0 (SPSS Inc., Chicago, IL, USA) paket programı kullanıldı. Kontrol grubu ile deneysel gruplar arasındaki karşılaştırmada ANOVA (tek yönlü varyans analizi; one-way ANOVA) testi ve grupların kendi aralarındaki karşılaştırılmasında ise LSD testi

kullanıldı. Sonuçlar mean ± SEM olarak verildi. İstatistiksel anlamlılık düzeyi için p değerleri dikkate alınarak $p<0.05$ olarak kabul edildi.

BULGULAR ve TARTIŞMA

Bu başlık altında, yalnızca araştırmadan elde edilen bulgular sunularak, konuyla ilgili daha önceden gerçekleştirilmiş benzer ve dolaylı çalışmalarla atıf yapmak kaydıyla bulgular karşılaştırılır. Benzer ve farklı yanlar vurgulanır ve yayına sunulan çalışmada diğer çalışmalara göre neden farklı bir bulgu elde edildiği tartışılır. Sonrada bu tartışma üzerinden araştırmada elde edilen bulgular istikametinde alanın uzmanı olarak yorum yapılır. Bu bölümde, deneysel sonuçların net bir sunumu yapılmalıdır (Çizelge 1).

Çizelge 1. T4 grubu fosfazenlerin GST aktivitesive total protein değerlerinin kontrol grubuna göre değişimi
Table 1. Changes in GST activity and total protein values of T4 group phosphasens compared to the control group

	Kontrol	FOS	T4A	T4B	T4C	H_2O_2
Total Protein (mg/g pellet)	95.46±0.57	128.46±3.40^{d}	110.94±1.03^{d}	94.81±0.98^{a}	109.07±2.04^{d}	105.51±1.72^{c}
GST (U/mg)	0.26±0.00	0.96±0.00^{d}	0.56±0.00^{d}	0.43±0.00^{d}	0.47±0.00^{d}	0,46±0.01^{d}

a: p>0.05 Gruplar arasındaki farklılıklar istatistiki açıdan önemli değil
b: p<0.05 Gruplar arasındaki farklılıklar istatistiki açıdan kısmen önemli
c: p<0.01 Gruplar arasındaki farklılıklar istatistiki açıdan önem derecesi yüksek
d: p<0.001 Gruplar arasındaki farklılıklar istatistiki açıdan belirgin düzeyde önemli

Fosfazen Gruplarının *Saccharomyces cerevisiae*'da Yağ Asidi Profili Üzerindeki Etkisi

T3 grubu fosfazenlerin yağ asidi değerleri kontrol grubu karşılaştırıldığı zaman, kaprilik ve laurik asitlerin kontrol grubuna göre fosfazen ilave edilen gruplarda azaldığı belirlendi ($p<0.05$, $p<0.01$, $p<0.001$). 14:0 miktarının, fosfazen T3A grubu ile H_2O_2 gruplarında yüksek düzeyde olduğu saptandı ($p<0.05$, $P<0.001$). 16:0 düzeyinin; T3, T3A ve T3B gruplarında yüksek düzeyde olduğu halde H_2O_2 ile T3C grubunda farklılık olmadığı belirlendi ($p<0.05$, $p<0.001$). Palmitoleik asit miktarının ise T3A, T3B ve T3C gruplarında azaldığı tespit edildi ($p<0.01$, $p<0.001$). 18:0 miktarı, T3, T3B ve T3C gruplarında, 18:1 n-9 miktarının ise T3, T3A ve T3B gruplarında yüksek olduğu saptandı ($p<0.05$, $p<0.001$) (Çizelge 2).

Çizelge 2. T3 grubu fosfazenlerin yağ asitleri değerlerinin kontrol grubuna göre değişimi (%)
Table 2. *Change of fatty acid result of T3 group phosphazene molecules compared to the control group (%)*

Yağ asitleri	Kontrol	T3	T3A	T3B	T3C	H_2O_2
8:0	4.25±0.53	2.27±0.04^{d}	4.65±0.16^{d}	3.02±2.71^{d}	3.10±3.52^{b}	4.45±0.24^{a}
12:0	3.34±0.12	2.34±1.37^{b}	2.73±0.10^{c}	2.40±0.31^{b}	1.34±0.06^{d}	3.26±0.17^{a}
14:0	4.51±0.15	4.38±0.08^{a}	7.08±0.19^{d}	4.55±0.04^{a}	4.42±0.15^{a}	5.22±0.15^{b}
16:0	42.52±0.60	43.31±0.51^{b}	45.25±0.24^{d}	47.83±0.41^{d}	42.45±0.39^{a}	41.80±0.47^{a}
16:1, n-7	12.46±0.32	11.13±0.13^{a}	8.62±0.25^{c}	9.79±0.11^{c}	7.07±0.45^{d}	12.29±0.28^{a}
18:0	14.64±0.13	16.19±0.87^{b}	15.30±0.11^{a}	27.15±0.67^{d}	22.46±0.1^{d}	14.01±0.46^{a}
18:1, n-9	17.22±0.26	19.73±0.24^{b}	14.97±0.14^{b}	14.83±0.30^{b}	18.07±0,19^{a}	17.71±0.08^{a}
18:2, n-6	0.39±0,14	0.32±0.21^{a}	0.47±0.02^{a}	0.58±0.06^{b}	0.35±0.03^{a}	0.54±0.12^{b}
18:3, n-3	0.67±0.14	0.60±0.14^{a}	0.56±0.14 ^{a}	0.44±0.14^{b}	0.74±0.14 ^{a}	0.86±0.14 ^{b}
∑ Doymuş	69.29	68.69	75.01	84.95	85.03	68.74
∑Doymamış	30.74	31.31	28.99	15.05	14.97	31.26

a: p>0.05 Gruplar arasındaki farklılıklar istatistiki açıdan önemli değil
b: p<0.05 Gruplar arasındaki farklılıklar istatistiki açıdan kısmen önemli
c: p<0.01 Gruplar arasındaki farklılıklar istatistiki açıdan önem derecesi yüksek
d: p<0.001 Gruplar arasındaki farklılıklar istatistiki açıdan belirgin düzeyde önemli

T4 grubu fosfazenlerin yağ asidi değerleri kontrol grubu karşılaştırıldığı zaman, kaprilik (8:0) ve laurik (12:0) asitlerin kontrol grubuna göre fosfazen ilave edilen gruplarda azaldığı belirlendi ($p<0.05$, $p<0.01$, $p<0.001$). Kaprilik asitin (8:0) T4A grubunda kontrol grubuna göre kısmen yüksek olduğu saptandı ($p<0.05$). 14:0 (miristik asit) miktarının, fosfazen grupları ile H_2O_2 grubunda değişik oranlarda azaldığı saptandı ($p<0.01$, $p<0.001$). 16:0 (palmitik asit) düzeyinin; T4A, T4C ve H_2O_2 gruplarında azaldığı tespit edildi ($p<0.001$). Palmitoleik asit miktarının ise T4A, T4C ve H_2O_2 gruplarında yüksek olduğu tespit edildi ($p<0.05$, $p<0.01$). 18:0 (stearik asit) ve 18:1 n-9 (oleik asit) düzeylerinin, kontrol grubu dışında H_2O_2 ile fosfazen gruplarında değişik oranlarda yüksek olduğu belirlendi ($p<0.05$, $p<0.001$) (Çizelge 3).

T3 gurubu fosfazen moleküllerin ortak bir şekilde palmitoleik ve oleik asitlerde azalmaya neden olmaları; hücredeki Delta 9 Desatüraz enzim aktivitesi üzerinde engelleyici bir etkiye sahip olmalarından kaynaklanabileceği sonucunu düşündürmektedir. Çünkü bu yağ asitlerin

sentezinde Delta 9 enzimi kullanılmaktadır. Bazı fosfazenler lipit seviyesini azaltırken bazıları arttırmaktadır. Bu azalış ve artış uygulanan konsantrasyon seviyesinden etkilenmektedir. Bazıları belirli konsantrasyonda maya hücresindeki yapıları etkilerken bazıları ise her konsantrasyonda etkilemektedir (Özşahin & Bozhan 2018). Duan ve ark. (2015)' nin sonuçları ışığında; hücresel lipid içeriğinde, farklı maddelerin eklenmesi doymamış yağ asitlerinde artışa neden olduğu gibi bazı şartlarda da azalışa neden olmaktadır. *S. cerevisiae*'nin anaerobik koşullar altında büyümesi için doymamış yağ asitleri (UFA'lar) gereklidir. Bunlar sadece membran bütünlüğünü ve fonksiyonunu korumak için değil, aynı zamanda yüksek şeker ve etanol toksisitesi gibi fermentasyon streslerine uyum sağlamak için de gereklidir. *S. cerevisiae* uzun zincirli yağ asiterini besi ortamından alabilir. Çünkü bu yağ asitlerini sentezleyen yağ asidi sentez enzimleri maya hücresinde bulunmamaktadır.

Çizelge 3. T4 grubu fosfazenlerin yağ asitleri değerlerinin kontrol grubuna göre değişimi (%)
Table 3. Change of fatty acid result of T4 group phosphazene molecules compared to the control group (%)

Yağ asitleri	Kontrol	FOS	T4A	T4B	T4C	H_2O_2
8:0	2.01±0.31	1.88±0.27[a]	1.51±0.15 [b]	1.36±0.16 [b]	1.32±0.40[b]	0.92±0.02 [c]
12:0	4.28±0.17	2.16±0.28 [d]	3.64±0.12 [b]	4.03±0.19 [a]	5.67±0.15[b]	4.09±0.31 [a]
14:0	6.46±0.11	4.05±0.17 [c]	3.66±0.13 [d]	4.25±0.06 [c]	4.22±0.12[c]	2.51±0.14 [d]
16:0	44.88±1.05	44.28±0.61	39.87±0.88 [d]	44.21±1.05	38.22±0.55[d]	37.53±0.81[d]
16:1. n-7	8.35±0.43	8.70±0.10 [a]	9.29±0.28 [b]	8.37±0.11 [a]	9.22±0.28 [b]	10.83±0.22[c]
18:0	20.29±0.43	23.20±0.19 [b]	22.42±0.39 [b]	22.11±0.53 [b]	22.49±0.40[b]	22.77±0.29[b]
18:1. n-9	13.03±009	14.81±0.13 [b]	18.96±0.29 [d]	14.77±0.34 [b]	18.06±0.21[d]	20.60±0.18[d]
18:2. n-6	0.43±0.02	0.53±0.02	0.52±0.02	0.63±0.02	0.48±0.02	0.61±0.02[b]
18:3. n-3	0.27±0.02	0.33±0.02	0.37±0.02	0.27±0.02	0.32±0.02	0.34±0.02
∑Doymuş	77.92	75.57	71.1	75.96	71.92	67.82
∑Doymamış	22.08	24.43	28.9	24.04	28.08	32.18

a: p>0.05 Gruplar arasındaki farklılıklar istatistiki açıdan önemli değil
b: p<0.05 Gruplar arasındaki farklılıklar istatistiki açıdan kısmen önemli
c: p<0.01 Gruplar arasındaki farklılıklar istatistiki açıdan önem derecesi yüksek
d: p<0.001 Gruplar arasındaki farklılıklar istatistiki açıdan belirgin düzeyde önemli

Fosfazen Gruplarının *Saccharomyces cerevisiae*'da Lipofilik Moleküller Üzerine Etkisi

T3 grubu fosfazenlerin lipofilik moleküller üzerine etkisi kontrol grubu ile karşılaştırıldığında; ergosterol düzeyinin T3,T3B ile H_2O_2 gruplarında yüksek olduğu halde (p<0.05,p<0.001); diğer gruplarda farklılık göstermediği gözlemlendi. Stigmasterol ve betasitosterol miktarlarının ise kontrol grubuna göre bütün gruplarda azaldığı saptandı (p<0.05, p<0.01, p<0.001). Alfa tokoferol düzeyinin T3 ve T3A gruplarında yüksek olduğu (p<0.001), T3B ile T3C gruplarında ise azaldığı belirlendi (p<0.05) (Çizelge 4).

Çizelge 4. T3 grubu fosfazenlerin lipofilik moleküller üzerindeki etkisinin değişimi (µg /g)
Table 4. Variation of the effect T3 group phosphazenes on lipophilic molecules (µg /g)

Vitaminler	Kontrol	T3	T3A	T3B	T3C	H_2O_2
R Tok	0.04±0.00	0.08±0.00[a]	0.14±0.01[d]	0.06±0.00[a]	0.04±0.00[a]	0.04±0.00[a]
D2	0.16±0.00	0.31±0.00[a]	1.25±0.12[d]	0.74±0.11[d]	0.12±0.00[a]	0.19±0.00[a]
A Tok	9.80±0.27	12.00±0.40[d]	15.29±0.31[d]	7.47±0.20 [b]	7.58±0.54 [b]	9.03±0.14[a]
Ergosterol	310.84±0.68	608.87±4.25[d]	323.00±2.17 [a]	366.75±3.10 [b]	312.58±0.66[a]	429.17±19.61[d]
K1	5.37±0.78	9.98±0.61[d]	7.66±0.49[b]	2.50±0.18[b]	8.01±0.29[c]	6.33±0.54[a]
Stigmasterol (mg /g)	1.39 ±96.28	1.23±16.36[b]	1.16±14.28[c]	0.70±17.99[d]	0.93±12.47[d]	0.67±14.05[d]
Betasitosterol	5.04±0.21	2.77±0.30[d]	2.09±0.02[d]	1.50±0.16[d]	2.40±0.07[d]	1.23±0.02[d]

a: p>0.05 Gruplar arasındaki farklılıklar istatistiki açıdan önemli değil
b: p<0.05 Gruplar arasındaki farklılıklar istatistiki açıdan kısmen önemli
c: p<0.01 Gruplar arasındaki farklılıklar istatistiki açıdan önem derecesi yüksek
d: p<0.001 Gruplar arasındaki farklılıklar istatistiki açıdan belirgin düzeyde önemli

Lipofilik moleküller üzerine T4 grubu fosfazenlerin etkileri kontrol grubu kıyaslandığında; ergosterol düzeyinin fosfazen grupları ile H_2O_2 grubunda belirgin azaldığı saptandı (p<0.001). Stigmasterol miktarının kontrol grubuna göre FOS, T4A ve T4B gruplarında; betasitosterolün ise H_2O_2 ile fosfazen gruplarda azaldığı gözlemlendi (p<0.001). Alfa tokoferol seviyesinin kontrol grubu dışındaki

gruplarda yüksek olduğu belirlendi (p<0.001) (Çizelge 5).

Ergosterol bilindiği gibi başta *S. cerevisiae* olmak üzere maya hücrelerinin membran yapısının en önemli moleküler bileşenleridir. Hücredeki anormal durumlara karşı ergosterol miktarında artış olduğu ileri sürülmüştür. Mayaların; biyoetanol fermantasyonları sırasında farklı fiziksel, biyolojik veya kimyasal streslerin bir kombinasyonu ile karşı karşıya kaldıkları belirtilmiştir. Fermantasyonun başlangıcında; maya yüksek şeker seviyeleri nedeniyle ozmotik strese maruz kalır. Yapılan çalışmalarda; maya hücresinin yüksek konsantrasyonlarda kompleks moleküller içeren bir ortama bırakıldığında da ozmotik şokla karşılaşacağı ileri sürülmüştür (Spencer ve ark., 2014). Fermentasyon sırasında etanol birikimi, pH'nın azalması, anaerobik büyümeye geçiş ve besin sınırlaması gibi diğer stres koşullarının da önem kazandığı ve fermantasyon sırasında, etanol birikiminin maya büyümesini ve canlılığını engellediği vurgulanmıştır (Spencer ve ark., 2014). Fermentasyon ortamında oksijen bulunmasının önemli bir kriter olduğu ve doymamış yağ asidi sentezinde görev alan enzimler ile sterollerin sentezinin oksijenli ortamda gerçekleştiği belirtilmiştir (Spencer ve ark., 2014).

Çizelge 5. T4 grubu fosfazenlerin lipofilik moleküller üzerindeki etkisinin değişimi (µg /g)
Table 5. Variation of the effect T4 group phosphazenes on lipophilic molecules (µg /g)

Lipofilik moleküller	Kontrol	FOS	T4A	T4B	T4C	H_2O_2
R Tok	0.56±0.08	0.45±0.00^{a}	0.14±0.08^{d}	0.55±0.01^{a}	0.21±0.00^{d}	0.50±0.00^{a}
D2	0.25±0.01	0.28±0.00^{a}	0.31±0.00^{a}	0.12±0.00^{b}	0.32±0.01^{a}	0.43±0.11^{b}
A Tok	0.10±0.01	0.17±0.01 ^{b}	0.26±0.00 ^{d}	0.13±0.00 ^{d}	0.11±0.00 ^{d}	0.36±0.01 ^{d}
Ergosterol	445.82±14.27	119.36±3.19 ^{d}	76.72±11.34 ^{d}	123.69±2.48 ^{d}	151.37±3.64 ^{d}	269.60±3.09^{d}
K1	1.61±0.14	0.57±0.02^{d}	1.30±0.08^{a}	1.31±0.14^{a}	1.57±0.16^{a}	1.43±0.06^{a}
Stigmasterol(mg /g)	2.63±0.08	1.57±0.06 ^{d}	1.81±0.04 ^{d}	1.81±0.09 ^{d}	2.42±0.04 ^{a}	2.48±0.07^{a}
Betasterol	26.71±0.77	10.74±5.16^{d}	8.85±2.05^{d}	14.40±0.92^{d}	7.44±0.22^{d}	14.58±0.18^{d}

a: p>0.05 Gruplar arasındaki farklılıklar istatistiki açıdan önemli değil
b: p<0.05 Gruplar arasındaki farklılıklar istatistiki açıdan kısmen önemli
c: p<0.01 Gruplar arasındaki farklılıklar istatistiki açıdan önem derecesi yüksek
d: p<0.001 Gruplar arasındaki farklılıklar istatistiki açıdan belirgin düzeyde önemli

Yapılan bazı çalışmalarda fosfazenlerin hücre yoğunluklarını azalttığı tespit edilmiştir. Bununla beraber bakteri ve maya suşlarında yapılan fosfazen ilavelerinin E vitamin gibi antioksidanların miktarında artışa neden olduğu gözlenmiştir. Ayrıca herhangi bir stres karşısında ya da H_2O_2 gibi maddelere maruz kalma durumunda membran yapısında bulunan bazı doymamış yağ asitleri ile ergosterol sentezi düzenlenerek sıvı mozaik yapı yani mebranın temel yapısı korunduğu ileri sürülmüştür.

SONUÇ ve ÖNERİLER

Çalışma sonuçlarımız biyokimyasal ve moleküler çalışmalarda en önemli model olarak kullanılan *S. cerevisiae* kültür ortamına biyolojik etkinliği yüksek olan fosfazen moleküllerin ilave edilmesi sonucu yağ asidi ve ergosterol düzeylerinde önemli varyasyonlara neden olduğunu göstermektedir. H_2O_2 ve fosfazenler ilave edildiğinde; yağ asidi sentezi, yağ asidi zincir uzaması ve yağ asitlerinin hidrokarbon zincirine çift bağ girişi yapan enzimlerin son ürünlerinde artışlara ya da azalışlara neden olmuştur.

Yapılan uygulamalar ile; H_2O_2 ilavesinin hücrelerin gen düzeylerinde bazı genleri aktive ederek biyokimyasal olaylar üzerinde etkili olduğu belirlenmiştir. Bu sonuçların hem pozitif yönde hem de negatif yönde yürüdüğü belirlenmiştir.

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Macromycetes Determined in Silifke (Mersin) District

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ABSTRACT

This study was carried out on the macrofungi samples collected from Silifke district of Mersin province. As a result, 122 macromycete species belonging to 91 genera, 48 families, 18 orders, and 7 classes, within *Ascomycota* and *Basidiomycota* were determined. Including the previously reported 11 species, a total of 133 macromycete taxa were compiled from the region. The list of the taxa was provided together with their habitats, collection dates, voucher numbers, and the citations for those reported before.

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ÖZET

Bu çalışma Mersin ilinin Silifke ilçesinden toplanan makromantar örnekleri üzerinde gerçekleştirilmiştir. Sonuç olarak, *Ascomycota* ve *Basidiomycota* bölümleri içinde yer alan 7 sınıf, 18 takım, 48 familya ve 91 cinse ait 122 makromantar türü tespit edilmiştir. Önceden rapor edilmiş 11 tür de dahil edilerek bölgeden toplamda 133 makromantar taksonu derlenmiştir. Taksonların listesi, türlerin habitatları, toplanma tarihleri, toplayıcı numaraları ve önceden rapor edilenlerin atıflarıyla birlikte verilmiştir.

Botanik

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INTRODUCTION

Silifke is a district of Mersin province within Mediterranean Region of Türkiye (Fig. 1). It is situated between 36°05′-37°00′ northern latitudes and 33°32′-34°08′ eastern longitudes, and surrounded by Erdemli to the east, Mut to the north, Gülnar and Aydıncık to the west, and Mediterranean Sea to the south. The area falls in Mediterranean floristic region within Holarctic flora kingdom (Davis, 1965) with a mean annual temperature of 19.5°C and 584 kg/m² rainfall.

The altitude of the research area starts from sea level and goes up to 2050 m. The plant cover shows variations depending on altitude and the topography. Marshy and halophytic formations such as *Eucalyptus* L'Hér., *Juncus* L., *Limonium* Phil., *Phragmites* Adans., *Tamarix* L., *Tanecetum* L., *Chenopodium* L. are abundant at coastal dunes. Then immediately macqi formation starts generally with *Olea* L., *Arbutus* L., *Ceratonia* L., *Quercus* L., *Laurus* L., *Cistus* L., *Phillyrea* L., *Arbutus* L., *Erica* L. and *Pistacia* L. Naturally growing or planted *Pinus brutia* L. is the dominant tree vegetation especially at Göksu river basin. *Pinus nigra* Ten., *Abies cilicica* subsp. *isaurica* (Coode & Cullen) Silba, and *Juniperus excelsa* M.Bieb. populations are also visible at higher elevations. *Populus* L, *Salix* L. and *Platanus* L., species are abundant along stream and river sides.

Işıloğlu & Watling (1992) and Işıloğlu & Öder (1995) reported some macromycete taxa from Silifke district in their works both concerning with the Mediterranean region of Turkey. Some similar studies had also been carried out in neighboring regions (Öztürk et al., 2003; Doğan et al., 2007), but

the current checklist (Sesli et al., 2020) and the latest studies (Acar et al., 2021; Çetinkaya & Uzun, 2021; Doğan et al., 2021; Keleş & Kaya, 2021; Kesici & Uzun, 2021; Şelem et al., 2021) indicated that there isn't a specific work on the macrofungal biodiversity of Silifke district. The study aims to contribute to the mycobiota of Turkey by determining the macrofungal biodiversity Silifke district.

MATERIALS and METHOD

Field surveys were carried out within the boundaries of Silifke district between 2017-2019. Morphological features such as size, color, shape and texture of the sporocarp were recorded as these features might change with drying.

During the field study, information about the ecology and geographic position of the samples was collected and they were photographed at their natural habitats. GPS data for the localities of fungi were obtained using a Magellan SporTrack Pro GPS Receiver (Table 1), and color photographs were taken by a Sony HX-400V digital camera. Microscopic investigations were carried out on dried samples. Microscopic measurements were made from preparations mounted in water or 3% KOH. A Nikon Eclipse Ci-S trinocular light microscope was used for microscopic investigations. Identification of the collected materials was confirmed using the works of Caillet & Moyne (1980), Breintenbach & Kränzlin (1984-2000), Benkert (1995, 2007), Miller & Miller (1988), Candusso & Lanzoni (1990), Wang & Kimbrough (1992), Courtecuisse & Duhem (1995), Pegler et al. (1995), Bessette et al. (1997, 2007), Cappelli (1997), Ellis & Ellis (1997), Hansen & Knudsen (1992, 1997, 2000), Heilmann-Clausen et al. (1998), Montecchi & Sarasini (2000), Arroyo et al. (2005), Kränzlin (2005), Medardi (2006), Trappe et al. (2007), Hausknecht (2009), Jaklitsch (2009), Antonín & Noordeloos (2010), Philips (2010), Thompson (2013), Beug et al. (2014), Cripps et al. (2016) and Siegel & Schwarz (2016).

Names of fungi and author's abbreviations follow indexfungorum.org (accessed on 20 December 2021). Voucher specimens are deposited in Karamanoğlu Mehmetbey University, Science Faculty, Department of Biology, Karaman.

RESULTS

The determined taxa are listed in alphabetical order together with their habitats, localities, collection dates, and accession numbers. For the previously reported taxa, only the citations of the presenting paper were provided.

Ascomycota Whittaker

Leotiomcycetes O.E. Erikss. & Winka

Helotiales Nannf. ex Korf & Lizoň

Gelatinodiscaceae S.E. Carp.

1. ***Ascocoryne sarcoides*** (Jacq.) J.W. Groves & D.E. Wilson: On *Populus* sp. stump, locality 33, 13.11.2019, DerKap-357; locality 34, 13.11.2019, DerKap-370.

Rhytismatales M.E. Barr ex Minter

Calloriaceae L. Marchand

2. ***Stamnaria americana*** Massee & Morgan: (Kaplan et al., 2021).

Orbiliomycetes O.E. Erikss. & Baral

Orbiliales Baral, O.E. Erikss., G. Marson & E. Weber

Orbiliaceae Nannf.

3. ***Orbilia sarraziniana*** Boud.: On dead *Populus* sp. twigs, locality 34, 13.11.2019, DerKap-371.

Pezizomycetes O.E. Erikss. & Winka

Pezizales J. Schröt.

Ascobolaceae Boud. ex Sacc.

4. ***Ascobolus behnitziensis*** Kirschst.: On sandy soil under *Salix* sp., locality 29, 25.09.2019, DerKap-227.

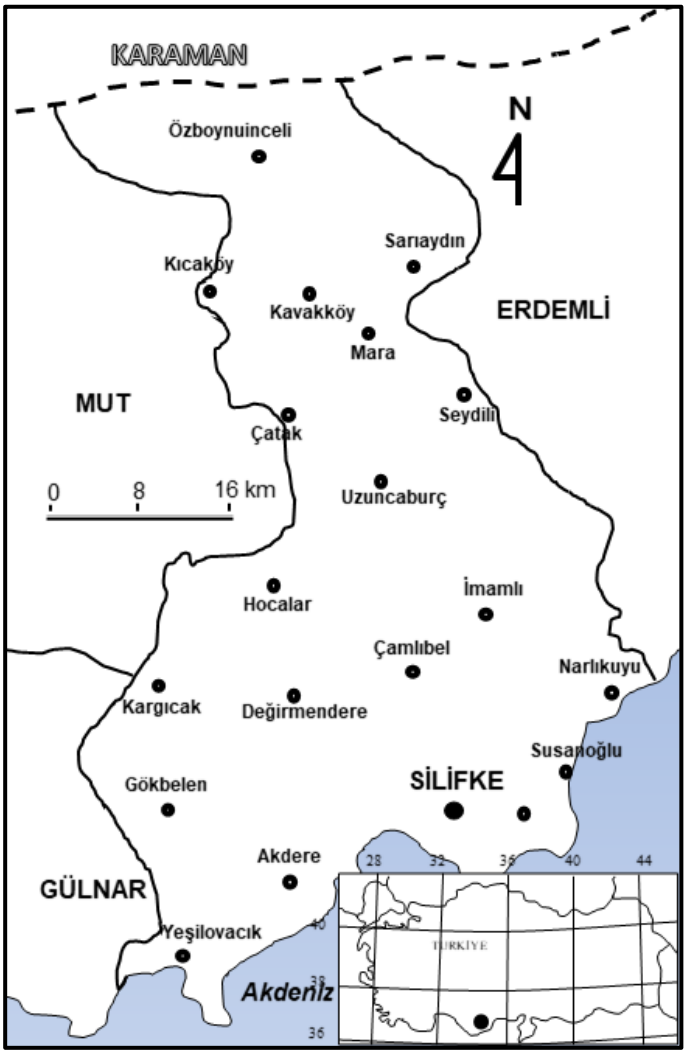

Figure 1. Map of the research area
Şekil 1. Araştırma alanının haritası

Table 1. Collection localities of the macrofungal samples
Çizelge 1. Makromantar örneklerinin toplanma lokaliteleri

Loc. No	Locality name	Coordinates	Altitude (m)
1	Altınkum village	36°19′N-34°04′E	1
2	Altınkum village	36°20′N-34°03′E	5
3	Altınkum village	36°21′N-34°03′E	5
4	Atayurt village	36°23′N-34°02′E	20
5	Bahçeköy village	36°31′N-34°00′E	10
6	Boğsak village	36°15′N-33°47′E	230
7	Boğsak village	36°15′N-33°48′E	85
8	Bozağaç village	36°36′N-33°52′E	1400
9	Cambazlı village	36°31N′-33°59′E	860
10	City Centre	36°22′N-33°55′E	20
11	Çadırlı village	36°21′N-33°45′E	790
12	Çalıbozkır village	36°30′N-33°52′E	1320
13	Çamlıca village	36°20′N-33°41′E	1100
14	Değirmendere village	36°23′N-33°48′E	185
15	Değirmendere village	36°23′N-33°49′E	76
16	Değirmendere village	36°24′N-33°48′E	40
17	Değirmendere village	36°26′N-33°46′E	50
18	Evkafçiftliği village	36°27′N-33°37′E	90
19	Evkafçiftliği village	36°27′N-33°38′E	260
20	Evkafçiftliği village	36°28′N-33°38′E	355
21	Gazi Çiftliği village	36°22′N-34°02′E	5
22	Gazi Çiftliği village	36°22′N-34°04′E	1
23	Hotamış village	36°41′N-33°57′E	1395
24	Kapızlı village	36°22′N-34°04′E	5
25	Kargıcak village	36°25′N-33°38′E	90
26	Kargıcak village	36°25′N-33°39′E	230
27	Kargıcak village	36°26′N-33°38′E	110
28	Kavak village	36°42′N-33°48′E	1340
29	Kavak village	36°43′N-33°48′E	1365
30	Kayabaşı village	36°20′N-33°45′E	725
31	Kayabaşı village	36°22′N-33°50′E	320
32	Keşli Türkmenli village	36°30′N-33°57′E	845
33	Kıca village	36°42′N-33°44′E	1270
34	Kıca village	36°43′N-33°46′E	1300
35	Kırobası village	36°44′N-33°55′E	1450
36	Kocaoluk village	36°43′N-33°55′E,	1300
37	Kocaoluk village	36°43′N-33°56′E	1430
38	Kocaoluk village	36°44′N-33°56′E	1320
39	Meydan village	36°31N′-33°57′E	915
40	Nuru village	36°23′N-33°34′E	850
41	Nuru village	36°24′N-33°34′E	830
42	Pelitpınarı village	36°21′N-33°35′E	990
43	Pelitpınarı village	36°22′N-33°35′E	925
44	Sarıaydın village	36°45′N-33°55′E	1320
45	Sökün village	36°19′N-34°01′E	1
46	Susanoğlu village	36°23′N-34°04′E	5
47	Uzuncaburç village	36°32′N-33°56′E	1010
48	Uzuncaburç village	36°33′N-33°55′E	1100

5. ***Ascobolus furfuraceus*** Pers.: On decaying cow dung, locality 2, 12.11.2018, DerKap-072.

Helvellaceae Fr.

6. ***Dissingia leucomelaena*** (Pers.) K. Hansen & X.H. Wang: Among needle litter, locality 26, 11.03.2019, DerKap-164; locality 14, 24.03.2019, DerKap-173; on soil among grass, locality 32, 24.03.2019, DerKap-178.

7. ***Helvella acetabulum*** (L.) Quél.: Among leaf litter under *Quercus* sp., locality 42, 19.03.2019, DerKap-169.

Incertae Sedis

8. ***Psilopezia nummularia*** Berk.: On sandy soil under *Populus* sp., locality 44, 21.09.2019, DerKap-202; 30.09.2019, DerKap-281; locality 36, 30.10.2019, DerKap-290.

Morchellaceae Rchb.

9. ***Morchella deliciosa*** Fr.: Among leaf litter under *Quercus* sp., locality 13, 22.04.2018, DerKap-027.

10. ***Morchella elata*** Fr.: Among needle litter under *Pinus* sp., locality 47, 07.03.2018, DerKap-024.

11. ***Morchella tridentina*** Bres.: Among leaf litter under *Quercus* sp., locality 47, 20.03.2019, DerKap-170.

Pezizaceae Dumort.

12. ***Phylloscypha boltonii*** (Quél.) Van Vooren & Hairaud: On sand and sandy soil along streamside, locality 25, 29.10.2019, DerKap-252; locality 25, 09.11.2019, DerKap-294.

13. ***Sarcosphaera coronaria*** (Jacq.) J. Schröt.: Among needle litter under *Pinus* sp., locality 14, 11.03.2019, DerKap-167.

Pyronemataceae Corda

14. ***Anthracobia melaloma*** (Alb. & Schwein.) Arnould: On ash and fired roots of *Juncus acutus* L. locality 3, 27.01.2018, DerKap-012; locality 3, 06.01.2019, DerKap-129.

15. ***Cheilymenia theleboloides*** (Alb. & Schwein.) Boud.: On cow dung, locality 25, 09.11.2019, DerKap-295.

16. ***Geopora arenosa*** (Fuckel) S. Ahmad: On sandy soil among moss, locality 34, 13.11.2019, DerKap-379.

17. ***Geopora sumneriana*** (Cooke ex W. Phillips) M. Torre: Among needle litter under *Pinus* sp., locality 40, 22.04.2018, DerKap-028.

18. ***Octospora gemmicola*** Benkert: On *Bryum* sp., locality 12, 21.09.2019, DerKap-204.

19. ***Octospora musci-muralis*** Graddon: On moss along riverside, locality 38, 30.10.2019, DerKap-265.

20. ***Pyronema domesticum*** (Sowerby) Sacc.: On ash under *Salix* sp., locality 44, 21.09.2019, DaerKap-195.

21. ***Pyronema omphalodes*** (Bull.) Fuckel: On ash, locality 4, 25.09.2019, DerKap-235.

22. ***Scutellinia scutellata*** (L.) Lambotte: On sandy soil among moss, locality 44, 21.09.2019, DerKap-198; locality 37, 07.10.2018, DerKap-053.

23. ***Smardaea planchonis*** (Dunal ex Boud.) Korf & W.Y. Zhuang: On sandy soil under *Populus* sp., locality 27, 09.11.2019, DerKap-298.

24. ***Tarzetta cupularis*** (L.) Svrček: On soil among leaf & needle litter under mixed forest, locality 16, 24.03.2019, DerKap-171.

25. ***Tricharina ochroleuca*** (Sacc.) Eckblad: On sandy soil under *Salix* sp., locality 44, 25.09.2019, DerKap-229.

26. ***Tricharina praecox*** (P. Karst.) Dennis: On sandy soil under *Populus* sp., locality 27, 09.11.2019, DerKap-299.

27. ***Trichophaeopsis bicuspis*** (Boud.) Korf & Erb: On sandy soil under *Populus* sp., locality 25, 25.09.2019, DerKap-218; locality 29, 25.09.2019, DerKap-228.

Sarcoscyphaceae Le Gal ex Eckblad

28. ***Pithya cupressina*** (Batsch) Fuckel: On dead *Cupressus* sp. leaves, locality 35, 09.11.2019, DerKap-320; locality 37, 13.11.2019, DerKap-367.

Tuberaceae Dumort.

29. ***Tuber nitidum*** Vittad.: In soil under *Pinus* sp., locality 14, 24.03.2019, DerKap-175.

Sordariomycetes O.E. Eriksss. & Winka

Diaporthales Nannf.

Valsaceae Tul. & C. Tul.

30. ***Valsa sordida*** Nitschke: On *Populus* sp. stump, locality 28, 09.11.2019, DerKap-313; locality 33, 13.11.2019, DerKap-362.

Hypocreales Lindau

Nectriaceae Tul. & C. Tul.

31. ***Nectria peziza*** (Tode) Fr.: On decaying *Salix* sp. stump, locality 44, 30.10.2019, DerKap-279; locality 34, 13.11.2019, DerKap-377.

Basidiomycota R.T. Moore

Agaricomycetes Doweld

Agaricales Underw.

Agaricaceae Chevall.

32. ***Agaricus bisporus*** (J.E. Lange) Imbach: On soil in *Juniperus* sp. forest, locality 37, 13.11.2019, DerKap-365.

33. ***Agaricus bitorquis*** (Quél.) Sacc.: On sandy soil among grass, locality 1, 17.12.2018, DerKap-110.

34. ***Agaricus campestris*** L.: On soil among mosses, locality 35, 09.11.2019, DerKap-321.

35. ***Agaricus sylvicola*** (Vittad.) Peck: On soil among needle litter under *Pinus* sp., locality 14, 23.12.2018, DerKap-117.

36. ***Apioperdon pyriforme*** (Schaeff.) Vizzini: On soil

among grass, locality 32, 24.03.2019, DerKap-176; on soil under *Juniperus* sp., locality 23, 09.11.2019, DerKap-353.

37. ***Coprinus comatus*** (O.F. Müll.) Pers.: On soil among grass, locality 41, 21.09.2019, DerKap-184; locality 28, 09.11.2019, DerKap-311; locality 27, 27.11.2019, DerKap-390.

38. ***Crucibulum laeve*** (Huds.) Kambly: On decaying woody remains in *Juniperus* sp. forest, locality 23, 09.11.2019, DerKap-348.

39. ***Cyathus olla*** (Batsch) Pers.: On decaying woody remains in *Juniperus* sp. forest, locality 23, 09.11.2019, DerKap-355.

40. ***Cystodermella cinnabarina*** (Alb. & Schwein.) Harmaja: On soil among needle litter under *Pinus* sp., locality 32, 07.01.2019, DerKap-132.

41. ***Lepiota cristata*** (Bolton) P. Kumm.: On soil in *Juniperus* sp. forest, locality 23, 09.11.2019, DerKap-339.

42. ***Lepiota helveola*** Bres.: (Işıloğlu & Öder, 1995).

43. ***Leucoagaricus leucothites*** (Vittad.) Wasser: In meadow, locality 41, 21.09.2019, DerKap-183.

44. ***Lycoperdon excipuliforme*** (Scop.) Pers.: On soil among needle litter under *Pinus* sp., locality 32, 05.11.2018, DerKap-071.

45. ***Lycoperdon molle*** Pers.: On soil among needle litter under *Pinus* sp., locality 39, 07.10.2018, DerKap-049; locality 32, 19.11.2018, DerKap-076.

46. ***Lycoperdon nigrescens*** Pers.: On soil among needle litter under *Pinus* sp., locality 11, 09.12.2018, DerKap-098.

47. ***Lycoperdon perlatum*** Pers.: On soil among needle litter under *Pinus* sp., locality 32, 05.11.2018, DerKap-062; 24.03.2019, DerKap-180; locality 23, 09.11.2019, DerKap-354.

48. ***Macrolepiota procera*** (Scop.) Singer: On soil among needle litter under *Pinus* sp., locality 32, 05.11.2018, DerKap-061.

49. ***Tulostoma brumale*** Pers.: On soil among mosses in *Pinus* sp. forest, locality 32, 29.01.2019, DerKap-149; 24.03.2019, DerKap-179; locality 35, 09.11.2019, DerKap-322.

50. ***Tulostoma fimbriatum*** Fr.: On soil at *Pinus* sp. forest clearing, locality 7, 14.01.2019, DerKap-142; locality 23, 09.11.2019, DerKap-345; on soil in *Juniperus* sp. forest, locality 35, 09.11.2019, DerKap-323; locality 37, 13.11.2019, DerKap-368.

51. ***Tulostoma squamosum*** (J.F. Gmel.) Pers.: On soil in *Juniperus* sp. forest, locality 36, 30.10.2019, DerKap-274.

Amanitaceae R. Heim ex Pouzar

52. ***Amanita ovoidea*** (Bull.) Link: On soil among needle litter in *Pinus* sp. forest, locality 19, 29.10.2019, DerKap-241.

Bolbitiaceae Singer

53. ***Conocybe apala*** (Fr.) Arnolds: On manured soil among grass, locality 10, 21.09.2019, DerKap-203.

54. ***Conocybe deliquescens*** Hauskn. & Krisai: On soil among grass, locality 9, 08.10.2018, DerKap-056.

55. ***Conocybe tenera*** (Schaeff.) Fayod: On soil among grass, locality 44, 30.10.2019, DerKap-285.

56. ***Galeropsis desertorum*** Velen. & Dvořák: On soil among mosses in *Juniperus* sp. forest, locality 35, 09.11.2019, DerKap-319.

Cyphellaceae Lotsy

57. ***Chondrostereum purpureum*** (Pers.) Pouzar: On decaying *Populus* sp. stump, locality 28, 09.11.2019, DerKap-314.

Hygrophoraceae Lotsy

58. ***Arrhenia spathulata*** (Fr.) Redhead: On mosses in *Juniperus* sp. forest, locality 36, 30.10.2019, DerKap-268; locality 35, 09.11.2019, DerKap-325.

Hymenogastraceae Vittad.

59. ***Hygrophorus chrysodon*** (Batsch) Fr.: (Işıloğlu & Watling, 1992).

60. ***Galerina pumila*** (Pers.) Singer: On soil in *Juniperus* sp. forest, locality 36, 30.10.2019, DerKap-267.

61. ***Hebeloma sinapizans*** (Paulet) Gillet: (Işıloğlu & Watling, 1992).

62. ***Psilocybe coronilla*** (Bull.) Noordel.: On soil among grass, locality 26, 02.12.2018, DerKap-081.

Inocybaceae Jülich

63. ***Crepidotus calolepis*** (Fr.) P. Karst.: On decaying *Populus* sp. stump, locality 18, 29.10.2019, DerKap-255; locality 25, 27.11.2019, DerKap-392.

64. ***Crepidotus epibryus*** (Fr.) Quél.: On decaying *Populus* sp. stump, locality 25, 27.11.2019, DerKap-396.

65. ***Inocybe lacera*** **(Fr.)** P. Kumm.: On sandy soil under *Populus* sp., locality 44, 1320m, 21.09.2019, DerKap-200; locality 25, 25.09.2019, DerKap-207; locality 34, 13.11.2019, DerKap-372.

66. ***Inosperma erubescens*** (A. Blytt) Matheny & Esteve-Rav.: (Işıloğlu & Öder, 1995).

67. ***Pseudosperma rimosum*** (Bull.) Matheny & Esteve-Rav.: On sandy soil among grass under *Salix* sp., locality 29, 25.09.2019, DerKap-222; locality 44, 25.09.2019, DerKap-234; locality 16, 09.11.2019, DerKap-302.

Marasmiaceae Roze ex Kühner

68. ***Marasmius oreades*** (Bolton) Fr.: On soil among grass, locality 26, 29.10.2019, DerKap-243.

Mycenaceae Overeem

69. ***Atheniella flavoalba*** (Fr.) Redhead, Moncalvo,

Vilgalys, Desjardin & B.A. Perry: On soil under *Juniperus* sp., locality 35, 09.11.2019, DerKap-330.

70. ***Mycena seynii*** Quél.: On decaying *Pinus* sp. cones, locality 32, 05.11.2018, DerKap-064; locality 19, 02.12.2018, DerKap-064.

Physalacriaceae Corner

71. ***Armillaria mellea*** (Vahl) P. Kumm.: Around decaying *Salix* sp. stump, locality 16, 29.10.2019, DerKap-242.

72. ***Cryptomarasmius minutus*** (Peck) T.S. Jenkinson & Desjardin: On decaying *Populus* sp. leaves, locality 16, 09.11.2019, DerKap-305.

Pleurotaceae Kühner

73. ***Hohenbuehelia petaloides*** (Bull.) Schulzer: On soil in *Pinus* sp. forest clearing, locality 31, 09.12.2018, DerKap-094

74. ***Pleurotus ostreatus*** (Jacq.) P. Kumm.: On decaying *Morus* L. sp. stump, locality 21, 05.11.2018, DerKap-069; on decaying *Ficus* L. sp. stump, locality 2, 05.11.2018, DerKap70; on decaying *Populus* sp. stump, locality 44, 25.09.2019, DerKap-231; locality 34, 13.11.2019, DerKap-382.

Psathyrellaceae Vilgalys, Moncalvo & Redhead

75. ***Candolleomyces candolleanus*** (Fr.) D. Wächt. & A. Melzer: Around decaying *Morus* sp. stump, locality 22, 03.12.2017, DerKap-002; around decaying *Populus* sp. stump, locality 29, 25.09.2019, DerKap-22; locality 27, 29.09.2019, DerKap-253.

76. ***Coprinellus disseminatus*** (Pers.) J.E. Lange: Among moss on decaying stump, locality 38, 07.10.2018, DerKap-050; locality 44, 21.09.2019, DerKap-194; locality 25, 25.09.2019, DerKap-210; on soil under *Populus* sp., locality 18, 29.10.2019, DerKap-256; locality 38, 30.10.2019, DerKap-263; locality 34, 13.11.2019, DerKap-381

77. ***Coprinellus micaceus*** (Bull.) Vilgalys, Hopple & Jacq. Johnson: On decaying *Morus* sp. stump, locality 3, 27.01.2018, DerKap-014; on decaying *Populus* sp. stump, locality 44, 21.09.2019, DerKap-189; locality 25, 25.09.2019, DerKap-209; locality 34, 13.11.2019, DerKap-364.

78. ***Coprinopsis atramentaria*** (Bull.) Redhead, Vilgalys & Moncalvo: On soil under *Populus* sp., locality 27, 29.10.2019, DerKap-254; locality 34, 13.11.2019, DerKap-374.

79. ***Coprinopsis lagopus*** (Fr.) Redhead, Vilgalys & Moncalvo: On decaying cow dung, locality 3, 03.12.2017, DerKap-004.

80. ***Coprinopsis nivea*** (Pers.) Redhead, Vilgalys & Moncalvo: On decaying cow dung, locality 3, 02.12.2017, DerKap-001.

81. ***Parasola galericuliformis*** (Losa ex Watling) Redhead, Vilgalys & Hopple: (Işıloğlu & Öder, 1995).

82. ***Parasola plicatilis*** (Curtis) Redhead, Vilgalys & Hopple: On soil among grass, locality 26, 02.12.2018, DerKap-084; on sandy soil under *Populus* sp., locality 25, 25.09.2019, DerKap-215.

83. ***Psathyrella ammophila*** (Durieu & Lév.) P.D. Orton: On sand, locality 17, 27.11.2019, DerKap-402.

84. ***Psathyrella longipes*** (Peck) A.H. Sm.: On soil under *Populus* sp., locality 28, 09.11.2019, DerKap-309.

Schizophyllaceae Quél.

85. ***Schizophyllum amplum*** (Lév.) Nakasone: On decaying *Populus* sp. stump, locality 25, 25.09.2019, DerKap-216; locality 38, 25.09.2019, DerKap-237; locality 28, 09.11.2019, DerKap-310; locality 33, DerKap-363; on decaying *Pinus* sp. stump, locality 17, 27.11.2019, DerKap-406.

86. ***Schizophyllum commune*** Fr: On decaying *Pinus* sp. stump, locality 16, 11.03.2019, DerKap-166; on decaying *Populus* sp. stump, locality 44, 25.09.2019, DerKap-232; locality 27, 29.10.2019, DerKap-251; locality 28, 09.11.2019, DerKap-312.

Strophariaceae Singer & A.H. Sm.

87. ***Cyclocybe cylindracea*** (DC.) Vizzini & Angelini: Around *Populus* sp. stump, locality 25, 25.09.2019, DerKap-206; locality 44, 25.09.2019, DerKap-230; locality 5, 12.10.2019, DerKap-238; on *Salix* sp. stump, locality 29, 25.09.2019, DerKap-221.

88. ***Deconica pratensis*** (P.D. Orton) Noordel.: (Işıloğlu & Öder, 1995).

89. ***Pholiota carbonaria*** *(Fr.) Singer*: (Işıloğlu & Watling, 1992).

90. ***Pholiota populnea*** (Pers.) Kuyper & Tjall.-Beuk.: On *Salix* sp. stump, locality 44, 30.10.2019, DerKap-287; locality 34, 13.11.2019, DerKap-384.

Tricholomataceae R. Heim ex Pouzar

91. ***Lepista nuda*** (Bull.) Cooke: Among grass in *Pinus* sp. forest clearing, locality 14, 23.12.2018, DerKap-121; on sandy soil along riverside, locality 15, 23.12.2018, DerKap-124.

92. ***Myxomphalia maura*** (Fr.) Hora: On soil among needle litter under *Pinus* sp., locality 27, 23.12.2018, DerKap-125.

93. ***Tricholoma fracticum*** (Britzelm.) Kreisel: Among leaf & needle litter in mixed forest, locality 6, 14.01.2019, DerKap-144; locality 35, 09.11.2019, DerKap-329; on soil under *Juniperus* sp., locality 23, 09.11.2019, DerKap-338.

94. ***Tricholoma caligatum*** (Viv.) Ricken: (Işıloğlu & Watling, 1992).

95. ***Tricholoma terreum*** (Schaeff.) P. Kumm.: On soil among needle litter under *Pinus* sp., locality 47, 07.01.2019, DerKap-135.

96. ***Tricholoma ustale*** (Fr.) P. Kumm.: (Işıloğlu & Öder, 1995).

Auriculariales Bromhead

Auriculariaceae Fr.

97. *Auricularia mesenterica* (Dicks.) Pers.: On decaying *Morus* sp. stump, locality 3, 18.03.2019, DerKap-168.

Boletales E.-J. Gilbert

Boletaceae Chevall.

98. *Boletus edulis* Bull.: Among leaf litter under *Quercus* sp., locality 43, 21.09.2019, DerKap-186.

99. *Xerocomellus chrysenteron* (Bull.) Šutara: On soil among grass in forest clearing, locality 32, 05.11.2018, DerKap-059.

Diplocystidiaceae Kreisel

100. *Astraeus hygrometricus* (Pers.) Morgan: On soil under *Juniperus* sp., locality 36, 30.10.2019, DerKap-273; locality 35, 09.11.2019, DerKap-328; locality 23, 09.11.2019, DerKap-333.

Gomphidiaceae Maire ex Jülich

101. *Chroogomphus rutilus* (Schaeff.) O.K. Mill.: Among needle litter under *Pinus* sp., locality 32, 05.11.2018, DerKap-066; locality 14, 24.03.2019, DerKap-172.

Rhizopogonaceae Gäum. & C.W. Dodge

102. *Rhizopogon luteolus* Fr.: In soil under *Pinus* sp., locality 32, 29.01.2019, DerKap-151.

103. *Rhizopogon roseolus* (Corda) Th. Fr.: In soil under *Pinus* sp., locality 39, 07.10.2018, DerKap-046; locality 32, 05.11.2018, DerKap-068; locality 20, 02.12.2018, DerKap-086; locality 19, 27.11.2019, DerKap-399.

Sclerodermataceae Corda

104. *Pisolithus arhizus* (Scop.) Rauschert: On sandy soil under *Eucalyptus* sp., locality 24, 01.10.2018, DerKap-040; 02.02.2019, DerKap-157; locality 3, 07.10.2018, DerKap-044; locality 46, 30.10.2019, DerKap-262; on soil under *Quercus* sp., locality 43, 29.10.2019, DerKap-247; locality 41, 29.10.2019, DerKap-250.

105. *Scleroderma cepa* Pers.: On sandy soil under *Eucalyptus* locality 45, 07.10.2018, DerKap-045.

Suillaceae Besl & Bresinsky

106. *Suillus bellinii* (Inzenga) Kuntze: (Işıloğlu & Watling, 1992).

107. *Suillus collinitus* (Fr.) Kuntze: Among needle litter under *Pinus* sp., locality 39, 07.10.2018, DerKap-047; locality 6, 14.01.2019, DerKap-145.

108. *Suillus granulatus* (L.) Roussel: Among needle litter under *Pinus* sp., locality 32, 05.11.2018, DerKap-060; locality 30, 09.12.2018, DerKap-102.

109. *Suillus luteus* (L.) Roussel: Among needle litter under *Pinus* sp., locality 19, 02.12.2018, DerKap-079.

Geastrales K. Hosaka & Castellano

Geastraceae Corda

110. *Geastrum fimbriatum* Fr.: On soil under *Juniperus* sp., locality 36, 30.10.2019, DerKap-266; on soil under *Pinus* sp., locality 8, DerKap-356.

111. *Geastrum floriforme* Vittad.: On soil under *Juniperus* sp., locality 23, 09.11.2019, DerKap-350.

112. *Geastrum pectinatum* Pers.: On soil under *Pinus* sp., locality 39, 07.10.2018, DerKap-048; on soil under *Juniperus* sp., locality 35, 09.11.2019, DerKap-327.

113. *Schenella pityophila* (Malençon & Riousset) Estrada & Lado: Under needle litter in *Pinus* sp. forest, locality 48, 27.11.2019, DerKap-407.

Gloeophyllales Thorn

Gloeophyllaceae Jülich

114. *Gloeophyllum odoratum* (Wulfen) Imazeki: On *Ceratonia* sp. roots, locality 16, 11.03.2019, DerKap-165.

Hymenochaetales Oberw.

Hymenochaetaceae Donk

115. *Phellinus igniarius* (L.) Quél.: On *Salix* sp. stump, locality 38, 07.10.2018, DerKap-055; locality 44, 21.09.2019, DerKap-187; locality 34, 13.11.2019, DerKap-373.

Incertae Sedis

116. *Trichaptum abietinum* (Pers. ex J.F. Gmel.) Ryvarden: On *Pinus* sp. stump, locality 23, 09.11.2019, DerKap-346.

Polyporales Gäum.

Fomitopsidaceae Jülich

117. *Antrodia albida* (Fr.) Donk: On decaying *Populus* sp. stump, locality 34, 13.11.2019, DerKap-369; locality 27, 27.11.2019, DerKap-397.

118. *Laetiporus sulphureus* (Bull.) Murrill: On *Salix* sp. stump, locality 17, 29.04.2018, DerKap-029.

Ganodermataceae Donk

119. *Ganoderma adspersum* (Schulzer) Donk: On dead *Eucalyptus* sp. stump, locality 24, 01.10.2018, DerKap-041.

Meruliaceae P. Karst.

120. *Bjerkandera adusta* (Willd.) P. Karst.: On decaying *Populus* sp. stump, locality 25, 25.09.2019, DerKap-217; locality 44, 30.10.019, DerKap-282; locality 18, 09.11.2019, DerKap-306.

121. *Phlebia tremellosa* (Schrad.) Nakasone & Burds.: On decaying *Populus* sp. stump, locality 33, 13.11.2019, DerKap-358; locality 27, 27.11.2019, DerKap-398.

Polyporaceae Fr. ex Corda

122. *Fomes fomentarius* (L.) Fr.: On decaying *Populus* sp. stump, locality 44, 21.09.2019, DerKap-188.

123. *Lentinus tigrinus* (Bull.) Fr.: Around *Salix* sp.

stump, locality 38, 07.10.2018, DerKap-054; locality 25, 25.09.2019, DerKap-208; locality 44, 25.09.2019, DerKap-233; locality 17, 27.11.2019, DerKap-403; around *Eucalyptus* sp. stump, locality 21, 07.11.2019, DerKap-292.

124. ***Trametes trogii*** Berk.: On decaying *Salix* sp. stump, locality 44, 21.09.2019, DerKap-196; on *Populus* sp. stump, locality 44, 30.10.2019, DerKap-286; locality 25, 25.09.2019, DerKap-211; locality 28, 09.11.2019, DerKap-315; locality 34, 13.11.2019, DerKap-383.

Russulales Kreisel ex P.M. Kirk, P.F. Cannon & J.C. David

Russulaceae Lotsy

125. ***Lactarius deliciosus*** (L.) Gray: Among leaf and needle litter in mixed forest, locality 11, locality 14, 09.12.2018, DerKap-096; 23.12.2018, DerKap-120.

126. ***Russula atropurpurea*** (Krombh.) Britzelm.: On soil among needle litter under *Pinus* sp., locality 11, 09.12.2018, DerKap-100.

127. ***Russula rosea*** Pers.: Among needle litter under *Pinus* sp., locality 32, 05.11.2018, DerKap-063.

Stereaceae Pilát

128. ***Stereum hirsutum*** (Willd.) Pers.: On decaying *Pinus* sp. stump, locality 8, 23.06.2019, DerKap-181.

Sebacinales M. Weiss, Selosse, Rexer, A. Urb. & Oberw.

Sebacinaceae K. Wells & Oberw.

129. ***Sebacina incrustans*** (Pers.) Tul. & C. Tul.: On dead *Juniperus* sp. twigs, locality 36, 30.10.2019, DerKap-271.

Thelephorales Corner ex Oberw.

Bankeraceae Donk

130. ***Hydnellum scabrosum*** (Fr.) E. Larss., K.H. Larss. & Kõljalg: (Işıloğlu & Watling, 1992).

131. ***Sarcodon imbricatus*** (L.) P. Karst: On soil among leaf & needle litter, locality 30, 09.12.2018, DerKap-104.

Dacrymycetes Doweld

Dacrymycetales Henn.

Dacrymycetaceae J. Schröt.

132. ***Dacrymyces variisporus*** McNabb: On decaying *Pinus* sp. twigs, locality 19, 27.11.2019, DerKap-400.

Pucciniomycetes R. Bauer, Begerow, J.P. Samp., M. Weiss & Oberw.

Pucciniales Caruel

Pucciniaceae Chevall.

133. ***Gymnosporangium clavariiforme*** (Wulfen) DC.: On dead *Juniperus* sp. stem, locality 8, 11.03.2019, DerKap-163.

DISCUSSION and CONCLUSION

A list of 133 macrofungi species was compiled from Silifke district. Thirty-one of the existing taxa (%23.31) belong to *Ascomycota* and 102 (%76.69) to *Basidiomycota*. The taxa are distributed in 7 classes (*Agaricomycetes* 100, *Pezizomycetes* 26, *Leotiomycetes* 2, *Sordariomycetes* 2, *Dacrymycetes* 1, *Orbiliomycetes* 1, *Pucciniomycetes* 1) and 18 orders. The order-wise distribution of the determined taxa is presented in Figure 2. The taxa are distributed in 48 families and 96 genera. Forty eight percent (64) of the existing taxa are distributed within the most crowded 5 families (*Agaricaceae*, *Pyronemataceae*, *Psathyrellaceae*, *Tricholomataceae* and *Inocybaceae* with 29, 14, 10, 6 and 5 taxa respectively). *Agaricus, Lycoperdon, Suillus* and *Tricholoma,* were found to be the most crowded genera each with 4 taxa. Six of the genera (*Conocybe, Coprinopsis, Geastrum, Morchella, Tulostoma*) are represented with 3 taxa, 15 of them (*Ascobolus, Coprinellus, Crepidotus, Geopora, Inocybe, Lepiota, Octospora, Parasola, Pholiota, Psathyrella, Pyronema, Rhizopogon, Russula, Schizophyllum, Tricharina*) are represented with 2 taxa while the other genera are represented in the region with only one taxon.

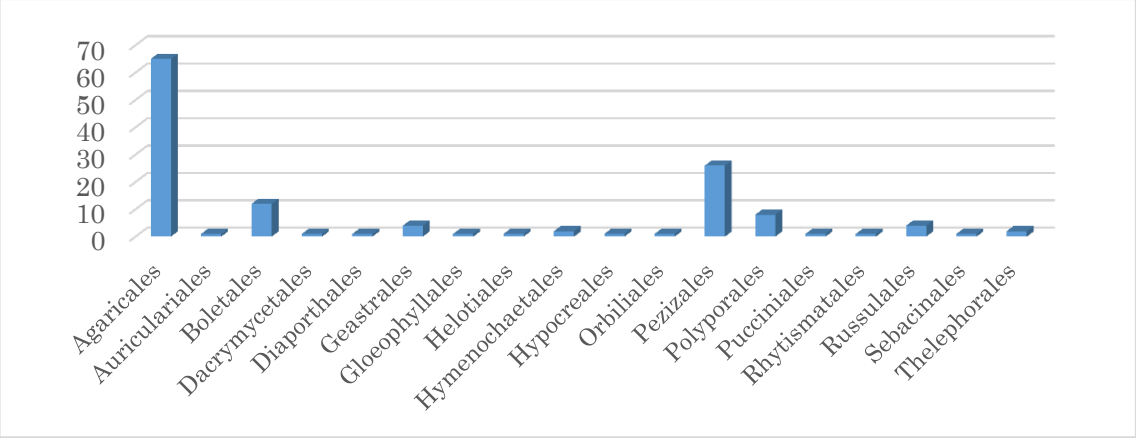

Figure 2. Order-wise distribution of the macromycete taxa existing in Silifke
Şekil 2. Silfke'de bulunan taksonların takım bazında dağılımı

One hundred and twenty two of the existing taxa are new for Silifke district while 11 of them (*Lepiota helveola, Hydnellum scabrosum, Hygrophorus chrysodon, Hebeloma sinapizans, Inosperma erubescens, Parasola galericuliformis, Pisolithus arhizus, Deconica pratensis, Pholiota carbonaria, Suillus bellinii, Tricholoma caligatum and Tricholoma ustale*) were presented in previous studies (Işıloğlu & Watling, 1992; Işıloğlu & Öder, 1995).

Thirty-nine (%29.32) of the taxa are edible. Five of them are collected and consumed by local public. Among the consumed taxa, *Pleurotus ostreatus* is known as "kavak mantarı", *Morchella deliciosa, M. elata* and *M. tridentina* are known as "kuzu göbeği", and *Lactarius deliciosus* is known as "çıntar mantarı". Except *P. ostreatus,* all of the locally consumed taxa also have regional economic importance. Eighty eight of the existing taxa are regarded as inedible while six (*Coprinopsis atramentaria, Hebeloma sinapizans, Inocybe lacera, I. rimosa, Lepiota cristata, L. helveola*) are more or less poisonous.

Seventy five of the total taxa are terricolous, 34 are lignicolous, 4 are bryophilous, 4 are coprophilous, 4 are herbicolous and 3 are pyrophilous, while five are hypogeous or semi-hypogeous.

The existing taxa were also compared with the studies carried out in mediterranean and neighboring regions and some similarities were observed. These studies and the similarity percentages are given in Table 2. The reason for this similarity may be the common climate and vegetation.

Table 2. Similarity percentages of Silifke district with the studies performed in neighbouring regions
Çizelge 2. Silifke ile komşu bölgelerde gerçekleştirilen çalışmaların benzerlik yüzdeleri

	Province/ District	# of Identical taxa	Total taxa	Similarity (%)
Gezer (2000)	Antalya	33	81	40.74
Aktaş et al. (2003)	Konya	23	74	31.08
Öztürk et al. (2003)	Antalya	31	188	16.49
Doğan & Öztürk (2006)	Karaman	43	202	21.29
Doğan et al. (2007)	Mersin	27	95	28.42
Kaya (2009)	Gaziantep	35	105	33.33
Kaya et al. (2009)	Kahramanmaraş	36	110	32.73
Doğan et al. (2012)	Adana	29	186	15.19
Solak et al. (2014)	Antalya	37	136	27.21
Kaya (2015)	Şanlıurfa	41	122	33.61

Author's Contributions

Authors declare the contribution of the authors is equal.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

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A New Distribution Area of the *Lathyrus undulatus* Boiss. (Fabaceae) in Türkiye and Taxonomic Contributions

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ABSTRACT
Lathyrus undulatus Boiss., an endemic species belongs to the Fabaceae family, is distributed in A1, A2, A3, A5 and B2 squares in the flora of Türkiye according to the literature records. In our researches, it has also been seen that the species is distributed in a narrow area at an altitude of 791-803 m around C2 Muğla: Menteşe, Yerkesik. *L. undulatus* has found for the first time by us in this locality and added as a new record for the C2 square with this study. By morphological examinations on the newly detected population, vegetative and generative characteristics such as stem, stipule, leaf, leaflets, inflorescence, peduncle, pedicel, flower, fruit, seed etc. have been revealed. The flowering time has been determined by conducting field studies on different dates. The morphological features of the new population have been compared with the descriptions of *L. undulatus* found in the Flora of Türkiye and the Flora Europaea, and similarities and differences have been revealed.

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Lathyrus undulatus Boiss. (Fabaceae)'un Türkiye'de Yeni Bir Yayılış Alanı ve Taksonomik Katkılar

ÖZET
Fabaceae familyasına ait endemik bir tür olan *Lathyrus undulatus* Boiss. literatür kayıtlarına göre Türkiye florasında A1, A2, A3, A5 ve B2 karelerinde yayılış göstermektedir. Yaptığımız araştırmalarda türün C2 Muğla: Menteşe, Yerkesik civarında 791-803 m rakımda, dar bir alanda da yayılış gösterdiği görülmüştür. *L. undulatus* bu lokalitede ilk defa tarafımızdan bulunmuş ve bu çalışma ile C2 karesine yeni bir kayıt olarak eklenmiştir. Yeni tespit edilen popülasyon üzerinde morfolojik inceleme yapılarak gövde, stipül, yaprak, yaprakçıklar, çiçek durumu, pedinkül, pedisel, çiçek, meyve, tohum gibi vejetatif ve generatif özellikleri ortaya konulmuştur. Farklı tarihlerde arazi çalışmaları yapılarak çiçeklenme zamanı belirlenmiştir. Yeni popülasyonun morfolojik özellikleri *L. undulatus*'un Türkiye ve Avrupa Florası'nda bulunan deskripsiyonları ile karşılaştırılarak benzerlik ve farklılıklar ortaya konulmuştur.

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INTRODUCTION
Lathyrus L., a genus from the Fabaceae family, is economically important. In Türkiye, the genus *Lathyrus* is typified by 79 taxa, 25 of which are endemic. (Davis, 1970; Davis et al., 1988; Güner et al., 2000; Genç and Şahin, 2008; Genç, 2009; Genç and Şahin, 2011; Güneş & Çırpıcı, 2012; Güneş, 2014; 2018; 2019). In different parts of the world, a few of these taxa are cultivated for different purposes. Their seeds are used as human foodstuff or the whole plant is used as fodder (Yamamoto et al., 1984; Campbell, 1997). In a study on the ethnobotanical use of plants, it was stated that the aerial parts of *L. undulatus* were also used as fodder (Kızılarslan & Özhatay,

2012). It has been stated that the tea of the plant is drunk due to its weakening feature and it is also eaten (Özkan, 2011).

L. undulatus species belongs to the genus *Lathyrus* is an endemic plant for Türkiye. Distribution areas of the species are A1 Kırklareli A2: İstanbul, Bursa, Kocaeli, Yalova A3: Sakarya, Düzce, Bolu A5: Sinop B2: Balıkesir, Kütahya in Türkiye. The species is found in different habitats such as under the deciduous forest, hedges, roadsides, slopes, field edges etc. (Davis, 1970; Aksoy & Uzun, 2011; Özkan, 2011; Kızılarslan & Özhatay, 2012; Tel, 2012; Güler, 2013; Güner & Akçiçek, 2014; Hekimoğlu, 2019; Öksüzoğlu, 2019; Açar & Satıl, 2021). In the work named Türkiye Bitkileri Listesi (Güner et al., 2012), it was stated that the *L. undulatus* also lived in the Konya section, which is a part of the Central Anatolian geographical region. However, no other work could be found indicating the squares or localities where the species is distributed in this section. Therefore, it has not been included in the map showing the distribution areas of the species.

Although it is registered in Flora Europaea, it is stated that the locality where the species is distributed is Türkiye (Tutin et al., 1968). In this study, it is aimed to add a newly detected distribution area of the species to the literature.

MATERIAL and METHODS

The materials of the study are the samples belong to the *L. undulatus* species. Samples were picked up from C2 Muğla: Menteşe, Yerkesik which is the natural spreading area of *L. undulatus* in May 2021. Flora of Türkiye (Davis, 1970) and Flora Europaea (Tutin et al., 1968) have been used for identification of the plant samples. In our study, the syntype specimen photograph of the *L. undulatus* (Anonymous, 2022) and the photographs taken by us in its natural habitat are also included.

The samples were examined and their morphological features such as stem, stipules, leaves, inflorescence, peduncles, pedicels, flowers, legumes etc. were determined. Plant parts like stipules, leaflets, calyx, corolla and legumes were examined using a stereo microscope and the measurements were made with digital caliper. It has been compared with the literature in terms of morphological features and flowering time. Whether or not the *L. undulatus* is new record to C2 square has been evaluated by investigating to the literature in this subject.

RESULTS and DISCUSSION

L. undulatus is a close species to *Lathyrus rotundifolius* Willd. taxonomically. In the European flora, *L. rotundifolius*; in the flora of Türkiye *L. rotundifolius* subsp. *miniatus* (Bieb. ex Stev.) Davis is distributed. *L. undulatus* is distributed intensively in the northwest of Türkiye (Figure 1) according to the literature.

Syntype specimen photograph (Anonymous, 2022) and the photographs of live specimen are given in Figure 2.

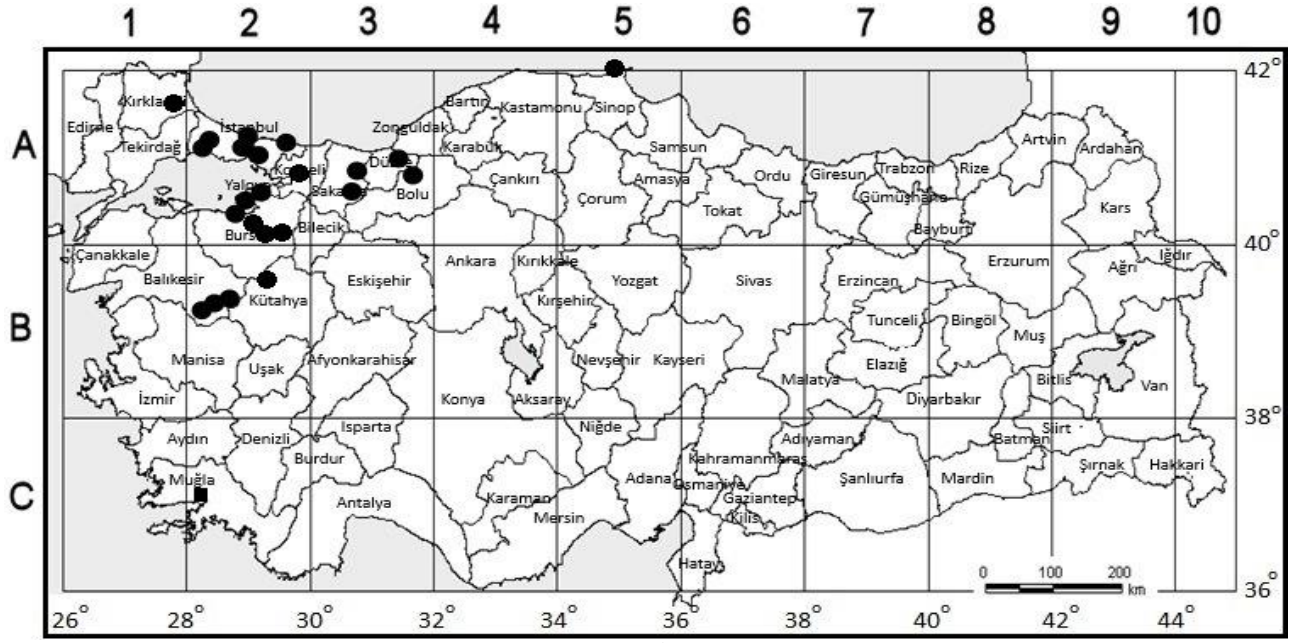

Figure 1. Distribution areas of *L. undulatus* in Türkiye (●Distribution areas according to the literature ■Newly detected distribution area)

Şekil 1. *Türkiye'de L. undulatus'un yayılış alanları (●Literatürde verilen yayılış alanları ■Yeni tespit edilen yayılış alanı)*

Figure 2. Syntype specimen of *L. undulatus* (a) and live specimen (b: habitus, c: flowers, d: legumes)
***Şekil 2**. L. undulatus'un sintip örneği (a) ve canlı örneği (b: genel görünüş, b: çiçekler, d: meyveler)*

An interesting new distribution area of the species has been determined with field surveys by us. In this research, it has been exhibited that the species also spread in a narrow area in C2 Muğla: Menteşe, Yerkesik (Figure 1). The species is distributed in the *Pinus brutia* Ten. forest in this locality. There are very few individuals in the population found in this newly detected locality. It has been determined by us that there are approximately 50 individuals in an area of approximately 1000 m². This population, which is already distributed in a narrow area, is exposed to grazing pressure. New field studies should be carried out to determine whether the distribution of the population is limited to this area.

It has been stated that *L. undulatus* is similar to *L. rotundifolius* in the Flora Europaea (Tutin et al., 1968) and to *L. rotundifolius* subsp. *miniatus* in the Flora of Türkiye (Davis, 1970). When samples of the newly detected *L. undulatus* population compared with the literature in terms of morphological characteristics, some similarities and differences were found.

The results of the examinations of the morphological characteristics performed on the species and the comparison with the literature data (Tutin et al., 1968; Davis, 1970) are given in Table 1.

According to the literature, *L. undulatus* is distributed in the Euro-Siberian phytogeographic region. It has been stated that it may be an Euxine element (Davis, 1970). The locality of the newly detected population is quite far from the localities given in the literature and is in the Mediterranean phytogeographic region. The bird flight distances between the new locality and the localities specified in the literature is 300-700 km. It is interesting that such long distances exist between the new locality and the localities given in the literature.

According to the Flora Europaea, *L. undulatus* differs from *L. rotundifolius* in that the some morphological differences of leaflets, inflorescence, calyx and legume (Tutin et al., 1968). A similar situation applies to the Flora of Türkiye. *L. undulatus* differs from *L. rotundifolius* subsp. *miniatus* in that the some morphological disparities of stipules, leaflets, flowering time, calyx and seeds according to the Flora of Türkiye (Davis, 1970).

In this study, samples of the *L. undulatus* population in the newly detected locality were examined in terms of morphological features and compared with the literature. Some morphological features show differences with literature information. It can be thought that morphological differences may have occurred under different ecological conditions. It is necessary to examine the factors that may affect the distribution of the populations of the *L. undulatus*.

CONCLUSION

Living species are evaluated in 9 groups in terms of IUCN categories: E (Extinct), EW (Extinct in the Wild), CR (Critically Endangered), EN (Endangered), VU (Vulnerable), NT (Near Threatened), LC (Least Concern), DD (Data Deficient) and NE (Not Evaluated). Although not in the CR and EN categories, plants that are under high threat in nature in the medium term are in the VU category. In Red Data Book of Turkish Plants, *L. undulatus* was specified in VU category (Ekim et al., 2000). This species has most recently been assessed for The IUCN Red List of Threatened Species a few years ago and listed as EN globally (Rowe et al., 2019). The change of the IUCN category of *L. undulatus* from VU to EN means that presence in nature of the species is endangered.

Table 1. Comparison of the morphological properties
Çizelge 1*. Morfolojik özelliklerin karşılaştırılması*

	L. undulatus		
	Flora Europaea (Tutin et al., 1968)	Flora of Türkiye (Davis, 1970)	Newly detected population (C2 square)
Life form	Perennial	Perennial	Perennial
Stem	40-80 cm, glabrous, winged	100-250 cm, very glabrous, winged, climbing	30-100 cm, glabrous, winged,
Stipules	10-25 x 3-6 mm, oblong or lanceolate, hastate	Usually narrower than *L. rotundifolius* subsp. *miniatus*, ovate-lanceolate or lanceolate, semi-sagittate	10-25x2-4 mm, lanceolate, sagittate to hastate
Leaves	n.i.	With branched tendrils	Petiole 10-25 mm, narrowly winged; rachis ending usually strongly branched tendrils, sometimes simple tendril.
Leaflets	1 pair, 30-70 x 16-35 mm, elliptical to ovate, up to 4 times as long as wide, the margin undulate-crispate	1 pair, (1.5-)2-4 x longer than broad, crisply undulate-margined, broadly elliptic, rarely suborbicular, parallel-veined	1 pair, 25-75x10-42 mm, length up to 3 x as long as width, elliptic to suborbicular
Flowering Time	n.i.	April-June	April-June
Inflorescence	5-10 flowered	3-13 flowered	(1-)3-9 flowered
Peduncle	n.i.	Much longer than leaves	60-85(-130) mm (without raceme)
Pedicels	n.i.	n.i.	3-8 mm, shorter than calyx
Calyx	Teeth unequal, lowest tooth c. 1.5 times as long as upper 2	Calyx teeth more attenuate according to *L. rotundifolius* subsp. *miniatus*, lowest tooth being 1-1.5 x as long as the tube,	5-9 mm, lower teeth longer than the others
Corolla	15-22 mm, purple-pink	18-25 mm, deep pink	10-20 mm, deep pink
Legume	60-80 x 7-11 mm, glabrous	50-70 x 7-10 mm, linear, glabrous, upper suture narrowly 3-keeled	50-70x7-9 mm, linear, glabrous, upper suture narrowly 3-keeled
Seeds	8-10 reticulate-rugose; hilum 1/5 of the circumference	6-10, faintly reticulate-rugulose, hilum shorter according to *L. rotundifolius* subsp. *miniatus* (1/7 of the seed's perimeter)	Mature seeds could not be collected

n.i.: No information

As the threat to populations of this species increases, it will be in danger of extinction. The danger of extinction is a momentous threat to all living species. The level of this threat is even higher, especially for species with narrow distribution areas and known from few localities. Endemic plants adapted to living in special habitats are more susceptible to threat factors. In particular, the presence of a population of an endemic species in more localities in nature is significant both for the continuation of the generation of that species and for biodiversity. For the reasons mentioned above, it is very important that a new distribution area of *L. undulatus*, an endemic species, has been found. But grazing pressure on the population is a serious threat in this area. It is necessary to eliminate this threat or to conduct studies to reduce its effects.

The differences between the morphological properties of the new population and the literature information show the necessity of new studies. Anatomical, palynological, molecular etc. studies should be done on the species. The taxonomic status of the population can be re-evaluated according to the results of the studies to be carried out.

Author's Contributions

The contribution of the authors is equal.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

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Mersin İli Tarla Koşullarında Yetiştirilen Farklı Domates Çeşitlerinde Domates Güvesi *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae)'nın Popülasyon Yoğunluğu ve Bulaşıklık Oranı

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ÖZET

Bu çalışma, Mersin ili Mezitli ilçesi Kale köyünde bulunan açık arazide yetiştirilen 500'er adet fidenin dikim arası 40 cm, sıra arası 60 cm olarak dikilen üç farklı domates çeşidi (Elibol, Süper Lapçin ve No:14397) üzerindeki *T. absoluta*'nın popülasyonu takip edilerek zarar durumunun belirlenmesi amaçlanmıştır. *T. absoluta*'nın 2015 yılında domates yaprakların da en fazla zararı Elibol, en az zararı No:14397 domates çeşidinde görülmüş, gövde de ise No:14397 çeşidindeki zararlanma Elibol ve Süper Lapçin çeşitlerine göre daha fazla olurken, meyvedeki zararlanmada Elibol çeşidinde başlangıçta diğer çeşitlere göre fazla olmasına rağmen, ileriki tarihlerde aynı düzeye ulaşmıştır. *T. absoluta*'nın 2016 yılında ise yapraklarda, gövde ve meyve de Elibol çeşidindeki zararlanma Süper Lapçin'e göre daha fazla olmuştur. Delta tipi eşeysel çekici feromon tuzaklarında 2015 yılında yakalanan ergin *T. absoluta* sayısı tuzak başına ortalama domates çeşitlerinde, Elibol 16.02, Süper Lapçin 15.46 olurken No:14397 de 14.94 olmuştur, 2016 yılında ise ortalama Elibol 6.43 olurken Süper Lapçin 4.87 olmuştur. Total yaprak, gövde ve meyve de çeşitler arasında yapılan tek yönlü varyans analizi ile istatistiksel olarak herhangi bir fark bulunmamıştır (P>0,05). Popülasyon durumuna göre kitlesel yakalama amaçlı tuzakların kullanılması ve geliştirilmesinin ve domates yetiştiriciliğinde yaprak ayası daha küçük olan çeşitlere yer verilmesinin *T. absoluta* ile mücadele de önemli olduğu belirlenmiştir.

Entomoloji

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 17.01.2022
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Anahtar Kelimeler
Tuta absoluta (Domates güvesi)
Domates çeşitleri
Delta tuzaklar
Popülasyon takibi

Population Density and Infestation Rate of *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) on Different Tomato Varieties in Mersin Field Conditions

ABSTRACT

This study was conducted to determine the effects of *T. absoluta* on three different tomato varieties (Elibol, Super Lapçin, and No: 14397) planted with 40 cm spacing and 60 cm row spacing of 500 seedlings grown in the open field in a kale village of Mezitli district of Mersin province. It is aimed to determine the damage status by following the population in 2015; the most damage of *T. absoluta* on tomato leaves was seen in Elibol, the minor damage was seen in tomato variety No:14397, while the damage on the stem was more in No:14397 than Elibol and Super Lapçin, while the fruit damage was seen in the Elibol variety at the beginning. Although it was more than the varieties, it reached the same level on the following dates. In 2016, damage to leaves, stems, and fruit of *T. absoluta* was more in Elibol variety than Super Lapçin. The average number of adult *T. absoluta* caught in delta-type sexual attractive pheromone traps in 2015 was 16.02 for Elibol, 15.46 for Super Lapçin, and 14.94 for No:14397 in tomato cultivars per trap. No statistically significant difference was found in the whole leaf, stem, and fruit with a one-way analysis of variance between cultivars (P>0.05). It has been determined that the use and development of traps for mass trapping according to the population status and the use of varieties with smaller leaf blades in tomato cultivation are also crucial in control against *T. absoluta*.

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GİRİŞ

Domates (*Lycopersicon esculentum* L.) Türkiye ekonomisi bakımından çok önemli bir yere sahiptir. Türkiye iklim şartlarının uygunluğu ve 1970'li yıllarda ketçap ve salça sanayisinin gelişmesiyle birlikte üretim alanı oldukça artmış ve dünyada domates üretimi bakımından söz sahibi konuma gelmiştir. Dünyadaki üretim miktarı 165 milyon ton olurken en fazla üretimi Çin (64.8 milyon ton) daha sonra sırasıyla Hindistan (20.5 milyon ton), ABD (12.2 milyon ton) yapmaktadır. Türkiye'de ise 1.744.372 dekarlık alanda 13.2 milyon tonluk üretim ile dünya sıralamasında 3. sırada yer almaktadır (Anonim, 2020a; Anonim, 2020b). Mersin ilinde ise domates üretimi 84.268 dekarlık alanda yapılmakta olup 930.128 tonluk bir üretim yapılmaktadır (Anonim, 2020b).

Bu kadar yoğun bir üretime sahip olan domates son yıllarda ortaya çıkan *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) zararlısı tarafından oldukça zarar görmüştür. Güney Amerika kökenli bir zararlı olan *T. absoluta* domatesin en önemli zararlılarından birisi konumuna gelmiştir (Barrientos et al., 1998; Miranda et al., 1998). Globalleşen dünyada kıtalararası ihracat ile birlikte çok hızlı bir yayılış gösteren *T. absoluta*, Avrupa'da ilk olarak 2006 yılında İspanya'da örtü altında yetiştirilen domateslerde tespit edilmiş, daha sonra ise İspanya'nın yanında tüm Akdeniz kıyı kesimindeki ülkelere yayılarak ciddi zararlara yol açtığı bildirilmiştir (EPPO, 2010). *T. absoluta* Türkiye'de ilk olarak 2009 yılı ağustos ayında İzmir ili Urla ilçesinde domates bitkileri üzerinde saptanmıştır. Aynı yıl diğer illerde yapılan surveyler sonucunda zararlının Çanakkale ve Muğla illerinde de görüldüğü bildirilmiştir (Kılıç, 2010). Akdeniz Bölgesi'nde ise 2010 yılında Antalya'nın Kumluca ilçesinde bir domates serasında saptanmıştır (Erler et al., 2010). Hatta aynı yıl Şanlıurfa'da yapılan bir survey çalışmasında açık alanda domates yetiştiriciliğinde bitkilerin zararlı ile %100 bulaşık olduğu belirlenmiştir (Mamay & Yanık, 2012). Uygun ekolojik koşullara sahip olan Türkiye'de hızla yayılmakta olan zararlı, 2010 yılı ağustos ayına kadar Akdeniz ve Ege Bölgelerine tamamen bulaşmış olup daha önce bulunmadığı bölgelere de hızla yayıldığı bildirilmektedir (Erler et al., 2010). Zararlının yaprak epidermisi altında galeriler açarak beslenmesinden dolayı kimyasal mücadelesi oldukça zordur (Cabello et al., 2009). Ayrıca yılda 10-12 gibi çok sayıda döl vermesi, bazı insektisitlere karşı çok çabuk dayanıklılık geliştirmesine sebep olmaktadır (Siqueira et al., 2001; Lietti et al., 2005). Bununla birlikte zararlının 2013-2014 yıllarında Hindistan, 2015-2016 yıllarında ise Çin'de ortaya çıkmasının muhtemel olduğundan bahsedilmekte olup 2050 ve 2100 yıllarında zararlının yılda 12-15 döl verebileceği bildirilmektedir (Bech, 2009; Abolmaaty et al., 2010; Ostrauskas & Ivinskis, 2010; Desneux et al., 2010, 2011; Abbes et al., 2012; Al-Jboory et al., 2012). Domates güvesi kısa bir sürede domatesin en önemli zararlısı konumuna gelmiştir. Ana konukçusu domates olmakla birlikte hem açık alanda hem de örtü altı yetiştiriciliğinde Solanacea familyasına ait patlıcan ve biber gibi diğer bitkilerde de zarar yaptığı bildirilmiştir (Gahramanovo & Mamay, 2020). Patlıcan, patates, biber, pepino, petunya gibi kültür bitkilerinde ayrıca köpek üzümü, şeytan elması gibi yabani otlarda da görüldüğü saptanmıştır (EPPO, 2005).

Bununla birlikte Türkiye'de önemli zararlara neden olan *T. absoluta*'nın açık alanlarda meydana getirdiği zararı belirlemek için Mersin ili, Mezitli ilçesi, Kaleköy beldesindeki açık tarla koşullarında yetiştirilen üç domates çeşidinde (Elibol, Süper Lapçin, No:14397) popülasyon yoğunluğu tuzaklarla takip edilerek yaprak, gövde ve meyve deki zarar durumunu belirlemek amacıyla ele alınmıştır.

MATERYAL ve METOD

Çalışma, bitkisel materyal olarak Mersin ilinde en yaygın kullanılan Elibol, Süper Lapçin ve No:14397 domates çeşitlerinden 500'er adet fide 2015 yılında denemede kullanılmıştır. Denemede kullanılan bu çeşitlerin fidelerinin ekimi 20 Nisan 2015 tarihinde dikim arası 40 cm, sıra arası 60 cm olarak dikilmiştir. Bitkiler toprağa dikildikten sonra 15 günde bir 6-7 kez sulanma ve gübreleme yapılmıştır. Bu çalışmada *T. absoluta*'ya karşı domates çeşitlerinde kimyasal mücadele yapılmamıştır. Denemede kullanılan Elibol domates çeşidi oturak cins olup 250-450 g meyve ağırlığında olurken hastalık dayanımı HR:Va,Vd,Fol 1.2'dir. Süper Lapçin domates çeşidi oturak cins olup 280-300 g meyve ağırlığında olurken hastalık dayanımı HR:ToMv,Vd,Va,Fol 0-1'dir. Diğer bir çeşit olan No:14397 domates çeşidi oturak cins olup 200-400 g meyve ağırlığında olurken hastalık dayanımı HR: ToMv, Vd, Va, Fol 0-1'dir (Çizelge 1).

Çalışmanın yapıldığı 2016 yılında ise Elibol ve Süper Lapçin domates çeşitleri kullanılırken domates çeşidi No:14397 çeşidi elde edilemediği için dikimi yapılamamıştır, 2015 yılında yapılan uygulamalar

2016 yılında aynı şekilde yürütülmüştür.

Domates çeşitleri Elibol, Süper Lapçin ve No:14397 domates bitkilerinin değişik fenolojik dönemleri göz önünde bulundurularak; genç fide, büyüme ve vejetatif gelişme, çiçeklenme ve meyve tutumu, meyve gelişimi, meyve olgunlaşması gibi fenolojik dönemleri 2015 ve 2016 yıllarında takip edilmiştir.

Çizelge 1. Denemede kullanılan domates çeşitleri ve özellikleri
Table 1. Tomatoes used in the experiment and their properties

Çeşit	Üretici Firma	Tipi	Meyve ağırlığı	Dayanıklılık
Elibol F1	Graines Voltz	Oturak	250 – 450 g	HR: Va, Vd, Fol 1.2
Süper Lapçin	Yüksel tohum	Oturak	280 – 300 g	HR: ToMv, Vd, Va, Fol 0-1
No: 14397	Sunny land	Oturak	200 – 400 g	HR: ToMv, Vd, Va, Fol 0-1

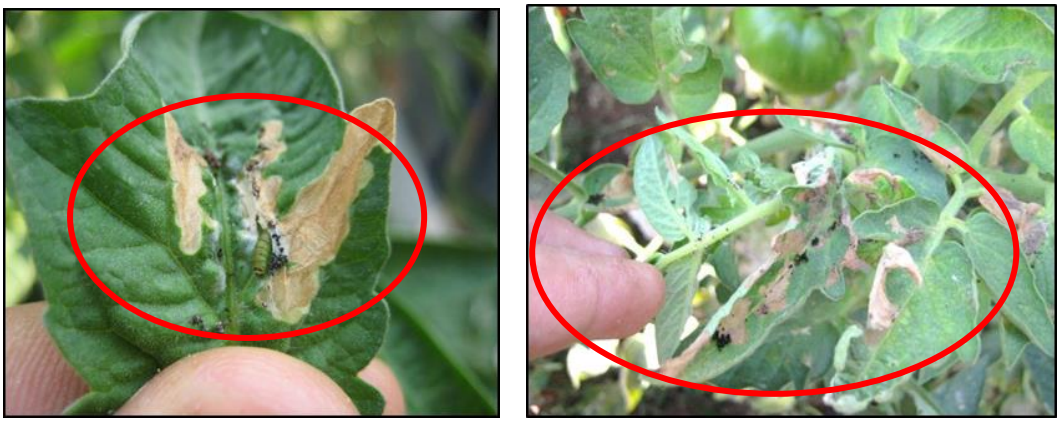
Nisan, mayıs, haziran ve temmuz ayları içerisinde *T. absoluta* larvalarının her domates çeşidindeki sayımlarda bileşik yapraklardan alt, orta ve üst olmak üzere 3 yaprak ve 100 bitki kontrol edilmiştir. Her çeşitten 100 bitkinin gövde kısmı ve her fidede meyve oluşumuyla birlikte rastgele 100 meyve kontrol edilmiştir. Sayımlar periyodik olarak her hafta yapılmıştır. Deneme alanlarında *T. absoluta*'nın domates çeşitlerindeki ilk ergin çıkışını ve popülasyonunu takip etmek amacıyla delta tipi eşeysel çekici feromon tuzaklar kullanılarak ergin sayımları yapılmıştır. Her sayımdan sonra tuzaklardaki erginler uzaklaştırılmış ve yeniden tuzak asılmıştır. Delta tuzaklardaki yapışkan plakaların yapışkanlık özellikleri kaybolduğunda yenisi ile değiştirilirken içerisindeki kapsüller firmanın talimatına göre belirli aralıklarla (4-6 hafta) değiştirilmiştir.

BULGULAR ve TARTIŞMA

Çalışma sonucunda, 2015 yılında açık arazide bulunan Elibol, Süper Lapçin, No:14397 domates çeşitleri kullanılırken, 2016 yılında ise Elibol ve Süper Lapçin domates çeşitlerinin vejetasyon dönemlerinde *T. absoluta*'nın yaprak, gövde ve meyvedeki vuruk sayımı nisan, mayıs, haziran ve temmuz ayları içerisinde periyodik olarak yapılmıştır.

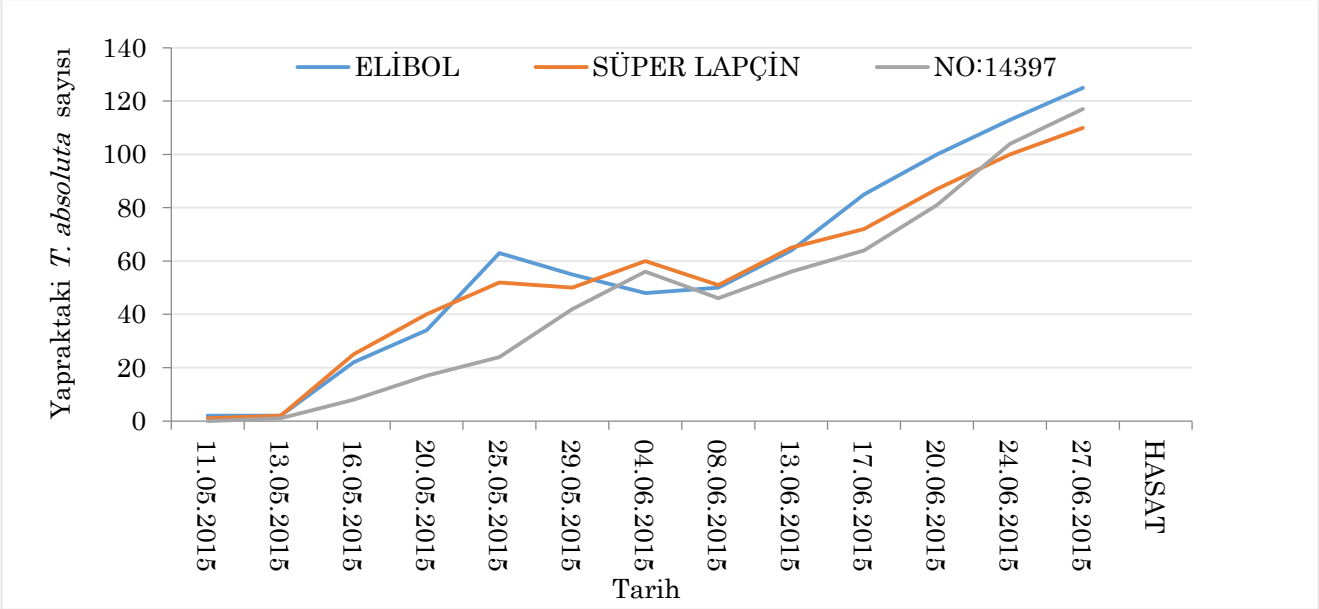
Tuta absoluta'nın domates yaprağındaki popülasyon takibi

Denemenin yapıldığı 2015 yılında domateste ilk zararlanma 13 Mayıs tarihinde Elibol, Süper Lapçin, No:14397 çeşitlerinde görülmeye başlamıştır (Şekil 1). Sıcaklığın artmasıyla birlikte yaprak üzerindeki zararlı popülasyonu artış göstermiştir. En fazla zararın 25 Mayıs tarihinde Elibol, en az zararın No:14397 domates çeşidinde görülmüştür (Şekil 2). Elibol çeşidinin yaprak ayasının geniş ve çalı yapısının kuvvetli olması nedeniyle zarar daha fazla görülürken, No:14397 çeşidinin gelişimi diğer çeşitlere göre daha yavaş ve yaprak ayası yapısının daha küçük olması nedeniyle daha az zararlanma olduğu gözlemlenmiştir.



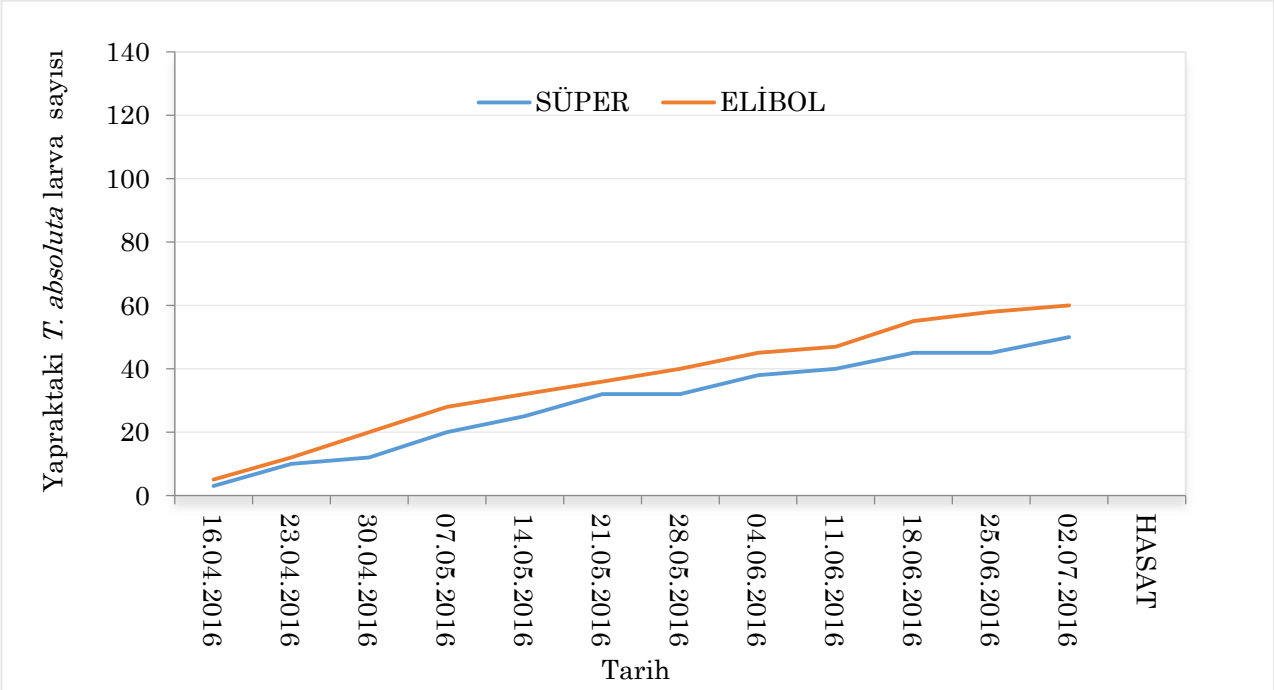
Şekil 1. *Tuta absoluta* larvasının domates yaprağındaki zararı
Figure 1. *Tuta absoluta* larva damage on tomato leaves

Deneme alanlarında 2016 yılında yapılan çalışmada ise domates çeşitlerinde yaprak üzerindeki ilk zararlanma 16 Nisan tarihinde Elibol ve Süper Lapçin çeşitlerinde görülmeye başlamıştır (Şekil 3). Elibol çeşidi Süper Lapçin domates çeşidine göre daha fazla zarar görülürken 2 Temmuz tarihine kadar artan periyotlarla zarar devam etmiştir. Elibol çeşidi 2015 yılında olduğu gibi yaprak ayasının geniş olmasından dolayı daha fazla zarar bulunmuştur; ancak 2016 yılındaki denemede No:14397 çeşidinin fideleri temin edilemediği için kullanılmamıştır.



Şekil 2. *T. absoluta*'nın 2015 yılında Elibol, Süper Lapçin, No:14397 çeşitlerindeki yaprak üzerindeki popülasyon yoğunluğu

Figure 2. Population density of *T. absoluta* on leaves of Elibol, Süper Lapçin, No:14397 cultivars in 2015



Şekil 3. *T. absoluta*'nın 2016 yılında Elibol ve Süper Lapçin çeşitlerindeki yaprak üzerindeki larva popülasyon yoğunluğu

Figure 3. Larval population development of *T. absoluta* on leaf in Elibol and Super Lapçin cultivars in 2016

Tuta absoluta' nın domates gövdesindeki bulaşıklık oranı

Tuta absoluta'nın 2015 yılında her çeşitteki 100 gövde kontrol edilerek gövde üzerindeki ilk zararı (Şekil 4) Elibol ve Süper Lapçin çeşitlerinde 13 Mayıs tarihinde görülmeye başlamıştır, domates çeşitlerinden No:14397 ise ilk zararlanma 25 Mayıs tarihinde görülmeye başlamıştır (Şekil 5). Sıcaklıkların artmasıyla birlikte 4 Haziran tarihinde No:14397 çeşidinin Elibol ve Süper Lapçin çeşitlerine göre gövde de daha fazla zararlanma görülmüştür ve 27 Haziran tarihine kadar artan periyotlarla zararlanma devam etmiştir. Bunun sebebinin ise diğer çeşitlerin gövdelerinin daha kalın ve sulu yapıda olmasından kaynaklı olduğu tespit edilmiştir.

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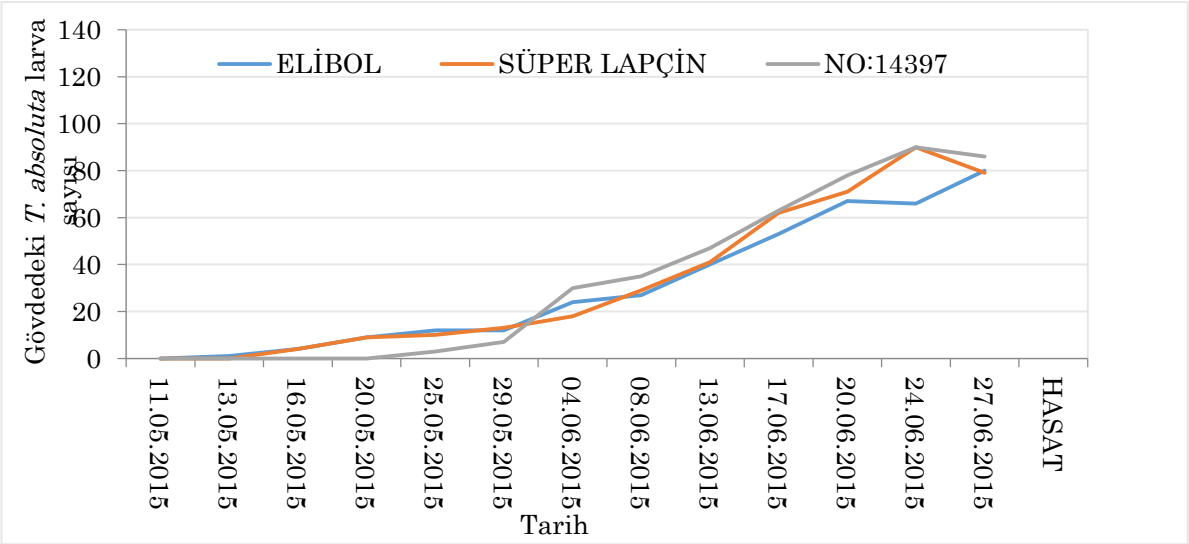
Şekil 4. *Tuta absoluta* larvasının domates gövdesindeki zararı
Figure 4. Damage of *Tuta absoluta* larva on tomato stem



Şekil 5. *T. absoluta'*nın 2015 yılında Elibol, Süper Lapçin, No:14397 çeşitlerindeki gövde üzerindeki popülasyon yoğunluğu
Figure 5. Population density of *T. absoluta* on stems of Elibol, Süper Lapçin, No:14397 cultivars in 2015

Tuta absoluta'nın 2016 yılında gövde üzerindeki ilk zararı ise Elibol çeşidinde 16 Nisan tarihinde görülmeye başlarken Süper Lapçin çeşidinde 23 Nisan tarihinde başlamıştır (Şekil 6). Elibol domates çeşidinde zararlanma Süper Lapçin çeşidine göre daha fazla olup, 2015 yılındaki gövde zararlanmasından daha az olduğu görülmüştür.

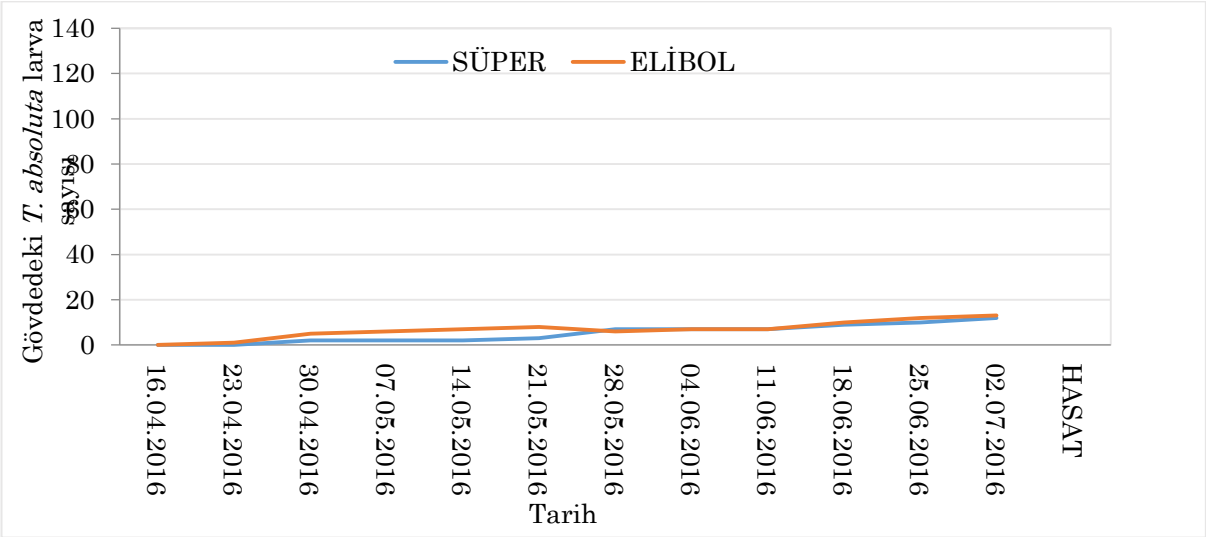
Tuta absoluta'nın domates meyvesindeki bulaşıklık oranı

Tuta absoluta'nın meyvedeki bulaşıklık oranını belirlemek için 2015 yılında her çeşitten 100 meyve kontrol edilerek meyvedeki zarar tespit edilmiştir (Şekil 7). Meyve üzerindeki ilk zararlanma 16 Mayıs tarihinde görülmeye başlamıştır (Şekil 8).

Domates çeşitlerinde meyvedeki en fazla zarar Elibol çeşidinde görülürken 20 Haziranda meyvedeki zararlanma Elibol, Süper Lapçin ve No:14397 hasata kadar aynı düzeyde devam etmiştir. Başlangıçta No:14397 çeşidinde zararın az olmasının nedeni diğer çeşitlere göre meyve sayısının daha az olmasından kaynaklanmaktadır. *T. absoluta*'nın 2016 yılında 11 Mayıs tarihinde meyve üzerindeki ilk zararı görülmüş ve 2015 yılı ile kıyaslandığında daha erken olduğu tespit edilmiştir. Meyvedeki zarar sayısı Elibol çeşidinde daha fazla görülürken, Süper Lapçin çeşidinde daha az görülmüştür (Şekil 9).

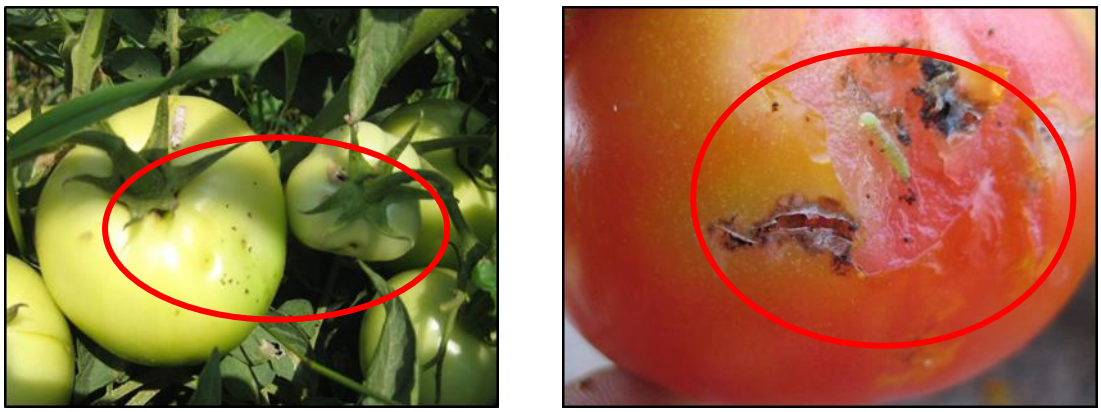
Tuta absoluta'nın domatesteki toplam zararlı sayımı

Domates çeşitlerinde 2015 yılında elde edilen toplam larva sayısı yapraklar da sırasıyla Elibol, Süper Lapçin ve No:14397 de 763, 715 ve 616 olmuştur. Gövde de ise 395, 426 ve 439 olurken, meyve de 444, 405 ve 439 olmuştur. Toplamda en fazla zararlı larva sayısı Elibol çeşidinde olurken bunu sırasıyla Süper Lapçin ve No:14397 takip etmiştir. Bu çalışmada zararlının daha çok yaprak kısmını tercih ettiği tespit edilmiştir (Çizelge 2).



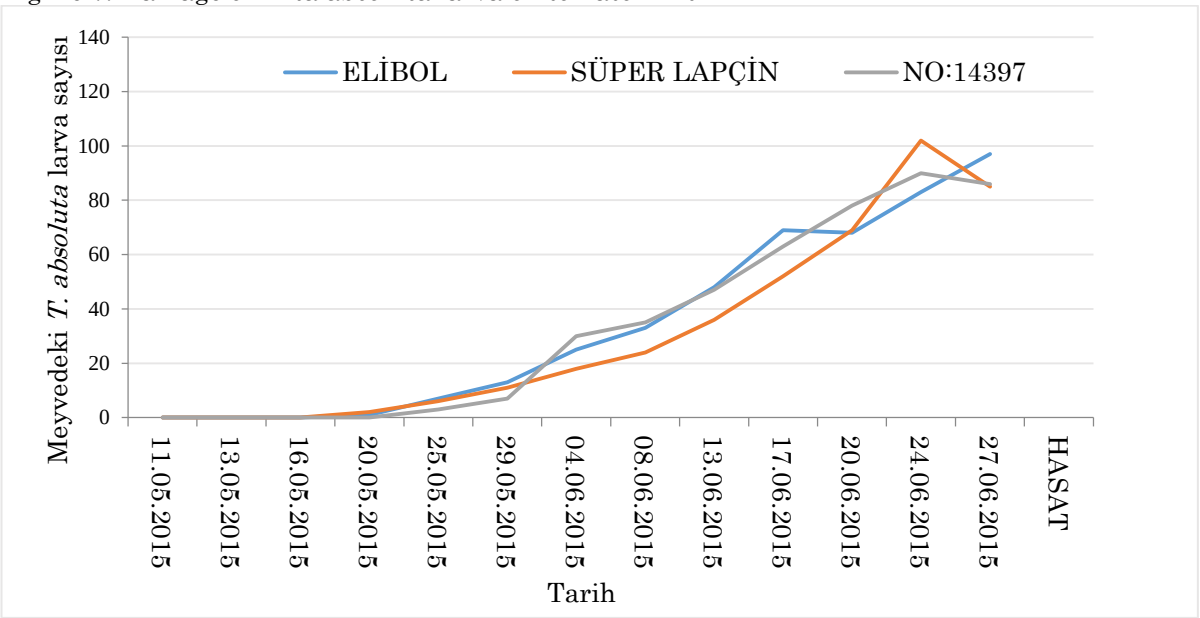
Şekil 6. *T. absoluta*' nın 2016 yılında Elibol ve Süper Lapçin çeşitlerindeki gövde üzerindeki popülasyon yoğunluğu

Figure 6. Population density of *T. absoluta* on stem in Elibol and Super Lapçin cultivars in 2016



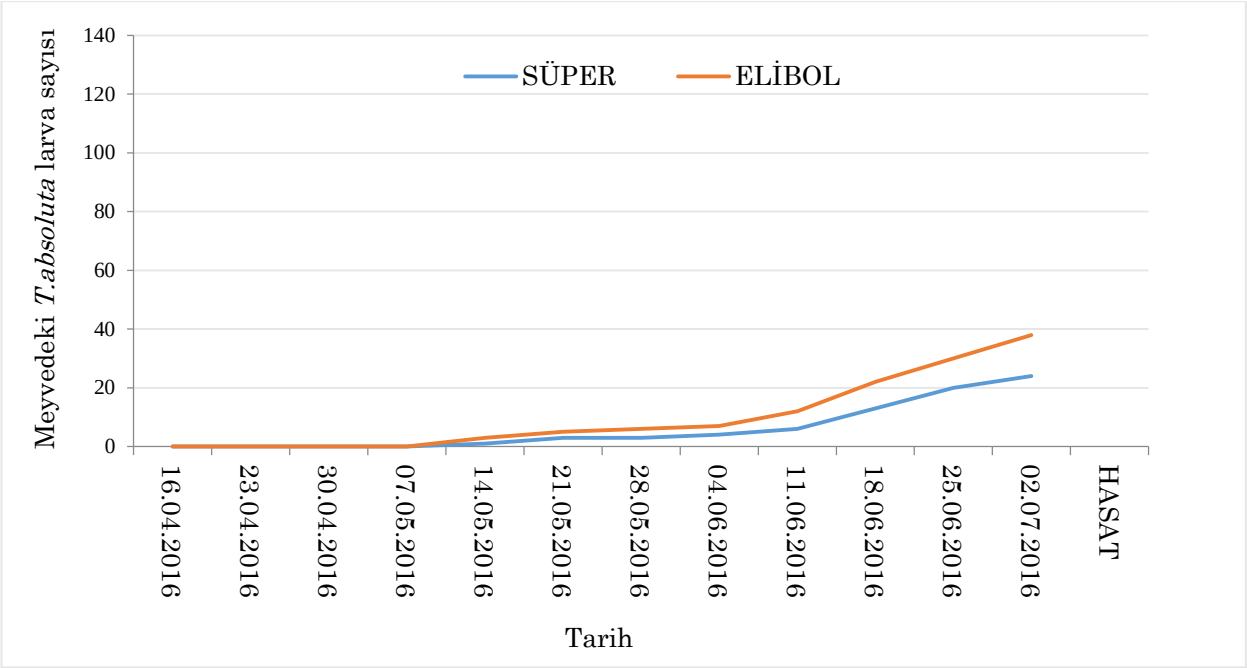
Şekil 7. *Tuta absoluta* larvasının domates meyvesindeki zararı

Figure 7. Damage of *Tuta absoluta* larva on tomato fruit



Şekil 8. *T. absoluta*'nın 2015 yılında Elibol, Süper Lapçin ve No:14397 çeşitlerindeki meyve üzerindeki popülasyon yoğunluğu

Figure 8. Population density of *T. absoluta* on fruit of Elibol, Super Lapçin and No:14397 cultivars in 2015



Şekil 9. *T. absoluta*'nın 2016 yılında Elibol ve Süper Lapçin çeşitlerindeki meyve üzerindeki popülasyon yoğunluğu

Figure 9. Population density of *T. absoluta* on fruit in Elibol and Super Lapçin cultivars in 2016

Table 2. Total numbers of *Tuta absoluta* on tomato plants in 2015

Çizelge 2. *Tuta absoluta*'nın 2015 yılındaki domates bitkisi üzerindeki toplam sayıları

	Elibol	Süper Lapçin	No:14397
Yaprak	763	715	616
Gövde	395	426	439
Meyve	444	405	439
Toplam	1602	1546	1494

Domates çeşitlerinde 2016 yılında ise elde edilen toplam larva sayısı yapraklarda sırasıyla Elibol ve Süper Lapçin de 438 ve 352 olmuştur. Gövde de ise 82 ve 61 olurken, meyve de 123 ve 74 olmuştur. Toplamda zararlı larva sayısı Elibol çeşidin de olurken bunu Süper Lapçin çeşidi takip etmiştir. *T. absoluta*'nın 2015 yılında olduğu gibi 2016 yılında zararlının daha çok yaprak kısmını tercih ettiği tespit edilmiştir (Çizelge 3).

Table 3. Total numbers of *Tuta absoluta* on tomato plants in 2016

Çizelge 3. *Tuta absoluta*'nın 2016 yılındaki domates bitkisi üzerindeki toplam sayıları

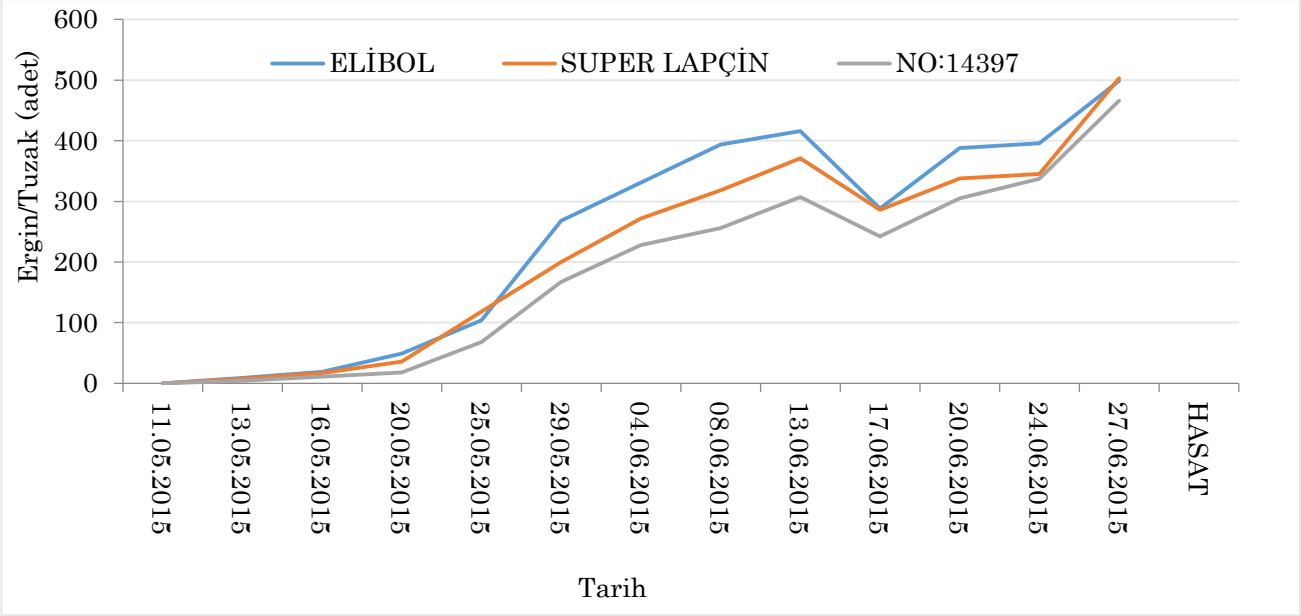
	Elibol	Süper Lapçin
Yaprak	438	352
Gövde	82	61
Meyve	123	74
Toplam	643	487

Tuta absoluta'nın ergin populasyon yoğunluğu

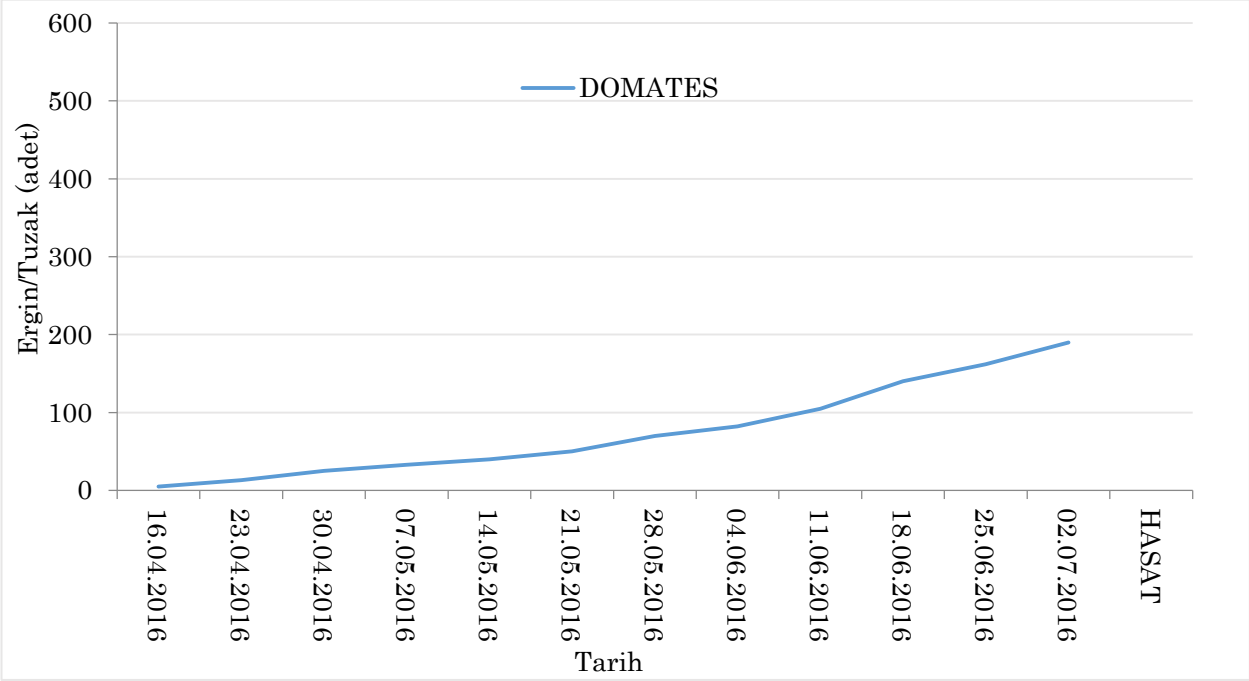
Çalışmada *Tuta absoluta*'nın 2015 yılındaki delta tuzaktaki zararlı sayımında sırasıyla Elibol sırasındaki tuzakta 1108, Süper Lapçin sırasındaki tuzakta 1098, No:14397 sırasındaki tuzakta 1026 adet ergin birey yakalanmıştır. Tuzaklarda yakalan ergin bireyler 17 Haziran tarihinde düşüş göstererek hasata kadar artan periyotlarla devam etmiştir (Şekil 10).

Çalışmada 2016 yılında ise *Tuta absoluta*'nın delta tuzaklardaki zararlı sayımı domates çeşitleri Elibol ve Süper Lapçin de takip edilmiştir. Sıra ortasında konan delta tuzaktaki yakalanan toplam *T. absoluta* ergin sayısı domates çeşitlerinde 915 olmuştur (Şekil 11).

Çalışma sonucuna göre çeşitler arasında *T. absoluta* zararı farklılık göstermiştir. 2015 yılında en fazla *T. absoluta* zararına uğrayan çeşit bitki başına ortalama 16.02 adet birey bulunan Elibol olmuştur. Bunun da nedeni Elibol çeşidi diğer çeşitlere göre yaprak ayalarının daha geniş ve meyvelerinin daha iri olmasından kaynaklanmaktadır (Şekil 12). Torres et al. (2001) yaptıkları çalışmada ise *T. absoluta*'nın çiçeklenme öncesi dönemde dişilerin bitkinin üst kısımlarındaki yaprak altlarını tercih ettiği ancak çiçeklenme sonrası ve meyve gelişimi sonrası dönemlerde bitkinin üst, orta ve alt kısımlarında yaprak saplarına bırakılan yumurta sayılarının eşit olduğunu bildirmişlerdir. Elibol çeşidine göre biraz küçük meyvelere sahip olan Süper Lapçin çeşidinde ise bitki başına 15.46 adet zararlı bulunmuştur. Yaprak ayası ve meyveleri en küçük olan No:14397 çeşit daha az zarara uğramıştır. Bitki başına ortalama 14.94 adet zararlı bulunmuştur (Şekil 12).



Şekil 10. 2015 yılında Eşeysel Çekici Feromon delta tuzaklarda yakalanan ergin *T. absoluta* sayısı
Figure 10. Number of adult *T. absoluta* caught in Sexual Attractive Pheromone delta traps in 2015

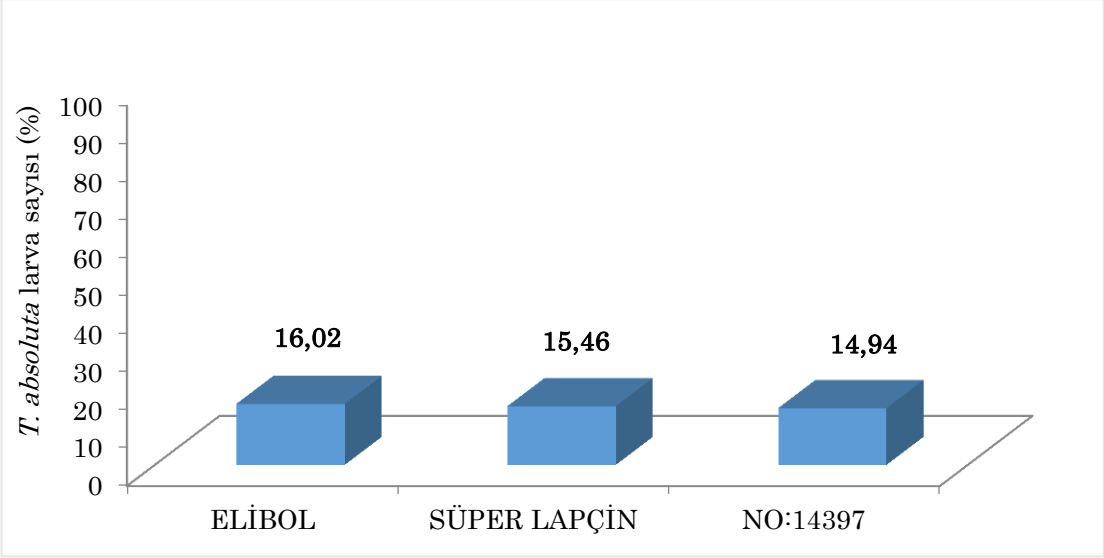


Şekil 11. 2016 yılında Eşeysel Çekici Feromon delta tuzaklarda yakalanan ergin *T. absoluta* sayısı
Figure 11. Number of adult *T. absoluta* caught in Sexual Attractive Pheromone delta traps in 2016

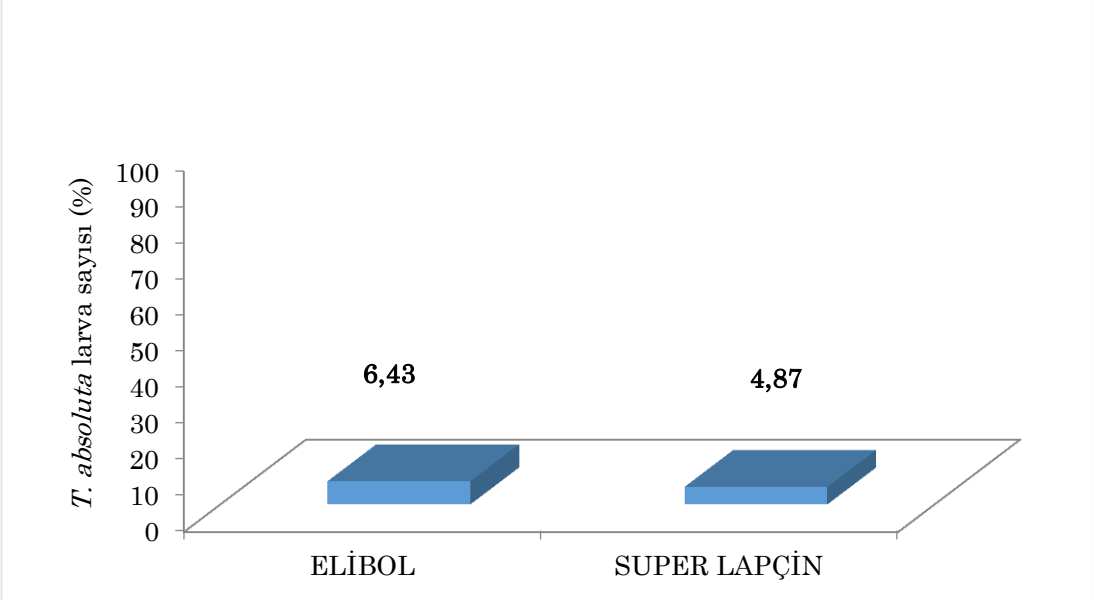
Tuta absoluta'nın 2016 yılında ise yine denemeye konu olan Elibol çeşidinde, Süper Lapçin çeşidine göre daha fazla *T. absoluta* zararlısı bulunmuştur. Elibol çeşidinde bitki başına ortalama 6.43 adet zararlı, Süper Lapçin çeşidinde ise bitki başına ortalama 4.87 adet larva bulunmuştur (Şekil 13).

Çekin & Yaşar (2015) çalışmasında *T. absoluta*'nın Newton, Caracas, Torry ve Şimşek domates çeşitlerinden en fazla Torry en az ise Şimşek domates çeşidini tercih ettiğini belirtmişlerdir. Milas (Muğla)'da tarla koşullarında yetiştiriciliği yapılan *Solanum lycopersicum* L. 5656 (VO-506), BT-236, BT-Tokat F1 çeşitleri ile geleneksel Pembe domates çeşidinin *T. absoluta*'nın ergin öncesi dönemlerinin popülasyon yoğunluklarına etkilerini belirledikleri çalışmada, tüm sonuçları bir arada değerlendirdiklerinde *T. absoluta*'ya karşın en dayanıklı çeşit Pembe olurken bunu 2014 yılı üretim sezonunda 5656, BT-236, BT-Tokat; 2015 yılı üretim sezonunda ise BT-236, 5656, BT-Tokat çeşitleri

izlemiştir (Türkmen & Kazak, 2021). Batı Akdeniz Bölgesi'nde *T. absoluta*'nın bulaşma alanlarını ve popülasyon dalgalanmasını Gazipaşa, Alanya, Manavgat, Serik, Antalya–Merkez, Kumluca, Demre ve Kaş ilçelerinde örtü altı ve açık domates üretim alanlarda delta tipi feromon tuzaklarda tuzak başına en az 5 ergin (20 Ağustos 2010, Manavgat) en fazla 240 ergin (22 Mart 2010, Antalya-Merkez) olduğunu saptamışlardır (Tatlı & Göçmen, 2011). Karut ve ark. (2011) ise 2009-2010 yılları arasında Mersin ilinin domates seralarını kontrol ederek 88 seradan 72'sinde tespit etmişler ve bitki başına vuruk meyve oranını en yüksek %38, 4 olarak bildirmişlerdir. Ayrıca Özkan & ark. (2017) sera üretim mevsiminde Çumra (Konya) ilçesinde 2011-2012 yıllarında *T. absoluta*'nın mücadelesinde feromon ve ferolite tuzaklarının etkinliğini tespit etmek amacıyla yürüttükleri çalışmada ferolite tuzaklarındaki ergin birey sayısının, eşey feromon tuzaklarındaki ergin birey sayısından iki kat fazla olduğunu ve sonuç olarak, ferolite tuzaklarına, hem erkek hem de dişi bireyler gelmesinden dolayı feromon tuzaklarına göre daha etkili olduklarını, *T. absoluta*'nın mücadelesinde ferolite tuzakların kitle yakalama tekniği için başarıyla kullanılabileceğini, eşey feromonlarıyla popülasyonunun belirlenip azaltılabileceği ve böylelikle bulaşıklık oranının, ergin sayıları azaltılarak düşürülebileceğini bildirmişlerdir.



Şekil 12. *T. absoluta*'nın domates çeşitlerinde 2015 yılında bitki başına bulaşma oranı
Figure 12. Infestation rate of *T. absoluta* per plant in tomato cultivars in 2015



Şekil 13. *T. absoluta*'nın domates çeşitlerinde 2016 yılında bitki başına bulunma oranı
Figure 13. Infestation rate of *T. absoluta* per plant in tomato cultivars in 2016

Genel olarak *T. absoluta*'nın domates bitkisini tercih ettiği görülmüştür. Gahramanovo & Mamay (2020) Azerbaycan'da örtüaltı domates ve patlıcan yetiştiriciliğinde *T.absulata*'nın zararlı olduğunu, domateste zararlının daha erken dönemde ve %100 gibi yüksek bir bulaşıklık oranına yükseldiğini, patlıcandaki zararının ise geç dönemde görülse de dikkatle takip edilmesi gerektiğini bildirmişlerdir. Nitekim Karabüyük et al. (2011) tarafından yapılan çalışmada *T. absoluta*'nın patlıcan, köpek üzümü ve tarla sarmaşığına nazaran domatesi daha fazla tercih ettiğini bildirmişlerdir. Sıcaklık ve nemin etkisinin zararlının popülasyon yoğunluğuna etkisi yüksek olmuştur. Nitekim Mamay & Yanık (2012) zararlının yaz aylarındaki zararının sıcaklıkla ilişkili olarak ve doğrusal bir şekilde arttığını bildirmişlerdir.

SONUÇ VE ÖNERİLER

Tuta absoluta zararlısının larvası yaprak, gövde ve meyvede açtığı galerilerde ileriki zamanlarda sekonder mikroorganizmaların gelişimine ortam hazırladığı, bunun sonucunda da meyvelerde çürüme ve bitkide kurumalara sebep olduğu belirlenmiştir.

T. absoluta ile mücadelede ancak entegre mücadele programının doğru bir şekilde uygulanması halinde başarıya ulaşabileceği tespit edilmiştir. Kültürel önlemler, biyoteknik mücadele, biyolojik mücadele ve kimyasal mücadele tek başına yeterli olmamaktadır. Bu çalışmada zararlı popülasyonu izleme amaçlı tuzaklar kullanılmıştır. Popülasyon durumuna göre kitlesel yakalama amaçlı tuzakların kullanılması ve geliştirilmesinin ve domates yetiştiriciliğinde yaprak ayası daha küçük olan çeşitlere yer verilmesinin *T. absoluta* ile mücadele de önemli olduğu belirlenmiştir.

Araştırmacıların Katkı Oranı Beyan Özeti

Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan ederler.

Çıkar Çatışması Beyanı

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

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Diyarbakır, Elazığ ve Muş İlleri (Türkiye) Sebze Alanlarında Saptanan Predatör Akar (Acari: Phytoseiidae) Türleri

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ÖZET

Bu çalışma Diyarbakır, Elazığ ve Muş illerinde 2018-2019 yıllarında; biber, domates, fasulye, hıyar, kabak, karpuz, kavun ve patlıcan yapraklarından predatör akar türlerini tespit etmek amacı ile yapılmıştır. Sebzelerden alınan 1063 adet bitki örneğinin 676 adedi akarlar ile bulaşık olarak tespit edilmiştir. Bu bitki örneklerinin %10,65' nde ise Phytoseiidae familyasına ait predatör akar türleri tespit edilmiştir. Tespit edilen faydalı akarlar; *Neoseiulus barkeri* Hughes, *Neoseiulus bicaudus* Wainstein, *Neoseiulus marginatus* (Wainstein), *Neoseiulus zwoelferi* (Dosse), *Neoseiulus* sp., *Phytoseius finitimus* Ribaga, *Proprioseiopsis messor* (Wainstein), *Typhlodromus* (*Anthoseius*) *rhenanus* (Oudemans) ve *Typhlodromus* (*Anthoseius*) *recki* (Wainstein) türleridir. *Neoseiulus barkeri* ise bu türler içerisinde %57.44 oranı ile tespit edilen en yaygın tür olmuştur. Araştırmada Phytoseiidae akarlarının en çok saptandığı kültür bitkileri ise %60,59 ve %15.94 ile sırasıyla *Cucumis sativus* ve ve *Solanum melongena* L.' dır. En az akar saptanan sebze ise % 1.06 ile *Capsicum annuum* L.' dur.

Bitki Koruma

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Anahtar Kelimeler
Predatör akar
Neoseiulus
Typhlodromus
Diyarbakır
Cucumis sativus

Predatory mites (Acari: Phytoseiidae) on Vegetable Fields in Diyarbakır, Elazığ and Muş Provinces, Turkey

ABSTRACT

This study was conducted to identify predatory mite species on pepper, tomato, bean, cucumber, pumpkin, watermelon, melon, and eggplant plant leaves between 2018 and 2019 Diyarbakır, Elazığ, and Muş provinces. 1063 plant samples were collected and 676 of these samples which were observed with mite species. Predatory mite species were identified belonging to the Phytoseiidae family, which constitues 10.65% of 676 plant samples. The identified predatory mites were listed as *Neoseiulus barkeri* Hughes, *Neoseiulus bicaudus* Wainstein, *Neoseiulus marginatus* (Wainstein), *Neoseiulus zwoelferi* (Dosse), *Neoseiulus* sp., *Phytoseius finitimus* Ribaga, *Proprioseiopsis messor* (Wainstein), *Typhlodromus (Anthoseius) bagdasarjani* Wainstein and Arutunjan, *Typhlodromus (Anthoseius) rhenanus* (Oudemans) and *Typhlodromus (Anthoseius) recki* (Wainstein) in this study. *Neoseiulus barkeri* wasdetected as the most common species with 57.44% total number of individuals in the three provinces. The most detected host plants were *Cucumis sativus* L. at 60.59%; *Solanum melongena* L. (Family name) at 15.94% while the least detected vegetable was *Capsicum annuum* L. at 1.06% percentages.

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INTRODUCTION

The vegetable yield in Turkey was 25,041,025 tonnes in the 592.937 hectares based on the Turkish Statistical Institute (TUIK) in 2019. Specifically,

Diyarbakır, Elazığ, and Muş provinces sustained 21.409 hectares of production areas and 667,755 tons' yield (TUIK 2019). These locations meet 2.66% of the country's overall demand. The production of vegetable is ordinarily carried out the in backyard of houses as a small business. Also, Diyarbakir is one of the most popular cities in Turkey concerning watermelon production (TUIK 2019).

The growers generally prefer the application of the chemicals because of getting higher yields in a short time. As known, the side effects of the chemical applications are harmful on non-target organisms, such as humans, animals, other beneficial pests and the environment. Therefore, the growers should be guided about management strategies in order to not disrupt the natural balance. Biological control is one of management strategies that is susceptible to environmental, human, and animal health. Besides, it is a suitable method for sustainable agriculture techniques. The main biocontrol agents for pest control are entomopathogens, predators, and parasitoids (Kılınçer et al. 2010). For this purpose, the Phytoseiidae predatory mites should be revealed and studied to increase their efficiency.

There are 2,692 identified species (including synonyms) belonging to the Phytoseiidae family so far (Demite et al. 2014). These species are included in the integrated pest management programs as a promising alternative source for pesticides. Phytoseiidae species are the largest group in commercially possible mite biocontrol agents, about 20 species offered globally.

Amblyseius swirskii Athias-Henriot *(Acari: Phytoseiidae), Phytoseiulus persimilis* Athias-Henriot *(Acari: Phytoseiidae), Neoseiulus cucumeris* (Oudemans) *(Acari: Phytoseiidae),* and *N. californicus* (McGregor) *(Acari: Phytoseiidae)* are the most recognized species, comprising the more significant part of the whole arthropod biocontrol agent market (Knapp et al. 2018).

Biocontrol-based studies show that *Phytoseiidae* sustains excellent potential for being used against *Tetranychus urticae* Koch (Acari: Tetranychidae), (Sarwar et al. 2011). *Neoseiulus* species are also promising predators as a part of such programs (Döker 2019). One of the most notable factors for suppressing phytophagous mites is Phytoseiidae species which was detailed by many researchers (Düzgüneş 1963; Çobanoğlu 1989; Çobanoğlu 2002; Zhang 2003; Kasap 2020; Yeşilayer & Çobanoğlu, 2011). In Turkey, 19 genera belonging to Amblyseiinae, Phytoseiinae, and Typhlodrominae subfamilies, and three sub-genera belonging to 85 phytoseiid mite species were determined (Döker et al. 2014; 2015). The number of species has exceeded 90 up until now (Döker 2019).

Objective of this study was to determine the phytoseiid species feeding on phytophagous mites in vegetable fields of Diyarbakır, Elazığ, and Muş territories. The samples collected sites and coordinates are shown in Table (1). The use of pesticides was rare, except some commercial production areas of Muş province. In general, farming wascarried out in the backyard of the houses. Which were considered valuable place for a comprehensive study on predatory mites for the first time in Elazığ, and Muş provinces.

There has been no study directly targeting predator mites in vegetable areas in related region. Yaman et al. (2018) identified *Phytoseius finitimus* (Ribaga) (Mesostigmata: Phytoseiidae) in the vegetable areas of Diyarbakır and Mardin provinces.

Table 1. The Coordinates of the studied locations of the Diyarbakır, Elazığ, and Muş provinces.
Çizelge 1. *Çalışmanın yapıldığı* Diyarbakır*, Elazığ ve Muş illeri' nin koordinatları.*

Province	Location	Geographic coordinates (N and E)	Altitude (m)
Diyarbakır	Bismil	37° 50′ 58′′ 40° 40′ 07′′	546
	Çermik	38° 08′ 06′′ 39° 27′ 21′′	688
	Çınar	37° 40′ 06′′ 40° 16′ 19′′	806
	Eğil	38° 15′ 26′′ 40° 04′ 51′′	848
	Ergani	38° 16′ 04′′ 39° 45′ 42′′	932
	Central district	37° 55′ 29′′ 40° 12′ 39′′	688
Elazığ	Baskil	38° 32′ 06′′ 38° 39′ 12′′	1.276
	Maden	38° 26′ 39′′ 39° 37′ 37′′	1.155
	Sivrice	38° 26′ 49′′ 39° 18′ 33′′	1.274
	Central district	38° 40′ 28′′ 39° 13′ 21′′	1.060
Muş	Hasköy	38° 41′ 00′′ 41° 41′ 26′′	1.278
	Korkut	38° 44′ 18′′ 41° 47′ 08′′	1.312
	Central district	38° 44′ 04′′ 41° 29′ 28′′	1.396

MATERIALS and METHODS

The materials of this study were *Capsicum annuum* L. (Solanales: Solanaceae), *Cucumis sativus* L. (Cucurbitales: Cucurbitaceae), *Cucumis melo* L. (Cucurbitales: Cucurbitaceae), *Cucurbita pepo* L. (Cucurbitales: Cucurbitaceae), *Citrullus lanatus*

(Thunb.) Matsum. & Nakai (Cucurbitales: Cucurbitaceae), *Solanum melongena* L. (Solanales: Solanaceae), *Solanum lycopersicum* L. (Solanales: Solanaceae) and *Phaseolus vulgaris* (Fabales: Fabaceae) fields in Diyarbakır, Elazığ and Muş provinces (Figure 1).

Samples were taken randomly from related areas. The primary material in this study was Phytoseiidae family predators collected from the vegetable leaves.

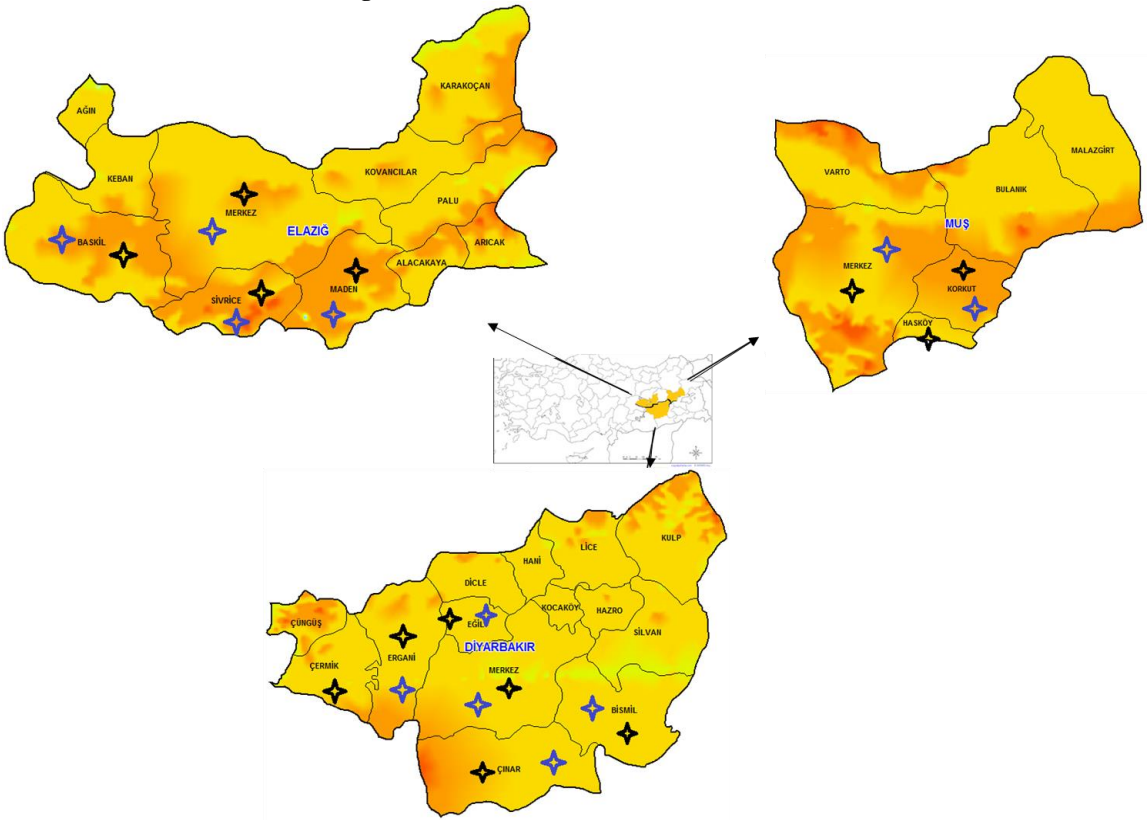

Figure 1. Sampling areas in Diyarbakır, Elazığ and Muş provinces (✦ Sampling districts✦, Phytoseiidae found districts).

Şekil 1: *Diyarbakır, Elazığ ve Muş illerindeki örnekleme alanları (✦ Örneklenen ilçeler,✦ Phytoseiidae bulunan ilçeler).*

The samples were collected during a 14 day vegetation period between April and October of 2018-2019.

The leaves were taken different level and direction of the plants including, lower, middle, and top of the host plants. If the plant morphology was consisted of the small plates, 50 or 60 samples were taken for each 0.1-hectare area. On the other hand, if plants have big leaves such as eggplant and cucumber, 20 or 30 leaves were taken for a 0.1-hectare area (Bora & Karaca 1970; Can & Çobanoğlu 2010; Çobanoğlu & Kumral 2014). Collected samples were first placed paper and then polyethylene bags and delivered to the Diyarbakır Plant Protection Research Institute laboratory (Diyarbakır) in the iceboxes. The plant materials were examined under the stereoscopic microscope to collect phytoseiid mite species. After first examination with stereoscopic microscope, overlooked leaf samples were transferred into Berlese system for extraction. The Acari class species were kept 70% ethyl alcohol until microscopic slides. Then, the individuals were transferred into Lactophenol solution for cleaning (Çobanoğlu & Kumral 2014; Kasap & Çobanoğlu 2009). After cleaning they were prepared with in Hoyer's medium and placed on the slides and incubated at 50-60 ºC' for 48 hours. The slides were sealed by nail polish to prevent possible air escape (Düzgüneş 1980).

Regardless of whether they were infected with *T. urticae* during the sampling. Mite species were identified with help of Prof. Dr. Sultan ÇOBANOĞLU at the Department of Plant Protection, Ankara University. Literatures were used in diagnoses: Kolodochka (1978); Çobanoğlu (1993 a,b,c); Moraes et al. (2004); Chant & McMurtry (2007); Papadoulis et al. (2009); Faraji et al. (2011).

RESULT and DISCUSSION

In this study ten predatory mites including *Neoseiulus barkeri* Hughes (Acari: Phytoseiidae), *N. bicaudus* Wainstein (Acari: Phytoseiidae), *N. marginatus* (Wainstein) (Acari: Phytoseiidae), *N.*

zwoelferi (Dosse) (Acari: Phytoseiidae), *Neoseiulus* sp. (Acari: Phytoseiidae), *P. finitimus*, *Proprioseiopsis messor* (Wainstein) (Acari: Phytoseiidae), *T. (A.) bagdasarjani* Wainstein and Arutunjan (Acari: Phytoseiidae), *T. (A.) rhenanus* (Oudemans) (Acari: Phytoseiidae) and *T. (A.) recki* (Wainstein) (Acari: Phytoseiidae) were identified (Table 2). The presence rate of the Phytoseiidae mites were calculated on randomly collected samples and formulated as bellow;

The presence rate= the individual numbers of Phytoseiidae species X 100 / Total number of individuals.

Also, five species relate to *Neoseiulus*, and three species relating to *T. (Anthoseius)* genera were identified as the most beneficial predatory mites (Table 2)., *N. barkeri* was detected as the most common species with 57.44% total number of individuals in the three provinces. *Neoseiulus bicaudus* was the second most common species with an 11.17%. The following percentages of species are *N. marginatus* 10.10%, *T. (A.) recki* 5.31%, *Phytoseius finitimus* 4.78%, *Neoseiulus sp.* 4.78%, *N. zwoelferi* 3.72%, *T. (A.) rhenanus* 1.59%, *P. messor* 0.53%, and *T. (A.) bagdasarjani 0.*53% in table 2. The table 2 shows that the most common host plant is *C. sativus* with 114 specimens, while the least preferred host is *C. annuum* with two samples. Also, the most common phytoseiid species is *N. barkeri*, with a 57.44 % ratio. Conversely, the rarest seen species are *T. (A.) bagdasarjani*, and *P. messor* 0.53% ratio.

Table 2. Phytoseiid mite species and range of the host plants.
Çizelge 2. *Phytoseiidae akar türleri ve kültür bitkilerinde bulunma oranları.*

Genera In	Mite Species	Host Plants*								Total Ratio (%)	
		1	2	3	4	5	6	7	8		
Phytoseiidae	*Neoseiulus barkeri*	76	14	1	-	1	11	-	5	108	57.44
	Neoseiulus bicaudus	-	13	-	-	6	-	2	-	21	11.17
	Neoseiulus marginatus	17	-	-	-	-	-	2	-	19	10.10
	Neoseiulus zwoelferi	7	-	-	-	-	-	-	-	7	3.72
	Neoseiulus sp.	4	2	-	-	-	1	2	-	9	4.78
	Typhlodromus (A.) bagdasarjani	1	-	-	-	-	-	-	-	1	0.53
	Typhlodromus (A.) recki	7	-	-	-	3	-	-	-	10	5.31
	Typhlodromus (A.) rhenanus	-	-	-	-	2	1	-	-	3	1.59
	Phytoseius finitimus	2	1	1	-	5	-	-	-	9	4.78
	Proprioseiopsis messor	-	-	-	-	1	-	-	-	1	0.53
In Total		114	30	2	0	18	13	6	5	188	100

*1. *C. sativus* 2. *S. melongena* 3. *C. annuum* 4. *S. lycopersicum* 5. *C. pepo* 6. *P. vulgaris* 7. *C. lanatus* 8. *C. melo*

Family: PHYTOSEIIDAE

Subfamily: AMBLYSEIINAE MUMA

Genus: *Neoseiulus* Hughes

Species: *Neoseiulus barkeri* Hughes

Examined Materials: Diyarbakır: Bismil, 5.07.2018, *P. vulgaris* (1♀); Diyarbakır: Bismil, 5.07.2018, *S. melongena* (1♀); Diyarbakır: Bismil, *C. sativus* (5♀♀ 2♂♂); Diyarbakır: Bismil, 19.07.2018, *S. melongena* (4♀♀ 1♂); Diyarbakır: Bismil, 19.07.2018, *C. melo* (1♀); Diyarbakır: Bismil, 19.07.2018, *C. sativus* (5♀♀); Diyarbakır: Bismil, 9.10.2018, *C. annuum* (1♀); Diyarbakır: Bismil, 2.08.2019, *C. sativus* (11♀♀ 3♂♂); Diyarbakır: Central District, 04.10.2018, *S. melongena* (2♀♀); Diyarbakır: Çınar, 5.07.2018, *C. sativus* (15♀♀ 2♂); Diyarbakır: Çınar, 5.07.2018, *C. melo* (4♀); Diyarbakır: Çınar, 5.07.2018, *S. melongena* (6♀♀); Diyarbakır: Çınar, 01.08.2018, *C. sativus* (3♀♀ 1♂); Diyarbakır: Çınar, 15.10.2018, *C. sativus* (26♀♀ 3♂♂); Diyarbakır: Ergani, 21.06.2018, *P. vulgaris* (6 ♀♀); Diyarbakır: Ergani, 12.09.2018, *P. vulgaris* (1♀); Elazığ: Baskil, 07.08.2018, *C. pepo* (1♀); Muş: Central District, 10.09.2019, *P. vulgaris* (2♀♀ 1♂).

Distribution of Turkey: Adapazarı, Ankara, Antalya, Aydın, Bursa, Çanakkale, Diyarbakır, Edirne, Istanbul, Izmir, Ordu, Samsun, Şanlıurfa (Çıkman 1995; İnal 2005; Faraji et al. 2011; Kılıç et al. 2012; Kasap et al. 2013; Çobanoğlu & Kumral 2014; Ölmez et al. 2015; Kutlu 2016; Soysal & Akyazı 2018).

Distribution the World: Algeria, Argentina, Australia, Benin, Brazil, Burundi, Cape Verde, Chile, Canary Islands, China, Cyprus, Egypt, Finland, France, former USSR (Georgia, Ukraine), England, London Dock, Germany, Ghana, Greece, Guinea, Hawaii, Iran , Israel, Italy, Japan, Jordan, Madagascar, Netherlands, Latvia, Malawi, Mayotte Island, Morocco, Mozambique, Oman, Portugal, Kenya, Japan, Norway, Nigeria, South Korea, South Africa, Spain, Sweden, Turkey, Yemen, Tunisia, Thailand, North Africa, New Jersey, Senegal, Rodriguez Island, Syria and the United States (California) (Athias-Henriot 1966; Hughes 1976; Papadoulis et al. 2009; Demite et al. 2020).

Species: *Neoseiulus bicaudus* Wainstein

Examined Materials: Diyarbakır: Çınar, 3.06.2018, *S. melongena* (11♀♀ 2♂♂); Elazığ: Baskil, 17.08.2018, *C. pepo* (5♀♀ 1♂); Elazığ: Maden, 30.07.2019, *C. lanatus*

(2♀♀).

Distribution of Turkey: Ankara, Aydın, Bursa, Izmir, Ordu, Samsun, Yalova (İnal 2005; Faraji et al. 2011; Çobanoğlu & Kumral 2014; Soysal & Akyazı 2018).

Distribution the World: France, former USSR (Armenia, Azerbaijan, Georgia, Caucasus Region, Kazakhstan, Moldova, Krasnodar region), Greece, Hungary, Iran, Latvia, Mexico, Portugal, Israel, Italy, Norway, Chile, Egypt, Saudi Arabia, Serbia, Slovakia, Spain, Switzerland, Syria, Tajikistan, Tunisia, Turkey and the United States (Papadoulis et al. 2009; Demite et al. 2020).

Species: *Neoseiulus marginatus* (Wainstein)

Examined Materials: Diyarbakır: Bismil, 19.07.2018, *C. sativus* (2♀); Diyarbakır: Bismil, 19.07.2018, *C. sativus* (1♀); Diyarbakır: Central District, 18.07.2019, *C. sativus* (1♀); Diyarbakır: Çınar, 15.10.2018, *C. lanatus* (1♀); Elazığ: Baskil, 17.08.2018, *C. lanatus* (1♀); Elazığ: Central District, 16.07.2018, *C. sativus* (7♀♀ 3♂♂); Elazığ: Central District, 11.10.2018, *C. sativus* (2♀♀); Elazığ: Maden, 23.07.2018, *C. sativus* (1♀).

Distribution of Turkey: Ankara (Faraji et al. 2011).

Distribution the World: Algeria, The Former Soviet Union (Armenia, Turkmenistan, Azerbaijan, Georgia, Kazakhstan, Moldova, Russia, Yaroslavl province, Ukraine), France, Greece, Hungary, Iran, Israel, Moldova, Moscow, Serbia, Turkey, Latvia and Kenya (Papadoulis et al. 2009).

Species: *Neoseiulus zwoelferi* (Dosse)

Examined Materials: Muş: Central District, 18.06.2019, *C. sativus* (6♀♀ 1♂).

Distribution of Turkey: Ankara, Erzurum, Hakkâri, Samsun (İnal, 2005; Faraji et al., 2011).

Distribution the World: Finland, the former USSR (Azerbaijan, Kazakhstan, Ukraine, Yaroslavl province, Russia, Moscow), the former Yugoslavia, Germany, Slovakia, Greece, Hungary, Latvia, Montenegro, Iran, Israel, Norway, Sweden, Switzerland, Turkey and the United States (Arizona, California, Ohio, Oregon, Pennsylvania and Wiskonsin) (Papadoulis et al. 2009; Demite et al. 2020).

Species: *Neoseiulus* sp.

Examined Materials: Diyarbakır: Bismil, 5.07.2018, *S. melongena* (2♀♀); Diyarbakır: Bismil, 19.07.2018, *C. sativus* (3♀♀1♂); Diyarbakır: Eğil, 4.09.2018, *C. lanatus* (2♀♀); Elazığ: Central District, 19.06.2018, *P. vulgaris* (1♀).

Genus: *Proprioseiopsis* Muma

Species: *Proprioseiopsis messor* (Wainstein, 1960)

Examined Materials: Elazığ: Sivrice, 10.09.2018, *C. pepo* (1♀).

Distribution of Turkey: Adapazarı, Aydin, Bursa, Çanakkale, Izmir (Faraji et al. 2011; Kasap et al. 2013; Çobanoğlu & Kumral 2014).

Distribution the World: Algeria, Argentina, Australia, former USSR (Armenia, Azerbaijan, Georgia, Turkmenistan, Ukraine), France, Egypt, New South Wales, Gaza Strip, Germany, Canary Islands, Greece, Hungary, Iran, Israel, Italy, Latvia, Morocco, Portugal, Saudi Arabia, Slovakia, Morocco, New Zealand, South Africa, Spain, Syria, Turkey and the United States. (Papadoulis et al. 2009; Demite et al. 2020).

Subfamily: PHYTOSEIINAE

Genus: *Phytoseius*

Species: *Phytoseius finitimus* Ribaga, 1904

Examined Materials: Diyarbakır: Bismil, 9.10.2018, *S. melongena* (1♀); Diyarbakır: Bismil, 9.10.2018, *C. annuum* (1♀); Diyarbakır: Eğil, 24.07.2019, *C. pepo* (2♀♀); Elazığ: Baskil, 17.09.2019, *C. pepo* (2♀♀ 1♂); Muş: Central District, 10.08.2018, *C. sativus* (2♀♀).

Distribution of Turkey: Adana, Adapazarı, Amasya, Ankara, Antalya, Amasya, Aydın, Balıkesir, Bolu, Burdur, Bursa, Çanakkale, Diyarbakır, Edirne, Erzincan, Giresun, Gümüşhane, Hakkâri, Icel, Isparta, Istanbul, Izmir, Kahramanmaraş, Kastamonu, Konya, Muğla, Niğde, Ordu, Rize, Samsun, Tekirdağ, Tokat (İnal 2005; Faraji et al. 2011; Özşişli & Çobanoğlu 2011; Kasap et al. 2013; Çobanoğlu & Kumral 2014; Soysal & Akyazı 2018; Yaman et al. 2018).

Distribution the World: Algeria, the Azores, Egypt, France, Greece, Iran, Israel, Italy, Montenegro, Morocco, Portugal, Slovenia, Spain, Syria, Tunisia, Turkey and the United States - California (Demite et al. 2020).

Subfamily: TYPHLODROMINAE

Genus: *Typhlodromus* (*Anthoseius*)

Species:
Typhlodromus (*Anthoseius*) *bagdasarjani* Wainstein and Arutunjan

Examined Materials: Diyarbakır: Ergani,15.08.2018, *C. sativus* (1♀).

Distribution of Turkey: Ankara, Hakkâri, Istanbul, Muğla, Van Lake (around). (Bayram & Çobanoğlu 2007; Inak & Çobanoğlu 2018; Kasap & Çobanoğlu 2009; Faraji et al. 2011; Özsisli & Çobanoğlu 2011; Yesilayer & Çobanoğlu 2011)

Distribution the World: Armenia, Azerbaijan, Iran, Turkey, Turkmenistan (Demite et al. 2020).

Species: *Typhlodromus* (*Anthoseius*) *recki* Wainstein, 1958

Examined Materials: Diyarbakır: Bismil, 19.07.2018, *C. pepo* (3♀♀); Diyarbakır: Bismil, 19.07.2018, *C. sativus* (2♀♀), Elazığ: Maden, 28.08.2018, *C. sativus* (1♀); Elazığ: Sivrice, 10.09.2018, *C. sativus* (3♀♀ 1♂).

Distribution of Turkey: Adapazarı, Amasya, Ankara, Amasya, Balıkesir, Burdur, Bursa, Çanakkale, Edirne, Gümüşhane, Içel, Isparta, Istanbul, Izmir, Kars, Kastamonu, Konya, Muğla, Nevşehir, Niğde, Samsun, Tekirdağ, Tokat, Yalova, Zonguldak (İnal 2005; Faraji et al. 2011; Kasap et al. 2013; Çobanoğlu & Kumral 2014; Kumral & Çobanoğlu 2015).

Distribution the World: Algeria, Cyprus, former USSR (Armenia, Azerbaijan, Caucasus Region, Georgia, Kazakhstan, Moldova, Russia, Ukraine), Austria, Slovenia, Cyprus, France, Greece, Hungary, Iran, Morocco, Portugal, Israel, Italy, Lebanon, Tunisia, Syria and Turkey. (Papadoulis et al. 2009; Demite et al. 2020).

Species: *Typhlodromus* (*Anthoseius*) *rhenanus* (Oudemans)

Examined Materials: Elazığ: Sivrice, 10.09.2018, *P. vulgaris* (1♀); Elazığ: Sivrice, 10.09.2018, *C. pepo* (2♀♀).

Distribution of Turkey: Antalya, Adapazarı, Erzurum (Faraji et al. 2011).

Distribution the World: Algeria, Belgium, Brazil, Canada, Cyprus, Denmark, England, Finland, France, former USSR (Azerbaijan, Belarus, Kazakhstan, Moldova, Russia, Ukraine), former Yugoslavia, Hungary, Germany, Greece, India, Iran, Ireland, Italy, Israel, Latvia, the Netherlands, Norway, Northern Ireland, Madeira Island, Portugal, Poland, Sweden, Switzerland, Slovakia, Slovenia, Spain, Syria, Turkey, and the USA (California, Illinois, Oregon, Virginia, Washington, Wisconsin) (Papadoulis et al. 2009; Demite et al. 2020).

CONCLUSION

As a result, 1063 plant samples were collected, and 676 of which were observed with mite species. On seventy-two plant samples predatory mite species were identified belonging to the Phytoseiidae family, which constitutes 10.65% of 676 plants. This ratio differs in each of the three provinces. When examined in terms of the total number of individuals; the distribution ratios of Phytoseiidae were 74.46%; 19.14%, and 6.38 % for Diyarbakır, Elazığ and Muş provinces, respectively (Table 3).

Table 3. Number of individuals of phytoseiid mite species in different Provinces in 2018 and 2019.
Çizelge 3. Phytoseiidae akar türlerinin 2018 ve 2019 yıllarında illerdeki birey sayıları.

Provinces	Years	1*	2	3	4	5	6	7	8	9	10	Total number of individuals	Ratio(%)
Diyarbakır	2018	90	13	4	-	8	1	5	-	2	-	123	74.46
	2019	14	-	1	-	-	-	-	-	2	-	17	
Elazığ	2018	1	6	14	-	1	-	5	3	-	1	31	19.14
	2019	-	2	-	-	-	-	-	-	3	-	5	
Muş	2018	-	-	-	-	-	-	-	-	2	-	2	6.38
	2019	3	-	-	7	-	-	-	-	-	-	10	
In Total		108	21	19	7	9	1	10	3	9	1	188	100

*1. *N. barkeri* 2. *N. bicaudus* 3. *N. marginatus* 4. *N. zwoelferi* 5. *Neoseiulus sp.* 6. *T. (A.) bagdasarjani* 7. *T. (A.) recki* 8. *T. (A.) rhenanus* 9. *P. finitimus* 10. *P. messor*

Overall, the host plants have a critical place in the study meaning that. Phytoseiidae species prefer different cultivated plants. At the same time, the rate of host plant preference reveals different numbers. For instance, *C. sativus*, with a 60.59% ratio, is the most preferred host plant. It is followed by; *S. melongena* 15.94%; *C. pepo* 8.96%; *P. vulgaris* 6.90%; *C. melo* 3.18%; *C. lanatus* 2.65% and *C. annuum* is 1.06 %(Figure 2). Interestingly, Phytoseiidae was not observed on the *Solanum lycopersicum* plants.

There is no other research for the identification of Phytoseiidae mite species in the region. However, some general fauna studies were completed prevously, such as Yaman et al. (2018); *P. finitimus* in Diyarbakır province, Ölmez-Bayhan et al. (2015); *N. barkeri* and *P. persimilis* in Diyarbakır province. Moreover, Kutlu (2016) was identified a related Phytoseiidae family since *P. finitimus*, *N. barkeri*, *Euseius finlandicus* Oudemans (Acari: Phytoseiidae) ve *N. californicus* in vegetable fields in Edirne, Turkey. Based on this study, the most common predatory mite species were described as *N. californicus* with a 42.15% appearance rate. Soysal & Akyazı (2018) identified 15 phytoseiid species and three predatory mite species including *N. barkeri*, *N. bicaudus*, *P. finitimus* in the vegetable fields in Ordu province. They indicated that predatory mites they found were comprise 21.8% of the general mite fauna.

Çobanoğlu (1989) reported *Amblyseius umbraticus* (Chant) (Acari: Phytoseiidae) in vegetable fields for the first time in Antalya. *Neoseiulus barkeri* and *T. (Anthoseius) rhenanus* species were also reported for the first-time in Turkey in the same study. Özşişli & Çobanoğlu (2011) were performed a Fauna study in vegetable and fruit fields in Kahramanmaras to extend their study. *Amblyseius andersoni* (Chant) (Acari: Phytoseiidae), *E. finlandicus*, *Paraseiulus triporus* (Chant and Yoshida-Shaul) (Acari: Phytoseiidae), *P. soleiger* (Ribaga) (Acari: Phytoseiidae), *Kampimodromus aberrans* (Oudemans) (Acari: Phytoseiidae), *P. subsoleiger*

Wainstein (Acari: Phytoseiidae), *T. (A.) bagdasarjani*, *P. finitimus,* and *T. (A.) intercalaris* (Livshitz-Kuznetsov) (Acari: Phytoseiidae) predatory species were reported. Based on this study, *E. finlandicus* was observed as the most common species in orchards. The same research has also shown that the phytoseiid mite fauna in Kahramanmaraş will contribute to future integrated management activities.

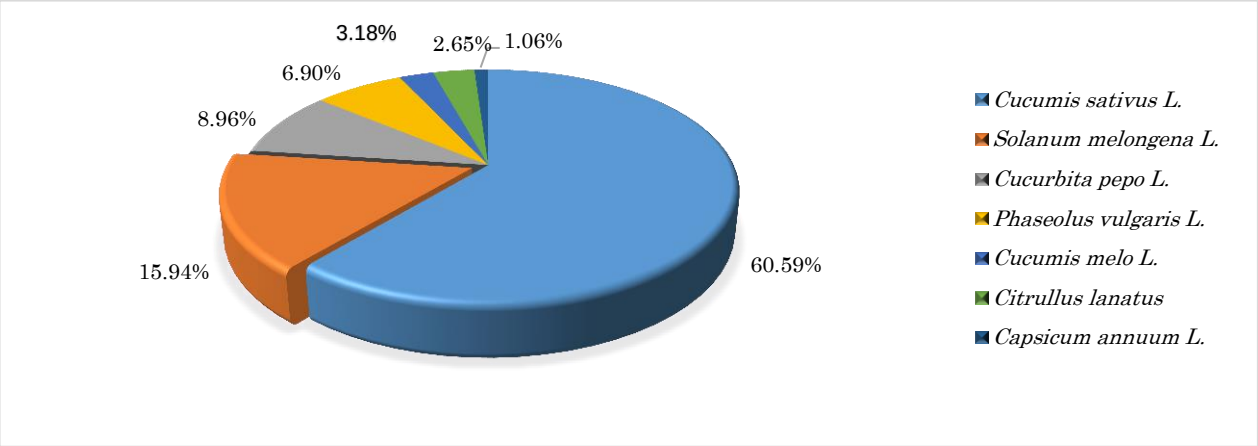

Figure 2. The host plants detected of Phytoseiidae species.
Şekil 2. *Phytoseiidae türlerinin saptandığı kültür bitkileri.*

This research proved that the predatory mite species are very promising for future biological management strategies, and these locations have significant predatory mite potential.

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Authors Contributions

The authors declare that they have contributed equally to the article.

Statement of Conflict of Interest

None of the authors had any financial or personal relationships with other individuals or organizations that might inappropriately influence their work during the submission process.

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Evaluations on Bioecology of *Contarinia pruniflorum* Coutin & Rambier, 1955 (Diptera: Cecidomyiidae)

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ABSTRACT
Apricot flower midge (*Contarinia pruniflorum* Coutin & Rambier Diptera: Cecidomyiidae) was first detected in *Prunus* species in the 1950s and did not become a significant pest for a long time. However, towards the 2000s, the pest caused damage to apricot and plum especially in Mediterranean countries and its distribution continued to increase. The population density of the pest is closely related to the climate of the agricultural area. In recent years, the fluctuation in climatic data causes the population fluctuations. The study was carried out in the Kale and the Yeşilyurt district of Malatya province (Turkey) in 2017-2018. Some biological stages of apricot flower midge were determined in Malatya province and these data were interpreted together with climatic data. It was determined that the pest overwinters as a pupa in the soil. In February, the adult emerges from the pupa with the warming of the air and soil temperatures. It was determined that the adult emerged from the pupa when the soil temperature was 13-14 °C and the air temperature was 7-15 °C. At the beginning of the pink-bud stage of apricot, the pests lay eggs into flower buds. The larval development is approximately 25-28 days. The average number of larvae in the damaged buds was 14, the maximum number of larvae in a bud was 26, and the pest gave an offspring annually and the ratio of male to female was 0.08.

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Contarinia pruniflorum Coutin & Rambier, 1955 (Diptera: Cecidomyiidae)'un Biyoekolojisi Üzerine Değerlendirmeler

ÖZET
Kayısı çiçek tomurcuğu sineği (*Contarinia pruniflorum* Coutin & Rambier Diptera: Cecidomyiidae) 1950 li yıllarda ilk olarak *Prunus* türlerinde tespit edilmiş olup uzun bir süre önemli bir zararlı olmamıştır. 2000'li yıllara doğru özellikle Akdeniz ülkelerinde kayısı ve erikde zarar oluşturmuş ve yayılış alanı artarak devam etmiştir. Zararlının popülasyon yoğunluğu yetiştiricilik yapılan alanın iklimi ile çok yakın ilişki içerisindedir. Son yıllarda iklimsel verilerdeki dalgalanma popülasyon seviyesinde de dalgalanmaya neden olmaktadır. Çalışma, 2017-2018 yıllarında Malatya ili Kale ve Yeşilyurt ilçelerinde yürütülmüştür. Çalışmada Malatya ilinde Kayısı çiçek tomurcuğu sineğinin özellikle mücadeleye esas bazı biyolojik dönemleri belirlenmiş ve bu veriler iklimsel verilerle birlikte yorumlanmıştır. Türün kışı toprakta pupa olarak geçirdiği, şubat ayı içerisinde hava (7-15 °C) ve toprak sıcaklıklarının (13-14 °C) ısınmasıyla birlikte erginin pupadan çıktığı tespit edilmiştir. Kayısının pembe tomurcuk döneminin başlarında çiçek tomurcukları içerisine yumurtasını bıraktığı, zarar görmüş tomurcuktaki ortalama larva sayısının 14 olduğu, bir tomurcukta görülen en fazla larva sayısının ise 26 adet olduğu ve yılda 1 döl verdiği, erkek bireylerinin dişi bireylere oranı ise 0.08 olarak belirlenmiştir.

Bitki Koruma

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INTRODUCTION

Contarinia pruniflorum Coutin & Rambier (Diptera: Cecidomyiidae) (Apricot flower midge) was firstly identified in *Prunus* species and some biological characters were determined (Coitin and Rambier, 1955). It was later seen in cultivated *Prunus* species in Czechoslovakia, Italy, Greece, and Turkey. Some morphological features, biological periods, pest control, and determination of cultivar preferences were carried out by researchers on this pest (Pollini and Bariselli, 1996; Montuschi et al., 2004; Tsagarakis and Mitsopoulus, 2007; Doğanlar et al., 2014; Gagne). , 20014; 2017). It was determined for the first time in Turkey that this pest damages apricots in Malatya (Kale district) and Mersin (Mut district) provinces (Doğanlar et al., 2014). It was firstly identified morphologically by molecular characterization using the COI gene sequence (Kaplan and İnal, 2021). The adults of the pest lay eggs on the flower buds of the tree, the hatched larvae feed on the bud wall, and the flower genitalia. The damaged flower does not turn into fruit.

Apricot is an important source of economic income for Malatya and its districts. Half of the 16 million apricot trees in Turkey are located in Malatya (Anonymous, 2020). This number is increasing every year. Turkey which produces 60% of the world's dried apricot is also the dominant country in apricot export (INC, 2019). Approximately 85% of dried apricot production in Turkey is produced in Malatya (Gündüz et al., 2021). In addition to many pest species threatening this source of income, species such as Apricot flower midge have recently emerged. It is not known whether the pest has been present in these areas before. It is also unknown whether the pest came from other regions to adapt to the ecology of this region or whether this pest is among the species found in the natural fauna of the region. Especially due to climatic reasons, the pest population has increased for a few years and it has caused serious damage, especially in the apricot fields of Malatya province and the necessity of pest control has emerged. The population density of the pest has changed due to the sudden temperature changes occurring in some biological ecological stages of the insect and the irregular climate over the years. The damage is very intense for some years, while some years are rarely seen. The fluctuation in the population worries producers and thus leads to unconscious pest control. Therefore, the study was carried out in order to determine some biological stages that can be the basis for pest control and to help control the pest correctly.

MATERIAL AND METHOD

The studies were carried out in 2017 and 2018 in an orchard with at least 20 Hacıhaliloğlu varieties (without pesticide application) between the ages of 12-15, in Kale district of Malatya, where the insect population is dense. It was carried out in the apricot orchard of the 15-year-old Hacıhaliloğlu variety in the Yeşilyurt Apricot Research Institute Central Campus in Malatya, Turkey, in 2018. In these orchards, a large number of mature larvae collected from the land in 2017 were placed on a certain area (2 m²) and 25x40 cm square prism above ground cages were established and observations and counts were made for a year. The time of adult emergence from the pupa, time to be seen in nature, time of egg-laying, time of larvae to exit from the bud, and the population growth under natural conditions were determined with survey studies, field observations, and taking the pest into culture cages. These studies were carried out under field conditions.

The determined biological stage data were compared with the meteorological data obtained from the climate station of the Meteorology Regional Directorate in Kale district and Yeşilyurt Campus of Apricot Research Institute. The results were analyzed and the relationship between climate data and biological stages was revealed.

Determining some biological terms

- Determination of adult emergence time from pupa; 25x40 cm square prism above-ground cages were placed on the soil surface at the crown level of apricot trees in January. The cages were checked daily in February, the counts were made and notes were taken. Four cages were used in the studies in the Kale district and eight cages were used in the Yeşilyurt district. In addition, soil temperatures (5 cm depth) were measured with Ituin KCB 300 Soil Survey Instrument between 1-2 pm which is the time when the soil temperature during the day may be high. The relationship between soil temperature and emerging time from pupa was revealed.

- Determination of time for the adult to be seen in nature; daily field observations were made and the trees were examined by eye to see when the insect began to appear in nature and how long it lasted in February-March.

- Determination of oviposition time; starting from the time before the insect was seen in nature, 200 apricot

flower buds were taken from the trees daily. The samples were brought to the laboratory and kept in 15x25 cm jars with water until the flowering term. In the laboratory, at room temperature (24 °C), at a relative humidity of 55-60%, the buds have been waited until the flowers were fully opened (10 days), then the flowers that did not bloom were checked and egg-laying dates were determined.

- Determination of the larval output from the apricot flower bud; the study was carried out on 3 apricot trees aged 12-15 years. Four 30x40 cm cylindrical chiffon branch cages were placed in 4 directions of the tree. The cages were checked daily. The larval exit time and duration were determined.

Population growth and number of offspring in natural conditions

Since there is no trap method for pests, mature larvae were collected from the soil in March 2017 in the garden where bioecological studies were carried out in Kale district and in the garden where bioecology studies were carried out in Yeşilyurt district of Malatya province in Turkey. 25x40 cm cages were placed and observed for 1 year. Adult insect emergences were recorded. The relationship between the adult emergence stage and the climate and phenological stages of the plant has been revealed.

RESULTS and DISCUSSION

Bioecology studies

The dates of some biological periods of *C. pruniflorum* are given in Table 1 and the dates of apricot phenology are given in Table 2. The number of adults obtained from cages by comparing pest biological stages with meteorological data is given in Figure 1-2-3.

When examined Table 1 and Table 2, it is seen that when the pest emerges from the pupa in nature, apricot is the beginning of the bud swelling period as a phenological period. It was determined that the time to complete the feeding of the insect and fall into the soil as a mature larva lasted from the middle of flowering (50%) to the end of flowering. The average flowering period of the plant is 11-12 days.

Table 1. Some biological stage and dates of Contarinia pruniflorum
Çizelge 1. *Contarinia pruniflorum*'un bazı biyolojik dönemleri ve tarihleri

Locations Lokasyonlar	Time the adult beetle leaves the pupa Ergin böceğin pupadan çıkış zamanı	Time for adult beetle to be seen in nature Ergin böceğin doğada görülme zamanı	Beginning of oviposition Yumurta bırakma başlangıcı	Larvae exit from the bud Larvanın tomurcuktan çıkışı
Kale (2017)	19.02.2017- 22.02.2017	22.02.2017	24.02.2017	22.03.2017-01.04.2017
Kale (2018)	07.02.2018- 12.02.2018	08.02.2018	09.02.2018	08.03.2018- 19.03.2018
Yeşilyurt (2018)	16.02.2018- 22.02.2018	18.02.2018	19.02.2018	15.03.2018-24.03.2018

Table 2. Some phenological periods and dates of apricot
Çizelge 2. Kayısının bazı fenolojik dönemleri ve tarihleri

Locations Lokasyonlar	Beginning of bud swell period Tomurcuk kabarma zamanı başlangıcı	Beginning of flowering (5-10%) Çiçeklenme başlangıcı(%5-10)	Mid-blooming (50%) Çiçeklenme ortası(%50)	Full blooming (90-100%) Tam çiçeklenme(%90-100)	End of Flowering Çiçeklenme sonu
Kale (2017)	20.02.2017	20.03.2017	22.03.2017	24.03.2017	31.03.2017
Kale (2018)	07.02.2018	05.03.2018	08.03.2018	10.03.2018	17.03.2018
Yeşilyurt(2018)	17.02.2018	12.03.2018	15.03.2018	18.03.2018	23.03.2018

When Table 1 and Figure 1 are examined, the first exit from the pupa started on February 19 when the soil temperature was 13 °C and it continued for 4 days and ended the emergence from the pupa. The highest air temperatures during the emergence period of the adult were 8.5-14.6 °C, average daily temperatures were 1.8-6.5 °C and the lowest daily temperature was -3.2 to 1.1 °C. When figure 1 is examined, it is seen that daily maximum and soil temperatures move in parallel. The adult insects lay eggs on the buds on February 24. On March 22, 26 days after the first egg-laying, the larva left the bud and began to fall into the soil. The fall of the larva into the soil lasted for 10 days until the flower petals were completely shed and ended on April 1st. Daily maximum temperatures were between 10-21.2 °C, average daily temperatures were between 7.4-15.2 °C and the lowest daily temperatures were between 2.3-9.7 °C.

When Table 1 and Figure 2 are examined, it was seen that the first emergence from the pupa started on February 7, when soil temperature was 14 °C and

continued for 6 days. During the adult emergence period, the maximum daily air temperatures were 8.3-15.8 °C, average daily temperatures were 4.8-11.3 °C and the lowest daily temperature was between 2.2 and 7.3 °C. When figure 2 is examined, it is seen that daily maximum and soil temperatures move in parallel. The adult insects lay eggs on the buds on February 8. On March 8, 28 days after the first egg-laying, it was observed that the larvae left the buds and began to fall into the soil. The fall of the larva to

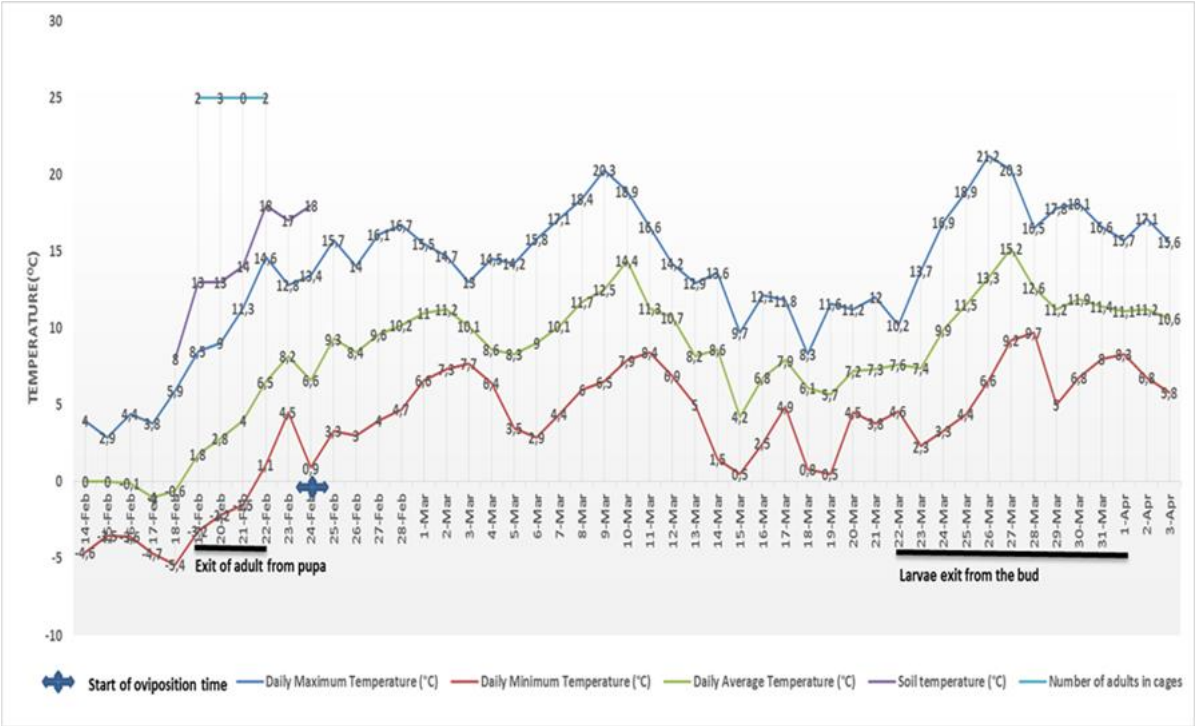

Figure 1. Relationship between some biological periods of Contarinia pruniflorum and climate data (Kale-2017)
Şekil 1. *Contarinia pruniflorum*'un bazı biyolojik dönemleri ve iklim verileri arasındaki ilişki (Kale-2017)

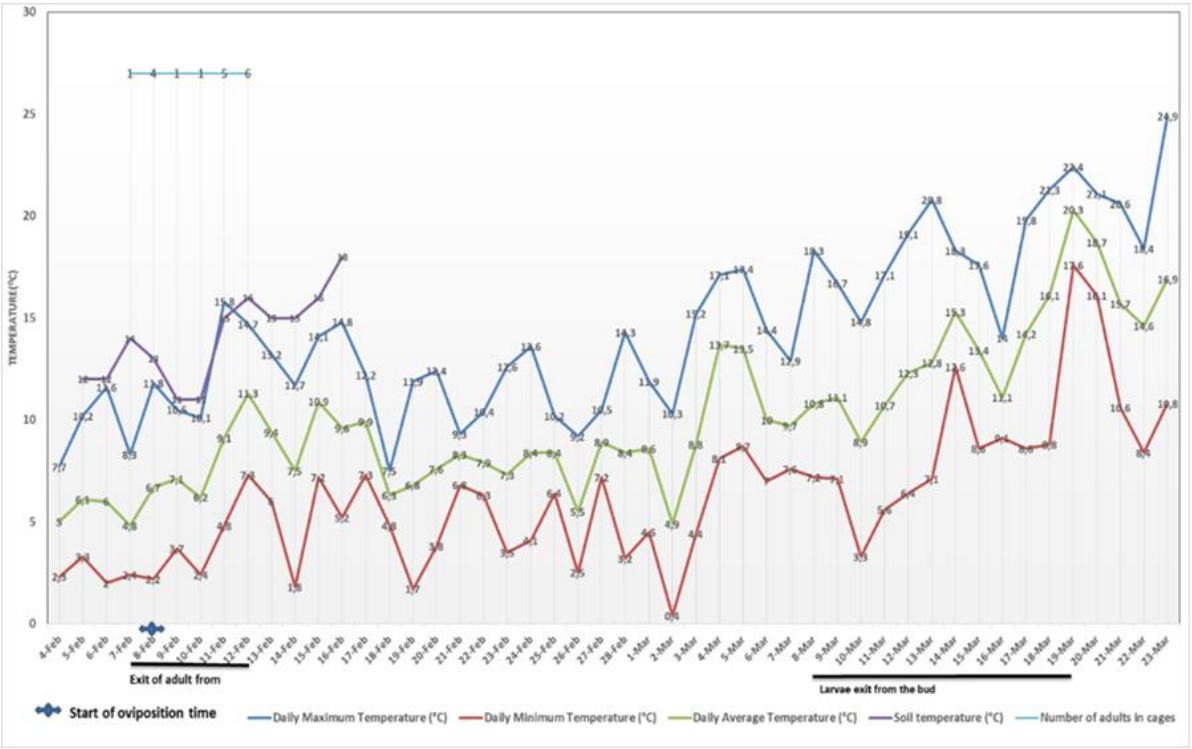

Figure 2. Relationship between some biological periods of Contarinia pruniflorum and climate data (Kale-2018)
Şekil 2. *Contarinia pruniflorum*'un bazı biyolojik dönemleri ve iklim verileri arasındaki ilişki (Kale-2018)

the soil ended on March 19 and continued for 11 days until the flower petals were completely shed. In the larval fall period, the highest daily temperatures were between 14-22.4 °C, the average daily temperatures were between 8.9-20.3 °C and the lowest daily temperatures were between 3.3-17.6 °C.

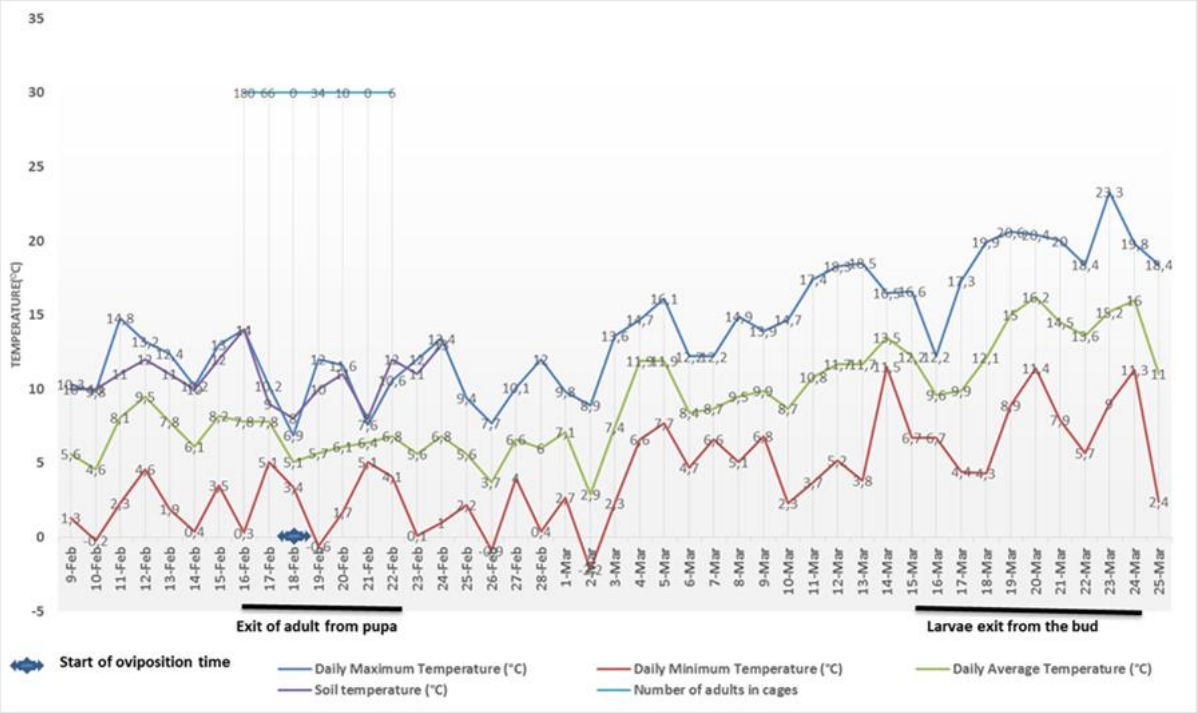

Figure 3. Relationship between some biological periods of Contarinia pruniflorum and climate data (Yeşilyurt-2018)

Şekil 3. *Contarinia pruniflorum*'un bazı biyolojik dönemleri ve iklim verileri arasındaki ilişki (Yeşilyurt-2018)

When Table 1 and Figure 3 are examined, it was seen that the first emergence from the pupa started on February 16, when soil temperature was 14 °C and continued for 7 days. However, on February 18 and February 21, when the soil temperature fell below the norms, no adults were found in the cages. The highest air temperatures during the emergence of the adult period were 6.9-14 °C, average daily temperatures were 5.1-7.8 °C and the lowest daily temperature was between -0.6 and 5.1 °C. When figure 3 is examined, it is seen that daily maximum and soil temperatures move in parallel. The adult insects lay eggs on the buds on February 18. On March 24, 25 days after the first egg-laying, it was seen that the larva left the buds and began to fall into the soil. The fall of the larva to the soil ended on March 24 and continued for 9 days until the flower petals were completely shed. It was seen that the maximum daily temperatures were 12.2-23.3 °C, the average daily temperatures were 9.6-16.2 °C and the lowest daily temperatures were 4.3-11.4 °C.

When the studies of both years are evaluated;

Adult emergence time from pupa and time of egg laying are the stages where the most effective pest control should be done. This period varies depending on the climatic conditions of the region. It was determined that the adult beetle emerged 12 days earlier in 2018 compared to the previous year in the Kale district. This is because the weather conditions in 2018, especially the February temperatures, were more temperate than in 2017. The same situation was observed in the phenology of the plant. The plant entered the early pink-bud stage and entered the early flowering period. When the climate data of both years are analyzed, it is seen that there is a parallel between air and soil temperatures. Soil temperature increases with increasing air temperatures. The most important factor in the emergence of adult insects from the pupa is the increase in soil temperatures. There is a direct correlation between adult emergence from the pupa and soil and air temperatures. The adult emerges from the pupa when the soil temperature is 13-14 °C and the air temperature is 7-15 °C. It is seen that there is no adult emergence on the days when soil temperature falls below 8 °C after the beginning of adult emergence. The time for the adult insect to occur in nature varies depending on the regions, climate, and phenology of the host plant. It occurs in the first week of February in some places, but in the middle of March in other regions. Pierre

and Chauvin-Buthaud (2001) stated that adult activity started in the apricot orchards in Drome on the first days of February. Gomez et al. (2006) reported that the first emergence of the insect was relatively late (around mid-March). Alford (2007) stated that adults were seen in early February or early March.

The adult emergence from the pupa continued for a maximum of 7 days. Even if the weather conditions were appropriate, it was observed that there was no adult emergence after that time. In other words, the period of adult emergence is very limited and the adverse weather conditions that may occur during this period directly affect the insect density. In particular, adverse events such as sudden temperature drops will cause the population to fall.

The pest was seen in nature and had flight within 1-3 days following the first emergence from the pupa. During this time, the insect mates and prepares to egg-laying into the buds.

It has been determined that the pests start to lay eggs 1-2 days after they appear in nature. The oviposition period of the pest is the beginning of the pink bud period of the plant. During this period, the females lay eggs between the sepals of the unopened flower. It was observed that more than one insect lay eggs in one bud. Alford (2007) stated that the adults deposit eggs in the outermost petals of the unopened flower buds and then the eggs were opened after a few days. Kyttariolou and Tsagarakis (2013) stated that adults lay eggs in the sepals of closed flower buds and that hatching larvae damage the flower organs. In addition to the air temperature, rainfall and the severity of the wind also affect egg-laying. Rainy and extremely windy weather reduces egg-laying activity. It was observed that adverse weather conditions during this period caused a decrease in the population. Another factor affecting egg-laying is the phenology of the plant. The oviposition time of the pest and the plant phenology must be in harmony with each other. Low air temperatures at the time of emergence from the pupa cause a decrease in the insect population and consequently a decrease in the number of damaged flowers. In addition, air temperatures above seasonal norms after the insect was seen in nature cause an acceleration in plant phenology. As a result, the harmony between the oviposition time and the phenology is impaired and the egg-laying period is shortened. As the flowering process accelerates, the eggs will die before the hatching or the newly emerged larvae do not cause damage to the bud. In 2018, with the sudden increase in air temperatures in February, the insect population increased and plant phenology accelerated. Therefore, although the insect population is high, it is not reflected in the damage rate of flowers.

It was determined that 25-28 days after the adult beetle was seen in nature, the first larvae completed their larval development and started to fall into the soil as mature larvae. Under laboratory conditions (24 °C and 55-60% relative humidity), the time between adult egg-laying and mature larvae emerging from the bud was determined for 9-10 days. Pierre and Chauvin-Buthaud (2001) reported that larval development lasted about 3 weeks. It is normal for this period to vary for several days depending on the weather temperatures. The larvae completed their development and continued to fall into the soil for 9-11 days. This period, starting from a 50% flowering period of the plant as the phenological period, continued until the period when all the flower petals were shed. After the mature larva falls into the soil, it goes to a suitable depth (2-3 cm). It was observed that it waited there for a while as a mature larva and became a pupa in summer. The insect overwinters as a pupa in the soil. Towards the end of winter, as the weather warmed in February, adults began mating and laying eggs.

Population growth and number of offspring in natural conditions

It was found that the insect gave offspring once a year. Pollini & Bariselli (1996) noted that *C. pruniflorum* gave one offspring in a year and overwintered at the pupa stage in the soil in Bologna Imola, Italy. Alford (2007) stated that the larvae fed in flower buds for about 3 weeks, then the larvae fell into the soil for being pupa. And the pest gives one generation a year, but some adults have emerged after the second winter. Tommasini (2006) stated that the pest was seen in Italy in 1996 in Emilia Romagna during the pink bud period in early March and that females laid their eggs into the flower with their ovipositor. The study showed that the larvae hatched out of the flower after 3 weeks and left themselves in the soil to become a pupa. Pierre and Chauvin-Buthaud (2001) indicated that mature larvae usually leave their hosts at the beginning of flowering and become pupae by burying themselves in the soil at a suitable depth. They also showed that a small percentage would wait until the next winter to become adults. Kyttariolou and Tsagarakis (2013) stated that the pest pupates in the soil and stays there until the next spring and gives offspring once a year when they become adults. In the same study, it was seen that some of the pests completed their life cycle in two years and that this was a biological safety measure for the survival of the species so that a population was transferred to the next year when unfavorable climatic conditions occurred.

In this study, of the 204 adult individuals identified, 188 were female and 16 were male. Based on this data, the ratio of male individuals to female

individuals was 0.0784. 7.84% of the individuals were male and 92.15% were female.

In the examination conducted on 1000 apricot flower buds, the number of buds infected with pests was 97 in 2017 and 42 in 2018. In 2017, the average number of larvae in each bud was 14 while in 2018 it was 13. The average number of larvae in the buds was 14 and the maximum number of larvae in a bud was 26. Kaplan (2014) stated that they leave 20-30 eggs in the flower buds of the pest species.

With this study, some bioecological features of the pest were revealed. It is important to determine the effects of changes in the climate on the insect by doing more studies on the pest.

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Statement of Conflict of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Author's Contributions

The contribution of the authors is equal.

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Biocontrol Potential of Turkish Entomopathogenic Nematodes Against the Citrus Mealybug, *Planococcus citri* (Risso, 1813) (Hemiptera: Pseudococcidae) Under Laboratory Conditions

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ABSTRACT
The citrus mealybug, *Planococcus citri* (Risso, 1813) (Hemiptera: Pseudococcidae) is one of the major pests of Citrus orchards in Turkey. Management of *P. citri* is quite challenging due to its cryptic and polyphagous feeding behavior. In the current study, the control potential of native entomopathogenic nematodes (EPNs) species (*Heterorhabditis indica* 216-H, *H. bacteriophora* FLH-4H, *Steinernema carpocapsae* E-76, *S. feltiae* KCS-4S, and *S. bicornotum* MGZ-4S) against *P. citri* was evaluated under laboratory conditions at different concentrations [80, 100, 150, 200 (Infective juveniles) IJs Adult^{-1}] and temperatures (20, 25, 30 ºC). The mortality rates ranged between 16 and 58% at the highest concentration 48 hours after treatment. The highest efficacy (68%) was obtained by *Heterorhabditis indica* 216-H at the highest concentration at 25 ºC. The mortality rates were generally higher at 25 ºC than other temperatures tested and *H. indica* 216-H performed better than other EPN species tested at this temperature at all concentrations. The results indicate that *H. indica* 216-H have a great potential in the control of *P. citri*.

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Yerel Entomopatojen Nematodların Laboratuvar Koşullarında Turunçgil Unlubiti, *Planococcus citri* (Risso, 1813) (Hemiptera:Pseudococcidae)'ye Karşı Biyokontrol Potansiyeli

ÖZET
Turunçgil unlubiti *Planococcus citri* (Risso, 1813) (Hemiptera: Pseudococcidae), Türkiye'deki turunçgil bahçelerinin en önemli zararlılarından biridir. *Planococcus citri*'nin mücadelesi, bu türün kriptik ve polifag beslenme davranışı nedeniyle oldukça zordur. Bu çalışmada, yerel entomopatojen nematod (EPN) türlerinin (*Heterorhabditis indica* 216-H, *H. bacteriophora* FLH-4H, *Steinernema carpocapsae* E-76, *S. feltiae* KCS-4S ve *S. bicornotum* MGZ-4S) *P. citri* mücadelesindeki kontrol potansiyeli laboratuvar koşullarında farklı konsantrasyonlarda [80, 100, 150, 200 Enfektif larva (EL) Ergin^{-1}] ve sıcaklıklarda (20, 25, 30 ^{o}C) araştırılmıştır. Ölüm oranları, uygulamadan 48 saat sonra en yüksek konsantrasyonda %16 ile 58 arasında değişmiştir. En yüksek etkinlik (%68), 25^{o}C'de en yüksek konsantrasyonda *Heterorhabditis indica* 216-H ile elde edilmiştir. En yüksek ölüm oranları genellikle 25 ^{o}C'de meydana gelmiştir. *Heterorhabditis indica* 216-H 25 ^{o}C'de tüm konsantrasyonlarda test edilen diğer EPN türlerine kıyasla en yüksek etkinliği göstermiştir. Elde edilen sonuçlar, *H. indica* 216-H'nin *P. citri*'nin kontrolünde önemli bir potansiyele sahip olduğunu göstermektedir.

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INTRODUCTION
Citrus cultivation occupies an important place in the Mediterranean Region of Turkey with an annual production of 4.3 million tons (TÜİK, 2020). Of the

various kinds of pests attacking citrus orchards, *Planococcus citri* (Risso, 1813) (Hemiptera: Pseudococcidae) is regarded as a major pest in Citrus orchards in the Mediterranean region of the world including Turkey (Blumberg & Van Driesche, 2001; Franco et al., 2004; Uygun & Satar, 2008; Morandi Filho et al., 2021). The citrus mealybug, *P. citri* was first discovered by Risso in 1813 from *Citrus* spp. samples in south France (Cox, 1981). The citrus mealybug is a wide spread, polyphagous and one of the the most devastating insect speices (Muştu et al., 2008). The population density of *P. citri* reaches its peak during early summer in the Mediterranean region. Newly emerged nymphs of *P. citri* settle in various parts of the plants such as the underside of leaves, twigs, and immature fruits, and start feeding by sucking plant and fruit juices. Immature fruits drop early due to feeding damages of *P. citri* on the growing fruits and fruit stalks. The sugary honeydew secreted by *P. citri* during the feeding also triggers the development of sooty mold on nearby leaves and fruits that limits the photosynthesis capacity of the leaves due to being coated with dark-colored mold. As a result of mold development and feeding damage, the yield quantity and quantity of the damaged trees drop severely. If not controlled properly, the mealybugs continue to breed and cause stunted growth, and the death of infected plants (Gill et al., 2012; Karacaoğlu & Satar, 2017). The management of *P. citri* is quite challenging. To date, attempts to control *P. citri* with chemical pesticides have met with limited success due to their cryptic feeding behavior and hidden feeding sites, coating with protective waxes, and inaccurate delivery of insecticides. Moreover, the intensive and inappropriate use of chemical insecticides lead to the development of resistance in *P. citri* populations and cause long-term severe negative effects on non-target organisms and environment (Venkatesan et al., 2016). Insecticide residues on the citrus fruits after harvest are also a cause for concern to exporters as violations of maximum residue limits (MRLs) may lead to rejected loads of product. Therefore, eco-friendly and sustainable control methods are needed in the control of *P. citri*.

Entomopathogenic nematodes (EPNs) are naturally occurring soil-borne insect pathogens and have been used successfully against many insect pest species (Karabörklü et al., 2015; Öğretmen et al., 2020; Mokrini et al., 2020; Čačija et al., 2021; Taşkesen et al., 2021; Gürkan & Çetintaş, 2022). A total of 82 species of EPN have been identified worldwide (65 belonging to *Steinernema*, 1 to *Neosteinernema*, and 16 to *Heterorhabditis*) (Kepenckci, 2014). First EPN belonging to *Steinernema* in Turkey was detected by Özer et al., (1995) as *S. feltiae* from soil samples collected from Rize. First nematode species belonging to *Heterorhabditis* in Turkey was detected by Kepenekci et al., (1999) as *H. bacteriophora* in *Aelia* population (*Aelia rostrata* Boh.) collected from Ekecik (Aksaray) winter quarters. Infective juveniles (IJs), which are the third larval stage of EPNs, are the only free-living stages outside of the host cadaver. Once a potential host is located, IJs move towards the potential host and actively penetrate into the insect body using their natural body openings/thin cuticle. Then, IJs proceed to the host's hemocoel to release the symbiotic bacteria within their intestine (*Xenorhabdus* sp. and *Photorhabdus* sp.) to assist them kill the insect in a short period time (Smart, 1995; Akhurst & Boemare, 2018; Stock, 2019). Bacterial toxins and other metabolites produced by symbiotic bacteria promote an infection process that results in the death of the host by septicemia (Griffin et al., 2005).

Although EPNs are well known to perform better in soil environment, recent studies have proven that they have also great potential in controlling the aboveground foliar pests of agricultural importance under favorable conditions (Arthurs et al., 2004; Schroer & Ehlers, 2005; Trdan et al., 2007; Laznik et al., 2010). But before initiating the field studies, EPNs are generally tested under laboratory conditions to determine the most pathogenic EPN species and isolates and achieve a successful control. In the current study, a pathogenicity screening study was conducted under laboratory conditions to reveal the control potential of different EPN species against the adults of *P. citri* at different temperatures and concentrations.

MATERIAL and METHOD

Source and Rearing of *Planococcus citri*

The initial population of *P. citri* was obtained from Assoc. Prof. Dr. Murat Muştu in the Department of Plant Protection, Faculty of Agriculture, Kayseri Erciyes University (Kayseri, Türkiye), and they were reared on sprouting potatoes (*Solanum tuberosum* L.) and pumpkin (*Cucurbita pepo* L.). Laboratory cultures were maintained in cages (650×350×590 mm) under controlled conditions (25±2°C, 60-70% RH). Only the adult female mealybugs were utilized in the experiments.

Source of nematodes

Experiments were carried out with five EPN species (*Steinernema feltiae* KCS-4S and *Heterorhabditis bacteriophora* FLH-4H, *Steinernema carpocapsae* E-76, *S. feltiae* KCS-4S, and *S. bicornotum* MGZ-4S) recovered from the same geographical region in the earlier studies (Canhilal *et al.*, 2016; 2017) (Table 1). The IJs of EPNs were reproduced last larval instar of *Galleria mellonella* L. (Lepidoptera: Pyralidae). The IJs were inoculated to Petri dishes containing 20 g autoclaved soil at the concentration of 200 IJs larva^{-1} and ten larvae were added to each Petri dish. The larvae of *G. mellonella* were cultured in 1 L glass jars under laboratory conditions (30±2°C, 60-70% RH)

using an artificial medium consisting of soybean flour, corn flour, dry bread yeast, honey, glycerin, milk powder and wheat flour (Metwally et al., 2012). Newly emerged IJs were harvested one week after inoculation and stored horizontally at the concentration of 1500 IJs ml^{-1} distilled water at 7-9ºC in culture flasks. During this period, flasks were agitated once a week to prevent the IJs from collapsing and agglomeration. Only three weeks old IJs were used in the experiments.

Table 1. Species, strain, habitat, locality, and GenBank accession numbers of entomopathogenic nematodes used in this study.

Çizelge 1. Çalışmada kullanılan entomopatojen nematodların türleri, izolatları, habitatları, lokaliteleri ve GenBank giriş numaraları.

Entomopathogenic nematodes species	Strain	Habitat	Locality	GenBank accession number
Steinernema feltiae	KCS-4S	Pine-poplar	Kocasinan-Kayseri	KX462908
S. bicornutum	MGZ-4S	Strawberry	Melikgazi-Kayseri	KX462912
S. carpocapsae	E-76	Grassland	Melikgazi-Kayseri	KX462907
Heterorhabditis indica	216-H	Olive	Dulkadiroğlu-Kahramanmaraş	KP970842
H. bacteriaphora	FLH-4H	Orchard	Felahiye-Kayseri	KX462939

Pathogenicity Bioassays

The bioassays were conducted in 24-well plates (Flat bottom, Nunc™, Cat.) (13 mm diameter) containing a circular piece of filter paper at the bottom of each well. Four multiwell plates, with each containing twelve adult female *P. citri*, were arranged (4 replicates; 48 insects) for each of the different nematode concentrations (80, 100, 150, and 200 IJs $insect^{-1}$ mealybug in 50 µl per well). Each mealybug in the control treatment was inoculated with only 50 µl of water by using a micropipette. Multiwell plates were covered with perforated parafilm to let air flow. Mealybugs were kept at 20°C after inoculation. Mortality was assessed at 24, 48, and 72 h. Previous processes were repeated for remaining temperatures 25 and 30°C. The impact of increasing concentrations of *H. indica*, *S. feltiae*, *H. bacteriaphora*, *S. carpocapsae*, and *S. bicornutum* was determined at humidity level of 95±5 RH. The bioassay was repeated on a separate date. The data of both bioassays were pooled for analysis.

Data Analysis

All data were tested for normality and the needed transformation (Arcsine) was carried out to obtain normal distribution and meet the assumptions of ANOVA. All statistical analyses were performed using Genstat software v12.1.0 (Genstat-VSNI international, 2009). Data were analyzed using RMANOVA, with a posthoc comparison of means using the Duncan method ($P \leq 0.05$).

RESULTS and DISCUSSION

All EPN species tested were able to infect and kill the adult female of *P. citri*. The analysis of data showed that the percentage mortality of *P. citri* was influenced by all main factors and their associated two-way interactions (Table 2). The virulence of tested EPN species was generally greater at 25ºC for all exposure times. The highest mortality (68.8%) was obtained after 72 hours of exposure to *H. indica* 216-H at the highest concentration (200 IJs) at 25 ºC. Only two EPN species, *S. carpocapsae* E-76 and *H. indica* 216-H were able to cause mortality over 60% at all concentrations and temperatures. *Heterorhabditis indica* 216H performed better than other EPN species at 25ºC at all exposure times and concentrations. *Steinernema carpocapsae* E-76 was the most pathogenic EPN species 24 hours post-inoculation at 20 ºC. The poorest efficacy was generally observed at *S. feltiae* KCS-4S isolate at all concentrations and exposure times.

Table 2. RMANOVA parameters for the main effects and associated interactions for mortality rates of *Planococcus citri*.

Çizelge 2. Planococcus citri'nin ölüm oranlarına ait ana faktörler ve interaksiyonlarının (Repeated Measure) ANOVA parametereleri.

Source*	df	F	P
N	4	294.12	<0.001
D	3	210.05	<0.001
T	2	79.3	<0.001
T	2	1009.33	<0.001
N x D	12	13.48	<0.001
N x T	8	17.15	<0.001
D x T	6	3.83	<0.001
N x t	8	16.77	<0.001
D x t	6	9.61	<0.001
T x t	4	4.34	0.002
N x D x T	24	0.73	0.857
N x D x t	24	0.61	0.950
N x T x t	16	1.37	0.129
D x T x t	12	0.17	0.999
N x D x T x t	48	0.09	1.000
Error	648		

*t: Time, N: Nematode species, D: Dose, T: Temperature, df: the degree of freedom, F: F-statistic, and P: Significance level (Duncan, $P \leq 0.05$).

Table 3. Mean percentage mortality (%) of *Planococcus citri* females after 24, 48, 72 hours of exposure to entomopathogenic nematode species at different concentrations and temperatures (20, 25, 30 °C).

Çizelge 3. Dişi Planococcus citri bireylerinin entomopatojen nematod türlerine farklı konsantrasyonlarda ve sıcaklıklarda (20, 25, 30 °C) 24, 48, 72 saat maruz kaldıktan sonraki ortalama ölüm yüzdeleri (%).

EPN species*	24 h				48 h				72 h			
	80 IJs	100 IJs	150 IJs	200 IJs	80 IJs	100 IJs	150 IJs	200 IJs	80 IJs	100 IJs	150 IJs	200 IJs
20°C												
216 H	4.0±2.5Aa*	4.2±2.1Aa	8.3±4.2 Aa	12.5±5.4 Aa	20.8±8.1Ba	22.9±4.5 Ba	22.9±5.1 Ba	41.7±7.3 Aa	25.0±6.3 Ba	27.1±4.3 Bab	33.3±6.5 Bab	47.9±7.4 Aa
KCS-4S	2.1±1.2Ba	2.1±1.2Ba	4.2±2.1 ABab	6.3±3.1 Aab	10.4±5.1 Cb	12.5±3.7BCb	16.7±7.2ABb	20.8±8.1Ac	16.7±7.2 Cb	20.8±8.1BCbc	25.0±5.5 ABb	31.3±6.1 Ab
FLH-4H	2.1±1.2Aa	6.3±2.5Aa	8.3±4.2 Aa	10.4±2.5 Aa	16.7±7.2 Bab	22.9±5.1ABa	25.0±5.5 Aa	31.3±6.5Aab	20.8 ±8.1Ca	25.0±5.5BCab	31.3±6.1ABab	39.6±8.1Aab
E-76	4.2±2.1Ba	8.3±4.2 ABa	10.4±2.5 ABa	14.6±5.4 Aa	18.8±8.1 Cab	25.0±6.3BCa	31.3±6.5ABa	39.6±8.1 Aa	27.1±4.3 Ca	31.3±6.1 BCa	39.6±8.1 Ba	47.9±7.4 Aa
MGZ-4	4.2±2.1Aa	6.3±3.1Aa	8.3±4.2 Aa	8.3±4.2 Aab	14.6±6.2 Bab	16.7±4.7 Bab	22.9±5.1ABab	29.2±3.2 Ab	20.8 ±8.1 Ca	25.0±5.5BCab	29.2±3.2 ABb	37.5±6.5 Ab
Control	0.0±0.0Aa	0.0±0.0Aa	0.0±0.0Ab	0.0±0.0Ab	8.3±4.2 Ab	8.3±4.2 Ab	8.3±4.2 Ac	8.3±4.2 Ad	14.6±6.2 Ab	14.6±6.2 Ac	14.6±6.2Ac	14.6±6.2 Ac
25°C												
216 H	8.3±4.2 Ca	12.5±2.5 BCa	18.8±6.3 ABa	25.0±6.3 Aa	25.0±6.3 Ca	31.3±6.1 Ca	47.9±7.4 Ba	58.3±4.2 Aa	35.4±5.7 Da	43.8±3.3 Ca	58.3±4.2 Ba	68.8±6.8 Aa
KCS-4S	2.1±1.2 Aa	4.2±2.1Abc	6.3±2.5Abc	8.3±4.2 Abc	10.4±2.5 Cb	14.6±6.2BCbC	20.8 ABc	25.0±5.4 Ac	18.8±5.4 Cb	22.9±3.9 BCc	27.1±7.3 ABc	33.3±6.1 Ac
FLH-4H	4.2±2.1Ca	8.3±4.2 BCab	14.6±5.5 ABab	20.8±8.1Aab	22.9±5.1 Ca	29.2±3.2 Ca	39.6±5.1 Ba	47.9±7.4 Aa	29.2±3.2 Ca	37.5±4.6 Cab	47.9±7.4 Bb	58.3±4.2 Ab
E-76	6.3±2.5Ca	10.4±5.4 BCab	16.7±6.1 ABab	22.9±5.1Aab	20.8±8.1Ca	29.2±3.2 Ba	37.5±6.5 Bab	52.1±8.2 Aa	31.3±6.1 Ca	39.6±4.7 Cca	52.1±6.3 Bab	62.5±8.4 ab
MGZ-4	6.3±2.5 Aa	8.3±4.2 Aab	10.4±2.5 Ab	14.6±6.2 Ab	18.8±4.2 Ba	20.8±8.1 Bb	27.1±7.3 Bbc	39.6±5.3 Ab	27.1±6.6 Ca	29.2±3.2 BCb	37.5±5.4 ABb	45.8±4.2 Abc
Control	0.0±0.0 Aa	0.0±0.0Ac	0.0±0.0Ac	0.0±0.0Ad	4.2±2.1Ac	4.2±2.1 Ac	4.2±2.1 Ad	4.2±2.1 Ad	10.4±3.2 b A	10.4±3.2 dA	10.4±3.2 Ad	10.4±3.2 Ad
30°C												
216 H	4.2±2.1 Ca	8.3±4.2 BCa	14.6±5.1 ABa	18.8±3.6 Aa	22.9±4.1 Ca	27.1±5.2 Ca	39.6±5.1 Ba	54.2±5.6 Aa	31.3±6.1 Ca	35.4±4.1 Ca	47.9±7.1 Ba	60.4±6.9 Aa
KCS-4S	0.0±0.0Aa	0.0±0.0 Aa	2.1±1.2 Abc	2.1±1.2 Abc	6.3±2.5 Bb	8.3±4.2ABCbc	12.5±5.4 Bcd	16.7 Ad	14.6±6.2Acd	16.7±6.3 Ad	18.8±4.9 Ad	22.9±5.1 Acd
FLH-4H	8.3±4.2 Ba	10.4±2.2 Ba	14.6±6.2 Aa	20.8±4.1 Aa	22.9±4.1 Ca	25.0±5.4BCab	31.3±3.5 Bab	43.7±7.4 Ab	27.1 Cab	31.3±6.1BCab	39.6±5.1 Bab	52.1±6.3 Aa
E-76	2.1±1.2 Bca	6.3±2.2 Bca	12.5±3.6 ABa	16.7±4.3 Aa	12.5±3.6 Cb	16.7±4.6 BCb	22.9±4.1ABbc	31.3±6.1 Ac	29.2±3.2Cbc	25.0±5.4BCbc	33.3±5.5ABbc	41.7±7.2 Ab
MGZ-4	0.0±0.0Aa	4.2±1.2 Aa	4.2±1.2 Abc	6.3±2.5Ab	12.5±3.6 Bb	14.6±5.2 ABb	18.8±3.6ABbc	22.9±4.3 Ad	18.8±5.2Bbc	20.8±4.7ABcd	25.0±5.4ABcd	29.2±3.2 Ac
Control	0.0±0.0Aa	0.0±0.0Aa	0.0±0.0 Ac	0.0±0.0Ac	6.3±2.1 Ab	6.3±2.1 Ac	6.3±2.1 Ad	6.3±2.1 Ae	12.5±3.5 Ad	12.5±3.5 Ad	12.5±3.5 Ae	12.5±3.5 Ad

*216-H: *Heterorhabditis indica* 216-H isolate; KCS: *Steinernema feltiae* KCS isolate; FLH: *H. bacteriaphora* FLH isolate; E-76: *S. carpocapsae* E-76 isolate; MGZ: *S. bicornutum* MGZ isolate. Different uppercase letters in the same line and different lowercase letters in the same column indicate significant differences according to Duncan test ($P \leq 0.05$).

In most cases, *Steinernema carpocapsae* E-76 showed superior performance at the lowest temperature (20 °C) compared to the other four nematode species tested (Table 3). At the highest temperature tested (30 °C), *H. bacteriophora* FLH-4H caused greater mortalities for only 24 hours of exposure and *H. indica* 216-H was the most efficient species for other exposure times. Pathogenicity screening study showed that susceptibility of the female of *P. citri* varies with EPN species under controlled conditions. The highest efficacy (68%) was obtained by *H. indica* (216 H) at the highest concentration (200 IJs) after 72 h of exposure. In most cases, *H. indica* (216 H) was also the most pathogenic nematode species, especially at 25 °C. Similar results obtained in this study, a moderate activity of EPN species against *P. citri* was reported in the earlier studies conducted by Negrisoli et al. (2013) and Stokwe and Malan (2016). Both studies reported the higher effectiveness of *Heterorhabditis* species than *Steinernema* species tested. The high virulence of *H. indica* could be explained by the small body size of the IJs of *H. indica* compared to other nematode species (Bhat et al., 2021) which is an important factor in the penetration process of EPNs into host insects. The penetration of IJs into the host body could be affected by the size of both host and IJs since most IJs use the natural body openings as an entrance (Bastidas et al., 2014). The results of this study clearly revealed that *P. citri* has a time and concentration-dependent susceptibility to both *Steinernema* species and *Heterorhabditis* species tested. The highest mortality was reached after 72 h of exposure when *P. citri* was inoculated with the highest concentration (200 IJs). These findings are in agreement with the studies conducted by van Niekerk and Malan (2012), Le Vieux and Malan (2013), Negrisoli et al. (2013), and Stokwe and Malan (2016). Increasing concentrations and exposure time to IJs had a positive effect on the mortality rates in these studies. In the current study, mortality rates were higher at 25ºC among other temperatures tested although mortality rates at 25ºC were quite similar to the ones at 30 °C. Earlier studies showed that temperature had a significant influence on the effectiveness of EPNs and the virulence of EPN species and isolates varies considerably at different temperatures (Hang et al., 2007; Radová and Trnková, 2010; Andaló et al., 2011; Yuksel et al., 2019). Low temperatures may give rise to a decrease in the movement of IJs and pathogenicity as reported in earlier studies (Dos Santos Ferreira et al., 2015). High temperatures may also cause to increase the movement of IJs leading to consumption of more energy to reach the target host (Sharmila and Subramanian, 2016).

Entomopathogenic nematode species have different foraging strategies and this is another factor causing variations in the mortality rates. Entomopathogenic nematode species with cruiser strategy search for their target host more actively following host's chemical cues compared to an ambusher nematode species (Dos Santos Ferreira et al., 2015; Lortkipanidze et al., 2016). Heterorhabditis indica is considered as a cruiser and this may have assisted them in locating their host (Lewis et al., 2006).

The overall results obtained during this study demonstrated that both *Steinernema* and *Heterorhabditis* have great potential in the control of *P. citri*. They differed in pathogencity to *P. citri*, with *Heterorhabditis* species showed more active than *Steinernema* species controlling *P. citri*. *Heterorhabditis indica* 216-H showed the more efficacy than other entomopathogenic nematodes used in this study.

CONCLUSION

All EPN species tested were pathogenic to adult females of *P. citri*. Among the EPN species tested *H. indica* 216-H and *S. carpocapsae* E-76 were the most virulent species in most cases. The results obtained in this study showed that entomopathogenic nematodes are eficcient biological control agents against *P. citri* under favorable conditions. However, further studies in field conditions are needed to evaluate the field potential of native EPN species and isolates.

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Author's Contributions

The contribution of the authors is equal.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

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Molecular Characterization and Phylogeny of *Meloidogyne chitwoodi* Golden, O'Bannon, Santo & Finley 1980 Obtained from Potato Production Areas in Turkey

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ABSTRACT

Meloidogyne chitwoodi is an invasive nematode that can cause economic damage to agricultural areas in many parts of the world. The identification of plant parasitic nematodes is basically carried out by observation of morphologic characteristics and morphometric parameters. However, it is not always possible to obtain certain results with the use of published original descriptions and diagnostic keys. Therefore, in this study, nematode samples isolated from potato tubers collected from the Central Anatolia region during the production season of 2018 and 2019 were determined molecularly by PCR-based diagnosis method using JMVhapla, JMV1 and JMV2 primer sets; and morphologically using morphometric measurements and perineal pattern. PCR reactions yielded 540 bp bands. As a result of both methods, the nematode species was determined as *Meloidogyne chitwoodi*. Phylogenetic analysis and pairwise distance were performed to evaluate the relationships of local populations with other *Meloidogyne* species. After phylogeny studies, it was determined that the populations were 99% similar to both the Turkish population and other populations. Compared to other sequences of published local isolates, the Niğde isolate in this study showed quiet similarity with Nevşehir (KF557791.1) isolate. As a result of this study, the data on *M. chitwoodi*, which causes damage in the potato growing areas of the Central Anatolia Region, has been updated.

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Türkiye'deki Patates Ekiliş Alanlarından Elde Edilen *Meloidogyne chitwoodi* Golden, O'Bannon, Santo & Finley 1980'nin Moleküler Karakterizasyonu ve Filogenisi

ÖZET

Meloidogyne chitwoodi, dünyanın birçok yerinde tarım alanlarında ekonomik zarara neden olabilen istilacı bir nematoddur. Bitki paraziti nematodların tanımlanması temelde morfolojik özelliklerin ve morfometrik parametrelerin gözlenmesiyle yapılmaktadır. Ancak yayınlanmış orijinal betimlemeler ve tanılama anahtarlarının kullanılmasıyla her zaman doğru sonuç elde edilememektedir. Bu nedenle, bu çalışmada 2018 ve 2019 yılı üretim sezonu boyunca İç Anadolu Bölgesi'nden toplanan patates yumrularından izole edilen nematod örnekleri JMVhapla, JMV1 ve JMV2 primer setleri kullanılarak PCR tabanlı tanı yöntemi ile moleküler tanılama ile morfometrik ölçümler ve perineal pattern kullanılarak morfolojik olarak tanılanmıştır. İki yöntem sonucunda da nematod türü *Meloidogyne chitwoodi* olarak tespit edilmiştir. Ayrıca çalışmada yerel popülasyonların diğer *Meloidogyne* türleri ile ilişkilerini araştırmak için filogenetik analiz ve ikili uzaklık değerlendirilmesi yapılmıştır. Filogeni çalışmalarından sonra popülasyonların hem Türkiye popülasyonuna

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hem de diğer popülasyonlara %99 benzer olduğu tespit edilmiştir. Yayımlanmış yerel izolatların diğer sekansları ile karşılaştırıldığında bu çalışmadaki Niğde izolatı, Nevşehir (KF557791.1) izolatı ile oldukça benzerlik göstermiştir. Bu çalışma sonucunda İç Anadolu Bölgesi patates yetiştirme alanlarında zarar yapan *M. chitwoodi* ile ilgili veriler güncellenmiştir.

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INTRODUCTION

Potato, *Solanum tuberosum* L. (Tubiflorales: Solanaceae) is an annual plant with more than 4.000 varieties grown in the world for its nutritionally rich content (Grzebisz et al., 2020). Potato contains higher amount of carbohydrates, proteins, fiber, many vitamins such as vitamin C, potassium, magnesium, phosphorus and iron. Also, potato is good industrial plant which is processed to chips, flour and alcohol (Çalışkan et al, 2010). Potato plant is adapted to grow well in many different climates and is the fourth most cultivated plant in the world with the product of 400 million tons yearly. China is leading producer and consumer while Türkiye ranked 14th with 4.800 tones (Anonymous, 2019).

Although many plant parasitic nematodes have been identified in potato growing areas in the world economically, most harmful species include in genera of *Meloidogyne* spp. Despite *M. hapla* Chitwood, 1949 and *M. chitwoodi* Golden, O'Bannon, Santo & Finley are considered as most damaging root-knot nematodes, *M. chitwoodi* is the most destructive species and is difficult to control by nematicides. (Tiilikkala et al., 1995). *M. chitwoodi* (Golden, O'Bannon, Santo & Finley) is one of the most destructive nematode in potato fields in the world. This species causes economic problems in potato growing areas in the northern, western and eastern regions of Türkiye (Devran et al., 2009; Özarslandan & Elekcioğlu, 2010; Evlice & Bayram, 2013; Özarslandan et al., 2013). Symptom caused by this nematode include small tuber galls, necrosis and above ground plant stunting (Anonymous, 2005). Related yield reduction and gall symptoms of tubers lead to significant reduction of market value of potato plants (Santo, 1994). The damage on plant roots and tubers leads to infection of several pathogens as well. The interactions between *Meloidogyne* spp. and *Fusarium* wilt and *Rhizoctonia solani* have been previously reported (Golden & Van Gundy, 1975; Siddiqui et al., 1999).

M. chitwoodi has two races and these have a wide host range from several families such as Brassicaceae, Cucurbitaceae, Lamiaceae, Liliaceae, Fabaceae, Umbelliferae and Vitaceae (Santo et al., 1980; O'Bannon et al., 1982; Evlice & Bayram, 2016). Despite the widespread distribution all over the world, the species was identified for the first time in Central Anatolia, Türkiye by Özarslandan et al. (2009) and later by Evlice and Bayram (2012).

Morphological methods are widely used in the identification of nematodes, but in some cases (dauer larvae) and due to the morphological proximity of the species, the accuracy of this method needs to be supported by other methods (Gerič Stare et al. 2018; Aslan & Elekcioğlu, 2022). Species identification using molecular technique give reliable and accurate results in a short time (Devran & Söğüt, 2009). Morphological and molecular diagnostic techniques are studies that support each other (Özarslandan & Elekcioğlu, 2010).

During the nematode surveys and inspections in 2018-2019, potato tubers with symptoms resembling *Meloidogyne* spp. damage were detected from samples collected from Central Anatolia. The species were identified by morphologic characteristics and perineal patterns of vulval cuttings. In order to prove morphologic identification, a study that aims development PCR based method with specific primers were carried out, the amplification product was sequenced and compared in universal gene bank to reveal phylogenetic relationships with other nematode species.

MATERIAL and METHODS

Sample collection, nematode isolation and morphologic characterization

Galled potato tubers were obtained from the infested potato fields located in Niğde and Konya province in 2018-2019 (Table 1). A total of 23 populations were brought to the laboratory and examined. Potato slides prepared from necrotic areas were examined with a Leica DM1000 microscope to verify the presence of *Meloidogyne* individuals. The female nematodes were hand-picked with pens after

examination at 10X magnification. Juveniles were isolated by modified Baermann Funnel method described (Baermann, 1917). The morphometrics of nematodes was measured using Leica Application Suite software and the images were taken with Leica ICC50 W camera (Figure 1).

Table 1. Isolates collected from potato production areas of Nigde and Konya province in Türkiye
Çizelge 1. Türkiye'de Niğde ve Konya ili patates üretim alanlarından toplanan izolatlar

Isolate	City	District	Isolate	City	District
MCN-1	Niğde	Edikli	MCN-13	Niğde	Alay
MCN-2	Niğde	Orhanlı	MCN-14	Niğde	Alay
MCN-3	Niğde	Orhanlı	MCN-15	Niğde	Alay
MCN-4	Niğde	Yeşilgölcük	MCN-16	Niğde	Ağcaşar
MCN-5	Niğde	Konaklı	MCN-17	Niğde	Ağcaşar
MCN-6	Niğde	Konaklı	MCK-18	Konya	Ovakavağı
MCN-7	Niğde	Konaklı	MCK-19	Konya	Ovakavağı
MCN-8	Niğde	Konaklı	MCK-20	Konya	Ovakavağı
MCN-9	Niğde	Konaklı	MCK-21	Konya	Ovakavağı
MCN-10	Niğde	Konaklı	MCK-22	Konya	Ovakavağı
MCN-11	Niğde	Konaklı	MCK-23	Konya	Ovakavağı
MCN-12	Niğde	Alay			

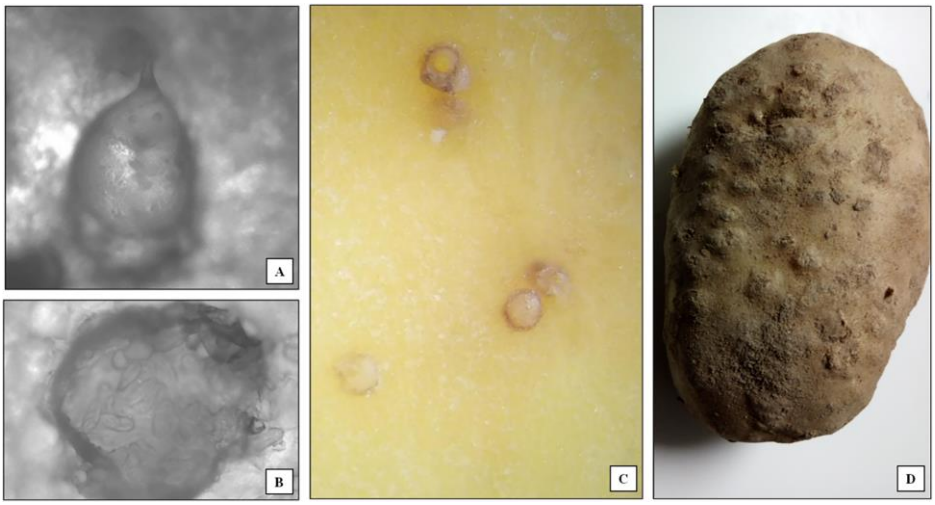

Figure1. *Meloidogyne chitwoodi*; A, C) Female, B) Egg mass of female, D) Galls on potato tuber
Şekil 1. Meloidogyne chitwoodi; A-C) Dişi, B) Dişi yumurta paketi, D) Yumruda bulunan galler

The MC-2 population was selected from the infected tubers. Morphologic identification of this population was carried out with juvenile and female perineal pattern morphology (Figure 2). In order to prepare nematode slides extracted *Meloidogyne* females, were cut from posterior body part and the posterior end of body with perineal patterns and were placed into glycerin. On the other hand, juveniles were collected from egg masses and the isolated individuals were heat killed at 60 ºC for one minute, fixed in double strengthen TAF solution and mounted on slides by wax-ring method (Seinhorst, 1959). Identifications were performed according to previous studies (Jepson, 1987; Karssen, 2002).

Molecular identification and phylogeny

A PCR based diagnostic method with primers set JMVhapla, JMV1 and JMV2 were performed to verify *M. chitwoodi*. Primer sequences used in PCR studies are given in Table 2 and used as stated in the literature (Wishart et al., 2002).

DNA was extracted from single female by using Sigma Aldrich Extract N Tissue PCR kit containing 2.5 µl tissue preparation and 10 µl extraction solution. Tubes were incubated at 55 °C for 10 minutes followed by 94 °C for 3 minutes. PCR assay was performed in a volume of 25 µl mixture containing 8 µl of water, 12 µl of master mix, 1 µl of each primer (JVM1, JVM2 and JVM hapla), 2 µl of DNA. The PCR reaction was performed at 94 °C, 3 min; (94 °C, 50 sec; 59 °C, 50 sec; 72 °C, 1 min) × 35; 72 °C, 7 min. Amplicons were controlled by agarose gel 2 % staining with ethidium bromide. The gel was run 50 minutes at 50 V and visualized UV Transilluminator. After UV visualization, the PCR product was sequenced for approval of identification. For DNA sequence, one population representing the area were chosen. The PCR products were sequences with specific forward primer of *M. chitwoodi.*

Table 2. The primers used for molecular identification for *M. chitwoodi*
Çizelge 2. M. cihwoodi'nin moleküler tanılamasında kullanılan primerler

Primer *Primer*	Primer Sequences (5'-3') *Primer Dizilimleri (5'-3')*	Species *Türler*	Fragment (bp) *Uzunluk (bp)*	Reference *Literatür*
JMV1	GGATGGCGTGCTTTCAAC	***M. chitwoodi***	540	**Wishart et al., 2002**
JMV2	TTTCCCCTTATGATGTTTACCC	***M. fallax***	670	
JMVhapla	AAAAATCCCCTCGAAAAATCCACC	***M. hapla***	440	

Phylogenetic analysis was conducted to evaluate relationships of *M. chitwoodi* populations with other local species and other published foreign species. On this purpose, the sequence data were subjected to GenBank sequence comparison with the BLAST records of NCBI. Neighbour joining with Kimura 2-parameter model was performed on Mega X software comparing *M. chitwoodi* with other sequences. Pairwise distance was calculated after aligning the sequences in ClustalW.

RESULTS and DISCUSSION

Meloidogyne chitwoodi Golden, O'Bannon, Santo & Finley, 1980 (Figure 2)

The measurements of second stage juveniles was; Female body is pyriform and white colour. Head region is offset and cephalic framework is distinct. Stylet is small with rounded knobs that slope posteriorly. Dorsal esophageal gland opens into esophagus lumen. Perineal pattern of female was round to oval with striae curved around anal area. Punctuation not present (Golden et al., 1980).

Male body is vermiform slender, tapering slightly at both extremities. Head is slightly offset, with large labial disc and post labial annule. Lateral field with four incisures. Testis one or two. Spicules are arcuate. Phasmids is located at or anterior to cloaca. Tail is short and rounded (Golden et al., 1980).

Juvenile body small and vermiform. Head not offset, cephalic framework weak, labial disc without striations. Lateral field with four incisures. Phasmids small and located in anterior part of tail. Tail hyaline short and tail terminus rounded (Golden et al., 1980).

The description and morphometrics of *M. chitwoodi* population in this study are compatible with original description of Chitwood (1949) and other reports from Türkiye (Devran et al., 2009; Özarslandan et al., 2009; Özarslandan & Elekcioğlu, 2010; Evlice & Bayram, 2016). Larval morphometric measurements were found similar to Karssen (2002) (Table 3).

Table 3. Second instar larvae measurements of *M. chitwoodi*
Çizelge 3. M. chitwoodi'nin ikinci dönem larva ölçümleri

	Niğde Population	Karssen (2002)
N	30	-
L (µm)	377.12±3.2 (365.8-389.2)	380±11.5 (362-394)
Greatest body diameter (µm)	12.7±0.62 (11.7-13.5)	13.1±0.5 (12.6-13.9)
Body diam. at stylet knobs (µm)	9.15±0.01 (8.9-9.3)	-
Body diam. at S-E pore (µm)	11.9±0.3 (11.6-12.0)	11.8±0.3 (11.4-12.0)
Stylet length (µm)	9.23±0.27 (8.9-9.8)	9.7±0.3 (9.5-10.1)
DGO (µm)	3.14±0.21 (2.89-3.5)	3.4±0.4 (2.5-3.83)
Anus uzunluğu (µm)	9.32±0.3 (8.2-10.2)	9.4±0.4 (8.9 -10.1)
Tail length (µm)	41.63±0.8 (38.8-44.6)	43.2±1.6 (39.8-44.8)
Tail terminus length (µm)	10.03±0.25 (9.2-11.5)	10.9±0.8 (8.9-12.0)
a	28.42±0.5 (26.65-30.52)	29.1±1.3 (26.0-31.0)
b'	6.9±0.1 (6.6-7.35)	7.6±0.9 (5.7-8.8)
c	8.69±0.12 (8.52-8.90)	-
c'	4.02±0.1 (4.89-5.1)	4.6±0.2 (4.2-5.0)

Molecular characterisation of *Meloidogyne chitwoodi*

PCR product of *M. chitwoodi* DNA with JMVhapla, JMV1 and JMV2 primers formed 540 bp bands at 2 % agarose gel electrophoresis. The results were similar with previous studies of *M. chitwoodi* (Wishart et al., 2002; Adam et al., 2007; Devran et al., 2009; Evlice & Bayram, 2016)(Figure 3).

After phylogeny studies, populations in this study showed 98 % Blast identity with Turkish populations from Niğde, Isparta and Nevşehir KF557828 (467/475), KF557817 (467/475), KF557779 (467/475), KF5599 (467/475), KF557793 (467/475), KF557791 (467/475), KF557771 (467/475), of Evlice & Bayram, (2013) (Figure 4). Compared to populations from other countries, the population in this study showed 97 % identity with populations AJ421701 (464/475) and AF013992 (512/541), 96 % identity with GQ395598 (452/460) from Holland, United Kingdom and USA.

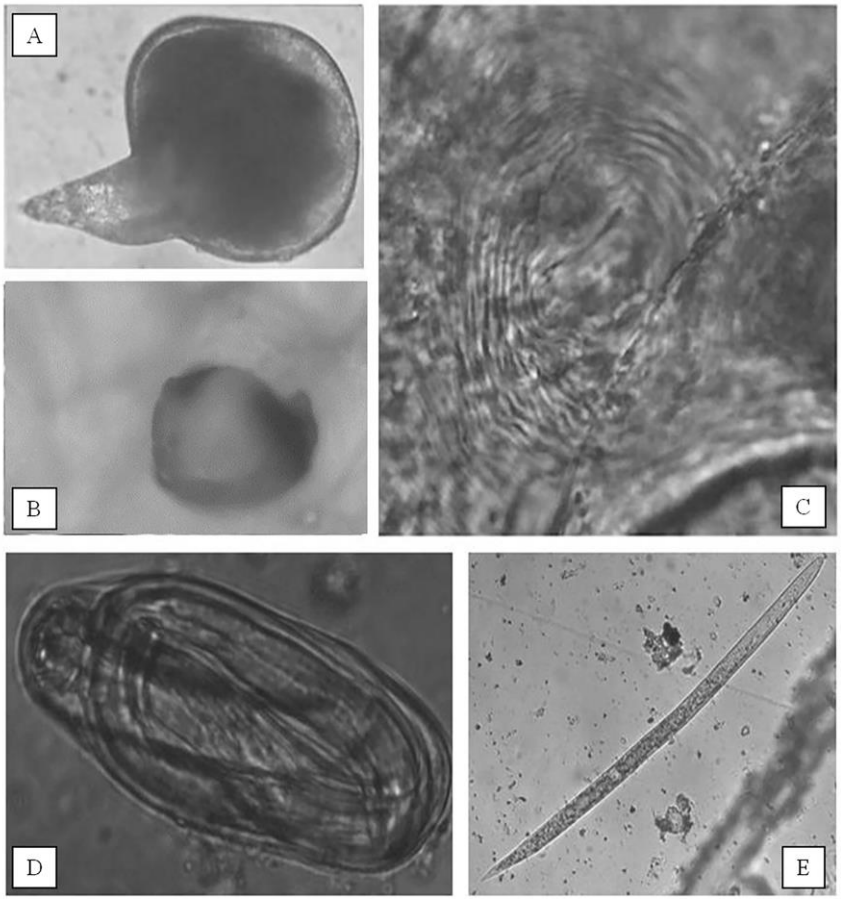

Figure 2. *M. chitwoodi*; A, B) Female body, C) Lateral line of perineal pattern, D) Egg, E) Juvenile
Şekil 2. M. chitwoodi; A-B) Dişi vücudu, C) Anal kesitin lateral hattı, D) Yumurta, E) Larva

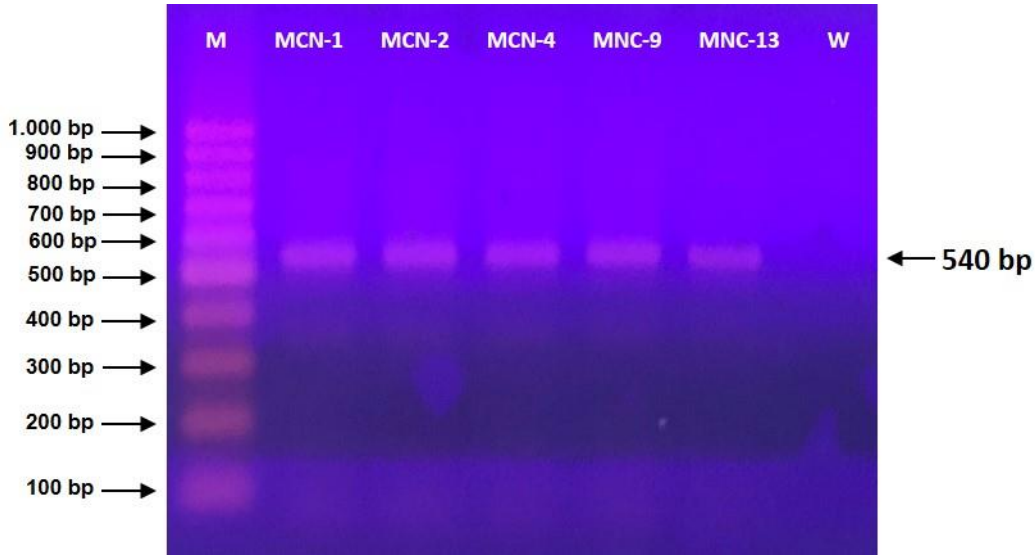

Figure 3. Electrophoresis of PCR products amplified with JMVhapla, JMV1 and JMV2 primers. MC: Sample region, W: water
Şekil 3. JMVhapla, JMV1 ve JMV2 primerleri ile amplifiye edilmiş PCR ürünlerinin elektroforezi. MC: Örnek bölge, W: su

Neighbour joining tree was designed by comparing *M. chitwoodi* isolates with AF013992.1, AJ421701.1, KC262253.1, GQ395598.1, KF557770.1, KF557774.1, KF557772.1, KF557816.1, KF557791.1 accessions of Canada, UK, USA, Switzerland, Türkiye and local isolate in this study. According to tree *M. chitwoodi* higher similarity between Canada, UK, USA, Switzerland in the rate of 99 % was observed and

compared to other Turkish isolates (Aksaray, Konya, Nevşehir, Niğde and Isparta), isolate in this study was found nearest with Nevşehir KF557791.1. The relationship between isolates which belongs to same species in the world was proved with phylogenetic analysis help.

Pairwise distance analysis was conducted on Mega X. differences among 9 *M. chitwoodi* sequences, and the sequences was shown in Figure 5. The distance was varied between 0 to 0,04. The difference was highest in accessions KF557816, AJ421701 and AF013992. P-distance of the samples varied between 0 % to 4 %.

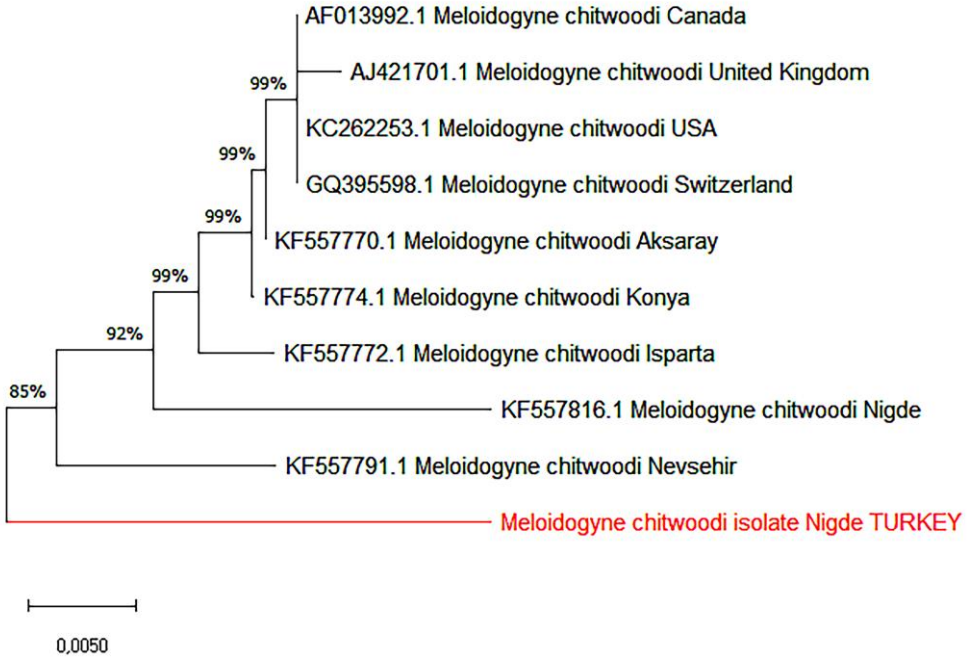

Figure 4. The phylogenetic analysis which belongs to *M. chitwoodi* isolates
Şekil 4. M. chitwoodi izolatlarına ait filogenetik analiz

	1	2	3	4	5	6	7	8	9	10
1. AF013992.1 Meloidogyne chitwoodi Canada										
2. AJ421701.1 Meloidogyne chitwoodi United Kingdom	0.002									
3. KF557791.1 Meloidogyne chitwoodi Nevsehir	0.021	0.023								
4. GQ395598.1 Meloidogyne chitwoodi Switzerland	0.000	0.002	0.021							
5. KC262253.1 Meloidogyne chitwoodi USA	0.000	0.002	0.021	0.000						
6. KF557774.1 Meloidogyne chitwoodi Konya	0.002	0.004	0.021	0.002	0.002					
7. KF557772.1 Meloidogyne chitwoodi Isparta	0.008	0.011	0.021	0.008	0.008	0.006				
8. KF557770.1 Meloidogyne chitwoodi Aksaray	0.000	0.002	0.021	0.000	0.000	0.000	0.004			
9. KF557816.1 Meloidogyne chitwoodi Nigde	0.023	0.026	0.030	0.023	0.023	0.019	0.021	0.017		
10. Meloidogyne chitwoodi isolate Nigde TURKEY	0.035	0.038	0.035	0.035	0.035	0.035	0.035	0.035	0.046	

Figure 5. Pairwise distance of local *M. chitwoodi* and other populations from the same species
Şekil 5. Yerel M. chitwoodi ve aynı türden diğer popülasyonların ikili uzaklıkları

Due to spread by irrigation water and infected seeds, *M. chitwoodi* infected areas increase continuously. Chemical control cannot be effective on individuals which are located and feed in tubers. Hence, toxic chemicals may not reach to nematodes colonized inside cells. Eradication can be achieved only by selection of proper species-specific management methods targeting *M. chitwoodi.*

Meloidogyne spp. are generally identified based on their morphology and morphometrics. Recently esterase and malate dehydrogenase were proven as applicable methods for reliable identification (Karssen et al., 1995). More recently several reports on molecular identification of nematodes were published. Multiplex PCR methods were applied for identification of *M. chitwoodi, M. fallax, M. hapla* and *M. incognita* while classic PCR was found reliable on *M. incognita*, *M. javanica, M. arenaria, M. mayaguensis, M. hapla, M. chitwoodi* and *M. fallax* (Adam et al., 2007; Meng et al., 2004; Devran et al. 2009). Molecular identification is reliable method which can be carried out even by unqualified staff to

any nematode specimen. Furthermore, nematode species at every developmental stage can be used for DNA isolation and diagnostic process.

CONCLUSION

In this study, we were able to identify *M. chitwoodi* from single juvenile sample with PCR using JMVhapla, JMV1 and JMV2 primers. 540 bp expected DNA band was observed on agarose gel electrophoresis. The application of other methods like RFLP analysis of *M. chitwoodi* was previously reported (Devran et al., 2009; Özarslandan et al., 2013). On this study, phylogenetic tree analysis provided to understand better relationship *M. chitwoodi* among other countries. It was determined that the phylogenetic analysis of the isolate showed great similarity with the Türkiye isolates, as expected. In addition, the isolate was highly similar to Canada, UK, USA and Switzerland isolates. These primers and PCR method can be used for identification of local nematode species.

Contribution of the authors as summary

Authors declares the contribution of the authors is equal.

Statement of conflict of interest

Authors have declared no conflict of interest.

Ethics committee decision

Ethics committee approval is not required for this study.

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Entomopatojen Nematod, *Steinernema carpocapsae* (Rhabditida: Steinernematidae)'nın İncir Kurdu *Ephestia cautella* (Walker) (Lepidoptera: Phycitidae) Üzerindeki Etkinliğinin Belirlenmesi

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ÖZET
İncir Kurdu *Ephestia cautella* (Walker) (Lepidoptera: Phycitidae) dünyanın birçok bölgesinde depolanmış ürünlerde zarar oluşturan başlıca zararlılardan birisidir. Bu çalışmada entomopatojen nematod *Steinernema carpocapsae* (Rhabditida: Steinernematidae) (Tokat-Bakışlı05)'nın laboratuvar koşullarında incir kurdu, *E. cautella* üzerindeki etkinliği araştırılmıştır. Denemeler 2 tekrarlı, 5 tekerrürlü olarak 2 ayrı sıcaklıkta (20 ve 25°C) ve %65 nem koşullarında yürütülmüştür. *S. carpocapsae* izolatı üç ayrı konsantrasyonda (250, 500 ve 1000 IJs ml^{-1}) uygulanmış, kontrol olarak saf su kullanılmıştır. Deneme sonucunda *S. carpocapsae* (Tokat-Bakışlı 05) izolatı 20°C'de 96 saat sonunda her üç dozda da %100 etkili bulunmuştur.

Bitki Koruma

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 16.12.2021
Kabul Tarihi : 15.03.2022

Anahtar Kelimeler
Steinernema carpocapsae
İncir kurdu
Ephestia cautella
Entomopatojen nematod

Determination Of The Activity Of Entomopathogenic Nematode, *Steinernema carpocapsae* (Rhabditida: Steinernematidae) on *Ephestia cautella* (Walker) (Lepidoptera: Phycitidae)

ABSTRACT
Ephestia cautella (Walker) (Lepidoptera: Phycitidae) is one of the main pests that cause damage to stored products in many parts of the world. In this study, the effectiveness of the entomopathogenic nematode *Steinernema carpocapsae* (Rhabditida: Steinernematidae) (Tokat-Bakışlı05) on the *E. cautella* under laboratory conditions was investigated. Experiments were carried out with 2 replications and 5 replications at 2 different temperatures (20 and 25ºC) and 65% humidity conditions. *S. carpocapsae* isolate was applied at three different concentrations (250, 500 and 1000 IJs ml^{-1}), and pure water was used as a control. As a result of the experiment, *S. carpocapsae* (Tokat-Bakışlı 05) isolate was found to be 100% effective in al doses after 96 hours at 20 °C.

Plant Protection

Research Article

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Keywords
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Almond moth
Ephestia cautella
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GİRİŞ
İncir güvesi *Ephestia cautella* (Walker) (Lepidoptera: Phycitidae) dünyanın özellikle sıcak, tropik bölgelerinde etkili önemli güve zararlılarından birisidir. Soğuk ve ılıman bölgelerde ise daha sınırlı zarar oluşturmaktadır (Cogburn, 1973; Sinha & Watters, 1985). Larvalar üründe beslenerek zarar yapmakta, çıkardıkları pislikler ve değiştirdikleri gömlek ve pupa kalıntıları ile de kaliteyi düşürmektedirler. En çok tercih ettikleri ve zarar verdikleri ürünler; kuru incir, kuru kayısı, fındık, kuru üzüm, yağlı tohumlar, hububat, un ve mamülleri, kakao, baharatlardır. Günümüzde depolanmış ürün zararlıları ile mücadelede en çok uygulanan yöntem hala pestisitler ile yapılan

fümigasyon uygulamalarıdır. Ancak fumigasyonda kullanılan kimyasalların çevreye, tüketicilere olan zararlı etkisi ve zararlılarda oluşturduğu dayanıklılık nedeniyle araştırmacılar alternatif metodlar üzerinde çalışmaya başlamışlardır (Lu & Wu, 2010). Alternatif mücadele yöntemlerinden olan biyolojik mücadele uygulamaları içerisinde entomopatojen nematod (EPN)'lar önemli bir yer tutmaktadır (Gaugler, 2002; Grewal ve ark., 2005). Böceklerde obligat parazit olarak yaşayan ve önemli birçok zararlıyı baskı altına alabilecek yüksek potansiyele sahip bazı EPN türleri oldukça geniş bir konukçu dağılımına, konukçuyu aktif arama ve enfekte edebilme özelliğine ve uygun koşullarda uzun süre enfektif larva (EL) [infektif juvenil (IJ)] denilen dayanıklı döneminde canlı kalabilme yeteneğine sahiptirler (Bedding ve ark., 1983; Kaya, 1985; Bedding, 1990). Ayrıca EPN'ler hedef dışı organizmalara zarar vermedikleri için çevre dostudurlar ve uygulamaları oldukça ekonomiktir (Gülcü ve ark. 2017).

Bu çalışmada, EPN'lerden *Steinernema carpocapsae* (Rhabditida: Steinernematidae) (Tokat-Bakışlı05)'nın laboratuvar koşullarında incir kurdu *Ephestia cautella* üzerindeki etkinliği araştırılmıştır.

MATERYAL ve METOD

Çalışmanın ana materyalini *Steinernema carpocapsae* (Tokat-Bakışlı05) izolatı, *Galleria mellonella* L. (Büyük balmumu güvesi) ve son dönem *Ephestia cautella* (Walker) (İncir kurdu) larvaları oluşturmuştur.

Galleria mellonella Larvalarının Yetiştirilmesi

Galleria mellonella larvaları içeriğinde 890 g un 222 g kuru ekmek mayası, 500 g gliserin, 500 g bal. 445 g süt tozu ve 445 g buğday kepeği bulunan özel besi ortamında yetiştirilmiştir. Steril edilen un ve buğday kepeği süt tozu ve maya karıştırıldıktan sonra bu karışıma bal ile gliserin eklenmiştir (Mohammed & Coppel, 1983). Yumurta kümeleri 1 L'lik cam kavanozlara hazırlanan besin ortamı üzerine yerleştirilmiş ve larvaların gelişmesi ve yumurtalardan çıkışı kavanozlar 28±2°C sıcaklık ayarlı 16/8 saat aydınlatmalı böcek yetiştirme dolabına yerleştirilmiştir.

Entomopatojen Nematod Kültürünün Oluşturulması

Entomopatojen nematod, *S. carpocapsae* (Tokat-Bakışlı05) kültürü olgun *G. mellonella* larvaları üzerinde üretilmiştir (Kaya & Stock, 1997). Bunun için 6 cm çapındaki petriler içerisine yerleştirilen distile su ile ıslatılmış Whatman üzerine 10'ar adet larva konulmuştur. Daha sonra EPN kültürüne ait IJ'ler damlalıkla alınarak (yaklaşık 5 ml) *G. mellonella* larvaları üzerine verilmiş ve petrilerin kapağı parafilm ile sarılarak 20-23°C'deki inkübatöre yerleştirilmiştir. İnkübatörde hergün kontrolleri yapılmış ve ölüm gerçekleştikten sonra ölü *G. mellonella* larvaları "White trap" düzeneğine alınarak yeni nesil IJ'ler toplanmıştır (White, 1927). Elde edilen bu IJ'ler hücre kültürü şişelerine alınarak inkübatörde +10°C'de muhafaza edilmiştir.

İncir Kurdu (*Ephestia cautella*) Kültürünün Oluşturulması

Ephestia cautella kültürü yetiştirilirken kepek, kuru maya ve gliserin karışımından oluşan besin ortamı kullanılmıştır. Besin ortamı hazırlanırken, kepeğe laboratuvar değirmeninde öğütülerek un haline getirilmiş kuru maya ve besinin nem içeriğini yükseltmek için ise gliserin eklenmiştir. Hazırlanan besin 2 L'lik fanuslara, tabanda 2-3 cm kadar yükseklik oluşturacak şekilde aktarılmış ve besin üzerine yaklaşık 200 adet yumurta bırakılmıştır. Fanusların kapaklarına hava girişini sağlamak için delikler açılmış ve bu deliklere plastik süzgeç teli yapıştırılmıştır. *E. cautella* üretimi yapılan böcek yetiştirme odasında, 25±1°C sıcaklık ve % 60±5 orantılı nem düzeyinde olmuştur.

Laboratuvarda Entomopatojen Nematod Uygulamaları

Denemeler 2 tekrarlı, 5 tekerrürlü, 2 ayrı sıcaklıkta (20 ve 25°C) ve %65 nem koşullarına sahip iklim odasında, plastik petrilerde (9 cm) yürütülmüştür. İçerisine Whatman kağıdı yerleştirilen petrilere 5 g steril edilmiş buğday kırığı koyulmuştur. *E. cautella*'nın son dönem larvaları her petriye 10'ar adet olacak şekilde yumuşak pens yardımı ile aktarılmıştır. Daha sonra 250, 500 ve 1000 Ijs ml^{-1} olacak şekilde saf su ile hazırlanan *S. carpocapsae* izolatı pipet yardımı ile petri içerisine her petriye 1 ml olacak şekilde uygulanmış ve petrilerin kenarları parafilm ile kapatılmıştır. Petrilerdeki böceklerin canlılık durumu 48, 72 ve 96 saatler sonunda düzenli olarak sayılarak ölüm oranları hesaplanmıştır. Ölü bireyler White trap düzeneğine alınmış ve stero mikroskop altında takip edilmiştir (White, 1927). Yaklaşık bir hafta sonra kadavralardan entomopatojen çıkışı izlenmiştir. Kontrolde saf su uygulanmıştır.

İstatistik Analizler

Abbott formülü kullanılarak düzeltilmiş ölümler hesaplanmış, MINITAB paket programı kullanılarak Tukey çoklu karşılaştırma testi ile uygulamalar arasındaki farklılıklar belirlenmiştir.

BULGULAR ve TARTIŞMA

Bu çalışmada yerel bir entomopatojen nematod (EPN) izolatı olan *Steinernema carpocapsae* (Tokat-Bakışlı05)'nın laboratuvar koşullarında incir kurdu

Ephestia cautella'nın son dönem larvaları üzerindeki etkinliği belirlenmiştir. Nematod inokülasyonundan 48, 72 ve 96 saat sonra elde edilen ölüm oranlarına göre yapılan istatistiki değerlendirmelere göre nematod konsantrasyonu ve sıcaklık parametrelerinin ve bu parametrelerin etkileşimlerinin önemli olduğu görülmüştür. Zararlının son dönem larvalarındaki ölüm oranları nematod konsantrasyonuna bağlı olarak artmıştır. En hızlı ve yüksek ölüm oranlarının 20°C'de meydana geldiği görülmüştür. *S. carpocapsae*'nin *E. cautella*'nın son dönem larvaları üzerinde 20 ve 25°C'de oluşturduğu ölüm oranları verilmiştir (Çizelge 1.)

Çizelge 1. *Steinernema carpocapsae'nın Ephestia cautella* larvaları üzerinde 48, 72 ve 96 saat sonunda oluşturduğu ölüm oranları.

Table 1. Mortality (%) of *Ephestia cautella* caused by *Steinernema carpocapsae* at the end of 48, 72 and 96 h.

	Ephestia cautella							
Sıcaklıklar	20 °C				25 °C			
Dozlar	250 IJ	500 IJ	1000 IJ		250 IJ	500 IJ	1000 IJ	Kontrol
48 saat/h	91.7±4.3a[1]	97.5±2.5a	100.0±0.0a	F=2.21;df=2.27;P>0.05	76.5±8.5a	88.0±6.1a	98.0±2.0a	F=3.06;df=2.27;P>0.05
72 saat/h	96.7±3.3a	100.0±0.0a	100.0±0.0a	F=1.0;df=2.27;P>0.05	84.2±6.9b	94.2±3.9a	100.0±0.0a	F=3.21;df=2.27;P<0.05
96 saat/h	100.0±0.0a	100.0±0.0a	100.0±0.0a	*	84.2±8.8a	94.7±3.7ab	100.0±0.0a	F=2.06;df=2.27;P>0.05

[1]Aynı sütunda ortalamaları izleyen farklı küçük harfler, istatistiki olarak birbirinden farklılığı gösterir (Anova P<0.05, Tukey testi)
*Tüm uygulamalarda %100 ölüme ulaşıldığı için hesaplanamamıştır.

Çizelge 1'de görüldüğü gibi kullanılan tüm konsantrasyonların kontrollere oranla etkili olduğu ve konsantrasyon arttıkça zararlının son dönem larvalarının ölüm oranının doğru orantılı olarak arttığı gözlenmiştir. 20°C'de 96 saat sonunda en düşük dozda (250 IJs ml^{-1}) %100 ölüm görülürken aynı sıcaklıkta 1000 IJs ml^{-1} dozda 48 saat sonunda tüm larvaların öldüğü görülmüştür.

72 saat sonunda incir kurdunun son dönem larvalarına karşı en yüksek ölüm oranını 20°C'de 500 IJs ml^{-1} sağlarken 25°C'de en yüksek konsantrasyon olan 1000 IJs ml^{-1} sağlamıştır. En düşük konsantrasyon olan 250 IJs ml^{-1}'de 20°C'de üç sayımda sırasıyla %91, 96 ve %100 ölüm görülmüştür. 96 saat sonunda en düşük doz olan 250 IJs ml^{-1}'de 20 ve 25°C'de sırasıyla %100.0 ve %84.2 ölüm gözlenmiştir.

EPN'lerin konukçuya girerek konukçuyu enfekte etmesi ve akabinde konukçuyu öldürmedeki başarısında en önemli faktörlerden birinin sıcaklık olduğu bilinmektedir (Grewal ve ark., 1994; Gouge ve ark., 1999; Kaya, 1990). Bu çalışma sonucunda denemeye alınan sıcaklıklar arasında entomopatojen nematodun en etkli olduğu sıcaklık 20 °C olmuştur ve bu sıcaklıkta en düşük dozda bile %100 etki görülmüştür.

Yapılan taramalarda EPN'lerin incir kurdu (*Ephestia cautella*) üzerinde etkinliklerinin araştırıldığı bir çalışmaya rastlanılamamıştır. Ancak depo zararlıarı ile yapılmış çalışmalar bulunmaktadır. *Steinernema feliae*'nin üç izolatının (UK 76 [=Nemasys], USA/SC ve Hawaii) Kırma biti (*Tribolium confusum*) ve Un güvesi (*Ephestia kuehniella*), üzerindeki etkinliğinin laboratuvar koşullarında belirlendiği bir çalışmada söz konusu olan entomopatojen nematod izolatları 3 konsantrasyonda (100, 300 ve 900 IJ/ böcek^{-1}) uygulanmış ve Hawaii izolatının *T. confusum*'un larva ve erginleri üzerinde en etkili izolat olduğu bildirilmiştir. Bu türün larvalarındaki ölüm oranının 900 nematod/böcek^{-1} dozunda uygulamadan 7 ve 14 gün sonra sırasıyla %79 ve %100'e ulaştığı ve *T. confusum*'un erginlerindeki ölüm oranının ise %66'yı geçmediği bidirilmiştir. *E. kuehniella* larvalarında ise USA/SC izolatının yine en yüksek dozunun uygulamadan 7 ve 14 gün sonra sırasıyla %52 ve %69 ölüme neden olarak en iyi performansı gösterdiği bildirilmiştir. *S.feliae* Hawaii ve USA/SC izolatlarının her iki tür için umut verici biyolojik kontrol ajanları olarak daha fazla araştırılması gerektiği sonucuna varıldığı belirtilmiştir (Athanassiou ve ark., 2008). Başka bir çalışmada ise *Steinernema* spp.'nin *Plodia interpuctella*, *Ephestia kuehniella*, *Oryzaephilus surinamensis*, *Tenebrio molitor*, *Tribolium castaneum* ve *Trigoderma variable*'nin larva, pupa, erginleri ve *Sitophilus oryzae* ve *Rhizopertha dominica*'nın erginleri üzerindeki etkinlikleri belirlenmiştir. Çalışmada *Steinernema* türlerinden birinin *P. interpunctella* larvalarına karşı %80 ve üzerinde ölüm meydana getirdiği bildirilmiştir (Ramos-Rodriguez ve ark., 2006). Negrisoli ve ark. (2013) yılında sekiz entomopatojen nematod izolatının, beş adet depolanmış ürün zararlısına karşı etkinliğini araştırmışlardır. Çalışma sonucunda tüm böcek türlerin EPN izolatlarına duyarlı olduğu belirlenmiştir. Özellikle *Anagasta kuehniella* ile *Tenebrio molitor*'un larvaları ve *Acanthoscelides obtectus*'un erginlerinin çoğu EPN türü ve/veya izolatının yüksek dozlarına hassas olduğu bildirilmiştir. Bunun yanı sıra *Sitophilus oryzae* ve

S. zeamais'in yetişkinlerinin, tüm EPN'lere nispeten daha az duyarlı olduğu rapor edilmiştir.

Rodriquez ve ark. (2007) yaptıkları başka bir çalışmada *Steinernema riobrave*'nin *T. castaneum* üzerindeki etkinliğini laboratuvar koşullarında araştırmış ve *T. castaneum*'un larva, pupa ve erginlerinde hayatta kalma oranının kontrollerde %77.9 iken uygulama yapılanlarda %27.4'e düştüğü görülmüştür. Çalışmada ayrıca EPN'lara en hassas dönemin larva dönemi olduğu ve sıcaklık (25 ve 30 C) ve bağıl nem (%43, 56–57, 75 ve 100)'in *S. riobrave*'nin etkinliğini önemli ölçüde etkilemediği bildirilmiştir. Ertürk ve ark. (2013) tarafından yürütülen başka bir çalışmada *Steinernema feltiae, S. carpocapsae* ve *Heterorhabditis bacteriophora*'nın Aydın izolatlarının *T. castaneum* ve *T. confusum* erginlerine karşı etkinlikleri yine laboratuar koşullarında araştırılmıştır. Çalışma sonucunda *S.feltiae* ve *H. bacteriophora* uygulamaları arasında önemli bir fark saptanmazken, *S. carpocapsae*'nin 2000 IJs dozunda *T. castaneum* (% 86.47 mortalite) ve *T. confusum* (% 85.35 mortalite) erginlerinde en etkili izolat olduğu bulunmuştur.

Javed ve ark. (2020) *Steinernema pakistanense* (LM-07), *S. bifurcatum* (LM-30), *S. affinae* (GB-14) ve *S. cholashanense* (GB -22)'nin laboratuvar koşullarında *T. confusum v*e *Rhyzopertha dominica* erginlerine karşı etkinliklerinin üç farklı sıcaklıkta (20, 25 ve 30°C) ve 3 farklı konsantrasyonda (50, 100 ve 150 IJs böcek^{-1}) araştırıldığı bir çalışmada ise *S. pakistanense* 150 IJs böcek^{-1} dozunda 30^{0}C % 100 ölüm meydana getirmiştir. Diğer yandan Erdoğuş'un 2021 yılında laboratuvar koşullarında yürüttüğü çalışmada 4 adet entomopatojen nematod izolatının [*Steinernema carpocapsae* (Tokat-Bakışlı05), *S. feltiae* (Tokat-Emir), *Heterorhabditis bacteriophora* (TOK20), *H. bacteriophora* (11KG)] *Tribolum castaneum* üzerindeki etkinlikleri araştırılmıştır. Denemeye alınan tüm nematod türlerinin 25°C'de etkili olduğu ancak en etkili türün *H. bacteriophora* (11KG) olduğu bildirilmiştir (Erdoğuş, 2021). Yapılan başka bir çalışmada Türkiye ve Kırgızistan'a ait Entomopatojen nematod izolatlarından *Heterorhabditis bacteriophora* türüne ait iki (11KG ve TOK20) ve *Steinernema feltiae* türüne ait iki (3KG ve Tokat-Emir) izolatın, fasulye bitkisinin önemli bir depo zararlısı olan fasulye tohum böceği (*Acanthoscelides obtectus* Say. Coleoptera: Bruchidae)'ne karşı etkinliği ortaya konmuştur. Tek doz denemelerinde, en yüksek etkiyi *S. feltiae*'nin her 2 izolatının (3KG ve Tokat-Emir) gösterdiği bildirilmiştir (%99,59±0,65). 3 farklı doz (25, 50 ve 100 IJ böcek^{-1}) ve 3 farklı sıcaklıkta (10, 15 ve 20°C) yürütülen deneme sonucunda en etkili izolatın 10 °C'de 100 IJ dozunda *H. bacteriophora* TOK20 izolatı (%73,09), 15°C'de 100 IJ dozunda *S. feltiae* 3KG izolatı (%100) ve 20°C'de 100 IJ dozunda *S.feltiae* 3KG izolatı (%100) olduğu bildirilmiştir. Doz ölüm denemeleri sonucunda; *A. obtectus*'a karşı *S. feltiae* 3KG izolatının 500 IJ böcek^{-1} konsantrasyonunda en yüksek etkiye sahip olduğu ortaya konmuştur (Ağım & Kepenekci, 2021). Yine Tülek ve ark. (2015), bir depo zararlısı olan *Rhyzopertha dominica* (F.)'ya karşı *Steinernema feltiae* (Aydın izolatı) türünün %28 ölüm oranıyla düşük etki gösterdiğini tespit etmişlerdir.

SONUÇ ve ÖNERİLER

Zararlılarla mücadelede başarılı ve etkili bir sonuç alınabilmesi için en önemli unsurlardan biri zararlı böcek türleri üzerinde en etkili olabilecek Entomopatojen nematod (EPN) türünün uygulanması gerekliliğidir (Gözel, 2016). Çünkü bazı nematod türleri oldukça geniş bir konukçu dağılımına sahipken, bazı türler sadece tek bir böcek takımını enfekte edebilmektedir. Sonuç olarak EPN türlerinin zararlı böcekler üzerindeki etkinlikleri farklılık gösterebilmektedir (Hazir & ark. 2003). Çalışmada kullanılan *Steinernema carpocapsae* (Tokat Bakışlı 05) (Rhabditida: Steinernematidae) izolatı 20 °C ve 25°C'de 1000 IJ ml^{-1} %100 etkili bulunmuştur. Bu izolatın laboratuvar koşullarında *Ephestia cautella* üzerinde etkili olduğu ve konu ile ilgili daha detaylı çalışmaların yapılmasının uygun olacağı düşünülmektedir.

TEŞEKKÜR

Entomopatojen nematod izolatını [*Steinernema carpocapsae* (Tokat-Bakışlı05)] sağlayan Doç. Dr. Turgut ATAY ve Ayşegül ÇAĞLAYAN'a (Tokat Gaziosmanpaşa Üniversitesi Ziraat Fakültesi Bitki Koruma Bölümü Tokat, TÜRKİYE) teşekkür ederiz.

Araştırmacıların Katkı Oranı Beyan Özeti

Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan eder.

Çıkar Çatışması Beyanı

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

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Kurutmalık Biber Meyvelerinde İç Çürüklüğüne Neden Olan Bazı Fungal Etmenlere Karşı Bitki Uçucu Yağlarının *in vitro* Antifungal Etkileri

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ÖZET

Hasat öncesi ve sonrası tarımsal ürünlerde meyve iç çürüklüğüne neden olan fungal etmenler ciddi kalite ve verim kayıplarına neden olurken, gıdalarda ürettikleri mikotoksinler tüketiciler için sağlık riski oluşturur. Bu çalışmada, Hatay ilinde yetiştirilen ve yerel pazarlarda satılan kurutmalık biber meyvelerinde iç çürüklüğü belirtisine neden olan fungal hastalık etmenlerinin, izolasyonu, morfolojik ve MALDI-TOF yöntemleri ile tanılanması, yaygın hastalık etmenlerine karşı farklı bitki uçucu yağlarının antifungal etkilerinin belirlenmesi amaçlanmıştır. Hastalık belirtisi gösteren meyve örneklerinden yapılan izolasyonları müteakiben yapılan morfolojik ve MALDI-TOF tanılama çalışmaları sonucunda *Alternaria alternata* ve *Aspergillus niger* en sık rastlanan fungal hastalık etmenleri olarak belirlenmiştir. Hastalık etmenlerine karşı kekik (*Thymus vulgaris* L., *Tymbra spicata* L. ve *Origanum syriacum L.*), rezene (*Foeniculum vulgare* Mill.), defne (*Laurus nobilis* L.) ve okaliptüs (*Eucalyptus camaldulensis* Dehnh) uçucu yağlarının buhar fazında farklı dozlarının antifungal etkinlikleri *in vitro* koşullarda araştırılmıştır. Fungus izolatlarına karşı en yüksek antifungal etkinlik (% 100 engelleme) *Thymbra spicata*, *Origanum syriacum* ve *Thymus vulgaris* uçucu yağlarının 4.0-6.0 µl petri^{-1} dozlarında gözlenmiştir. Test edilen fungal izolatlara karşı en düşük antifungal etkinlik ise *Eucalyptus camaldulensis* (16.0-40.0 µl petri^{-1}) uçucu yağı tarafından gösterilmiştir. Yapılan çalışmalarla uçucu yağların antifungal özellikleri ve EC_{50} değerleri belirlenmiştir. Elde edilen sonuçlara göre bitki uçucu yağları, doza bağlı bir şekilde test edilen izolatlara karşı antifungal etkinlik göstermişlerdir. Elde edilen sonuçlar bitki uçucu yağlarının, kurutmalık tarımsal ürünlerde biyofumigant olarak uygulanabileceğini göstermiştir.

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Anahtar Kelimeler
Uçucu yağlar
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Biber
Alternaria alternata
Aspergillus niger

In vitro Antifungal Effects of Plant Essential Oils Against Some Fungal Disease Agents Causing Internal Rot of Dried Pepper Fruits

ABSTRACT

Fungal disease agents that cause internal fruit rot in agricultural products before and after harvest cause serious quality and yield losses. At the same time, the mycotoxins they produce in foods pose a health risk for consumers. In this study, it was aimed to isolate fungal disease agents that cause internal rot symptoms in dried pepper fruits grown in Hatay province and sold in local bazaars, to identify by morphological and MALDI-TOF methods, and to determine the antifungal effects of different plant essential oils against the most common disease agents. *Alternaria alternata* and *Aspergillus niger* were determined as the most common fungal disease agents following the result of isolations, morphological and MALDI-TOF diagnostic studies from fruit samples showing signs of disease. Antifungal effects of different vapor doses of essential oils of different thyme (*Thymus vulgaris* L., *Tymbra spicata* L. and *Origanum syriacum* L.), fennel (*Foeniculum vulgare* Mill.), laurel (*Laurus nobilis* L.) and eucalyptus (*Eucalyptus camaldulensis* Dehnh) were investigated against fungal disease agents *in vitro* conditions. The

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highest antifungal activities (100% inhibition) against fungal isolates were displayed by essential oils of *Thymbra spicata* (2.0-4.0 µl Petri^{-1}), *Origanum syriacum* (4.0 µl Petri^{-1}) and *Thymus vulgaris* (4.0-6.0 µl Petri^{-1}). *Eucalyptus camaldulensis* (16.0-40.0 Petri^{-1}) exhibited the lowest antifungal activity against tested fungal isolates. In addition, the antifungal properties and EC_{50} values of essential oils were also determined. Plant essential oils showed antifungal effects against the tested fungal isolates in a dose-dependent manner. The results showed that plant essential oils might be applied as biofumigants in dried agricultural products.

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GİRİŞ

Solanaceae familyasında yer alan biber (*Capsicum annuum* L.), Dünyada ve Türkiye'de en fazla üretilen sebzelerden biri olup, gerek taze olarak gerekse kurutmalık veya baharat olarak oldukça fazla tüketilmektedir. 2020 yılı biber üretim verilerine göre Türkiye, sırasıyla Çin, Meksika ve Endonezya'dan sonra 2 636 905 ton üretim miktarı ile dünyada en çok taze biber üretimi gerçekleştiren 4. ülke olurken, aynı yıl 16 271 ton kurutmalık biber üretimiyle ise dünya genelinde 24. sırada yer almıştır (Wien, 1997; Anonymous, 2022).

Kurutmalık biber ve bunlardan üretilen baharatlar, fungal etmenlerin üretmiş oldukları mikotoksinlerden en fazla etkilenen bitkisel ürünlerden biridir (Özkaya ve ark., 1999; Duman, 2010). Kurutmalık olarak tüketilen biberler üzerinde gelişen ve insan sağlığına ciddi zararlar verebilen mikotoksinlerden kaynaklı ürün kayıpları son yıllarda önemli bir boyuta ulaşmıştır (Ham ve ark. 2016). Farklı ürünlerde toplamda 400'e yakın fungal etmen tarafından oluşturulan mikotoksinlerin, insanlarda ve hayvanlarda doğrudan veya dolaylı bir şekilde ciddi sağlık sorunlarına sebep oldukları farklı çalışmalarda bildirilmiştir (Hussein & Brasel, 2001; Smith, 2001; Weidenbörner, 2014; Hontanaya ve ark., 2015; Öksüztepe & Erkan, 2016).

Özellikle *Aspergillus, Alternaria*, *Penicillium* ve *Fusarium* cinslerine ait türlerinin en fazla mikotoksin oluşturan hastalık etmenleri oldukları farklı çalışmalarda bildirilmiştir (McKee, 1995; Kabak & Dobson, 2017).

Mikotoksinler, gıda ürünlerine bulaştıktan sonra çevresel koşullara dayanıklı olmalarından dolayı bu ürünlerden uzaklaştırılmaları oldukça zor olmaktadır. Bu nedenle bulaşıklık oluşmadan önce alınacak koruyucu tedbirler, ürünlerde mikotoksin oluşumunu sınırlandırmak adına önemlidir (Dvegowda ve ark. 1998; Smith, 2001). Mikotoksin oluşturan funguslarla mücadelede başvurulan yöntemlerin başında kimyasal fungisit uygulamaları gelmektedir. Ancak kullanılan kimyasallara fungal etmenlerin zamanla dayanıklılık kazanması, mücadele etkinliğini azaltabilmektedir (Smith, 2001; Dwivedy ve ark., 2016).

Son zamanlarda bu etmenlerle mücadelede kullanılan kimyasal pestisitlerin insan sağlığına, doğaya, çevreye ve hedef dışı canlılara olan zararlarının artması ve insanların bu maddelerin kullanımı konusunda bilinçlenmesinden dolayı kimyasal pestisit kullanımı konusunda daha mesafeli durulmaktadır. Bu yaklaşımlar neticesinde, bakteriyel ve fungal kökenli hastalık etmenleriyle kimyasal pestisitlere alternatif, insan sağlığına ve doğaya dost mücadele yöntemlerinin araştırılması ve geliştirilmesi zorunluluğu ortaya çıkmıştır (Soliman & Badeaa, 2002; Soylu ve ark., 2022).

Son yıllarda bilim insanları tıbbi ve aromatik bitkilerden elde edilen uçucu yağ, ekstrakt ve doğal bileşenlerinin bitkisel ürünlerde sorun olan fungal, bakteriyel hastalık etmenleri ve zararlıların yanısıra gıdasal ürünlerde mikotoksin oluşturan *Aspergillus, Alternaria, Botrytis, Fusarium, Penicillium* vb. cinslerine dahil farklı fungal türlere karşı kimyasal pestisitlere alternatif kullanılabilme potansiyellerinin araştırılmasına yönelik çalışmalara yoğunlaşmışlardır (Soylu ve ark., 2005; Askun ve ark., 2008; Sertkaya ve ark., 2010; Soylu ve ark., 2010; Kurt ve ark., 2011, Tyagi & Malik, 2011; Otoni ve ark., 2014; Nazareth ve ark., 2016; Hu ve ark., 2017; Kaya ve ark., 2018; Kara ve ark., 2022; Atay & Soylu, 2022). Yapılan literatür araştırmasında farklı baharat ve kuruyemişlerde mikotoksin oluşturan fungal tür(ler)e karşı pekçok sayıda çalışma mevcutken, biber meyvelerinde iç çürümelere neden olan fungal hastalık etmenlerine karşı bitki uçucu yağların antifungal etkinliği konusunda yapılmış oldukça sınırlı sayıda çalışmalar bulunmaktadır.

Bu çalışmada, (i) Hatay ili genelinde yetiştirilen ve yerel aktarlarla pazarlarda satılan kurutmalık biber

meyvelerinde iç çürüklüğün neden olan fungal hastalık etmenlerinin izolasyonu, (ii) morfolojik ve MALDI-TOF ile tanılanması, (iii) yaygın olarak belirlenen mikotoksin oluşturma potansiyeline sahip fungal etmenlerden *Alternaria alternata* ve *Aspergillus niger*'e karşı farklı bitki türlerinden (*Thymus vulgaris* L., *Tymbra spicata* L., *Origanum syriacum* L., *Foeniculum vulgare* Mill., *Laurus nobilis* L. ve *Eucalyptus camaldulensis* Dehnh) elde edilmiş bitki uçucu yağlarının antifungal etkileri araştırılmıştır.

MATERYAL ve METOD

Çalışmanın ana materyallerini oluşturan fungal izolatlar *Aspergillus niger* ve *Alternaria alternata* yapılan sörveylerde toplanan kurumuş veya iç çürüklüğü belirtisi gösteren biberlerden izole edilmiştir. Çalışmada antifungal etkileri farklı bitki pataojeni fungal ve bakteriyel hastalık etmenlerine karşı araştırılan *Thymus vulgaris, Thymbra spicata, Origanum syriacum, Laurus nobilis, Foeniculum vulgare* ve *Eucalyptus camaldulensis* uçucu yağlarının, kimyasal içerikleri önceden belirlenmiş olmaları nedeniyle seçilmişlerdir. Fungal hastalık etmenlerinin izolasyonu, tanısı ve uçucu yağların antifungal etkilerinin belirlendiği çalışmalarda kullanılan Patates Dekstroz Agar (PDA) besi yeri (Merck, Darmstad, Germany) kullanılmıştır.

Bitkilerden Uçucu Yağların Elde Edilmesi

Funguslara karşı antifungal etkileri araştırılan uçucu yağlar, kurutulan bitkilerin farklı kısımlarından elde edilmiştir. *Thymus vulgaris, Thymbra spicata, Origanum syriacum, Laurus nobilis* ve *Eucalyptus camaldulensis* uçucu yağları bitkilerin yapraklarından, *Foeniculum vulgare* uçucu yağı ise bitkinin tohumlarından daha önceden bildirildiği şekilde Clevenger tipi uçucu yağ çıkartma cihazı ile 3 saatlik buhar distilasyonu sonucu elde edilmiştir (Soylu ve ark., 2010). Elde edilen uçucu yağlar, çalışmalarda kullanılmak üzere içerisinde anhidroz sodyum sülfat bulunan koyu renkli cam şişelerde -20 °C'de muhafaza edilmiştir.

Fungal Etmenlerin İzolasyonu ve Tanısı

Hatay ilinin önemli biber ekim alanlarındaki tarlalarında hastalık belirtileri gösteren hasat olgunluğundaki biberler ile yerel aktar ve halk pazarlarından tesadüfen toplanmış kurutulmuş kırmızı baş biberleri hastalık etmenlerinin izolasyonunda kullanılmıştır. Hastalık belirtisi gösteren bitki dokuları yüzeysel olarak dezenfekte edildikten sonra 50 µg ml^{-1} streptomisin sülfat içeren PDA besi ortamına ekimleri yapılmış ve 3-5 gün boyunca 25 °C'de inkübasyona bırakılmıştır. İnkübasyon sonrası petrilerde gelişen fungus kolonilerinden saflaştırmalar yapılmış ve denemelerde kullanılmak üzere tek spor izolatları elde edilmiştir (Soylu ve ark., 2021). Saf kültürler sonraki çalışmalarada kullanılmak üzere +4 °C'de muhafaza edilmiştir. Fungal izolatların tür teşhisleri gerek morfolojik olarak (Dugan, 2006), gerekse MALDI-TOF (Soylu ve ark., 2020) analizleriyle belirlenmiştir.

Fungal Izolatların Patojenisite Testleri

Elde edilen fungal izolatların patojenisite testlerinde sağlıklı, taze kırmızı biber meyveleri kullanılmış ve testler 2 farklı şekilde uygulanmıştır. Birinci yöntem olarak, 5 günlük fungus kültürlerinin hazırlanan spor süspansiyonları (10^5 spor ml^{-1}), sağlıklı biber meyveleri (n=5) içerisine (50 µl $biber^{-1}$ olacak şekilde) doğrudan enjekte edilmiş ve enjeksiyon bölgesi parafilmle sarılmıştır. İkinci yöntem olarak ise, yine 5 günlük fungus kültürlerinden alınan misel diski (6 mm çapında), steril koşullarda kesilen sağlıklı biber meyvesi (n=5) içerisine yerleştirilmiş ve sonradan kesilen doku parafilm ile sarılmıştır. Her iki şekilde de inokulasyonu yapılmış biber meyveleri önce 15x25x15 cm ebatlarında steril plastik saklama kapları içerisine konulmuştur. Kapların tabanına önceden steril su ile ıslatılmış kurutma kağıtları yerleştirilerek gerekli nem koşulları sağlanmıştır. Bu şekilde hazırlanmış ve kapağı kapalı kutular daha sonra 16:8 aydınlık/karanlık foto periyoda, 24 °C sıcaklığa ayarlanmış inkübatörlerde 5-10 gün süre ile inkübasyona bırakılmıştır. Kontrol olarak sağlıklı biber meyvelerin inokulasyonunda steril su ve steril PDA diski kullanılmıştır. Bu süre sonunda inokulasyon noktalarında ortaya çıkan iç çürüklüğü-yumuşaması belirtilerinden re-izolatlar elde edilmiştir. Elde edilen re-izolatlar, benzer teşhis yöntemleri kullanılarak orijinal izolatlarla karşılaştırılmış ve tanıları tekrar teyit edilmiştir.

Bitki Uçucu Yağlarının *Alternaria alternata* ve *Aspergillus niger*'in Misel Gelişimi Üzerine Antifungal Etkilerinin Belirlenmesi

Uçucu yağların fungal izolatların misel gelişimi üzerine olan antifungal etkileri PDA besi yeri içeren cam petri kaplarında (90 mm çapında) *in vitro* koşullarda araştırılmıştır. Bu amaçla 7 günlük kültürden alınan miselyal agar diskleri (6 mm), PDA besi yeri içeren (20 ml $petri^{-1}$) petri kaplarına inokule edilmiştir. Petri kapağının iç yüzeyinin merkezine ise mikro pipet yardımı ile uçucu yağların farklı konsantrasyonları (0.25-40.0 µl $petri^{-1}$) konulduktan sonra hızlı bir şeklide kapak kapatılarak, petriler parafilm ile (2-3 kez) sarılmıştır. İşlem sonunda petriler ters çevrilerek (kapak altta kalacak şekilde) 25 °C'de 5-7 gün inkübasyona bırakılmıştır. Kontrol grubu petrilere aynı şekilde fungus diskleri yerleştirilmiş ancak uçucu yağ yerine sadece steril saf su emdirilmiştir. Kontrol petrilerinde fungus misel

gelişimi tüm petri yüzeyini kapladığında fungal koloni çapları ölçülerek değerlendirilmiş, her bir uçucu yağ için farklı konsantrasyonlarda engelleme oranı, (%) Abbott formülüne göre hesaplanmıştır.

Engelleme (%) = [(K_{FG}-U_{FG})/K_{FG}] x 100

K_{FG} = Kontrol petrilerdeki fungal gelişim (mm)
U_{FG} = Uygulama yapılmış petrilerdeki fungal gelişim (mm)

Uçucu yağların farklı dozlarına ait antifungal etkinlik çalışmaları 3 tekerrür olacak şekilde tesadüf parselleri deneme desenine göre kurulmuştur. Yapılan denemeler iki farklı zamanda tekrarlanmıştır.

Uçucu Yağlarının *in vitro* Koşullarda Fungisidal ve Fungistatik Özelliklerinin Belirlenmesi

Uçucu yağların en düşük engelleme konsantrasyonlarında (MIC)'daki antifungal etkilerinin fungisidal ya da fungistatik özellikle olup olmadığını belirlemek amacıyla, denemeler sonrası petrilerde gelişme göstermeyen misel diskleri, taze PDA besi yerine (n=3) aktarılmış ve 25°C'de 5 gün boyunca tekrar inkübasyona bırakılmıştır. Misel diskleri yeni aktarıldıkları PDA besi yeri üzerinde herhangi bir gelişme göstermemişse uçucu yağın antifungal etkisi fungisidal (tamamen engeleyen, öldürücü), misel gelişimi tekrar başlamış ise uçucu yağın antifungal etkisi fungistatik (geçici) olarak kayıt edilmiştir. Denemeler her uçucu yağın MIC dozu için 3 tekerrür olacak şekilde tesadüf parselleri deneme desenine göre kurulmuştur. Yapılan denemeler iki farklı zamanda tekrarlanmıştır.

Deneme Deseni ve Istatistik Analizler

Farklı uçucu yağ konsantrasyonlarında ölçülen koloni çapları SPSS istatistik programı (SPSS Statistics 17.0) kullanılarak tek yönlü ANOVA ile analiz edilmiş, uygulamalar (yağlar ve dozları) arasındaki farklılık Tukey HSD Testi ile karşılaştırılmıştır ($P \leq 0.05$). Uçucu yağların farklı konsantrasyonlarda misel gelişimini %50 düzeyinde engelleyen etkili konsantrasyonları (EC_{50}), her bir uçucu yağ için farklı konsantrasyonlardaki ortalamaları SPSS istatistik programının (Versiyon 11.5, SPSS Inc., Chicago, IL, USA) kullanıldığı Probit analiziyle değerlendirilmiştir.

BULGULAR ve TARTIŞMA

Kurutmalık Biberlerde Çürümelere Neden Olan Hastalık Etmenlerinin İzolasyonu, Tanılanması ve Patojenisite Testleri

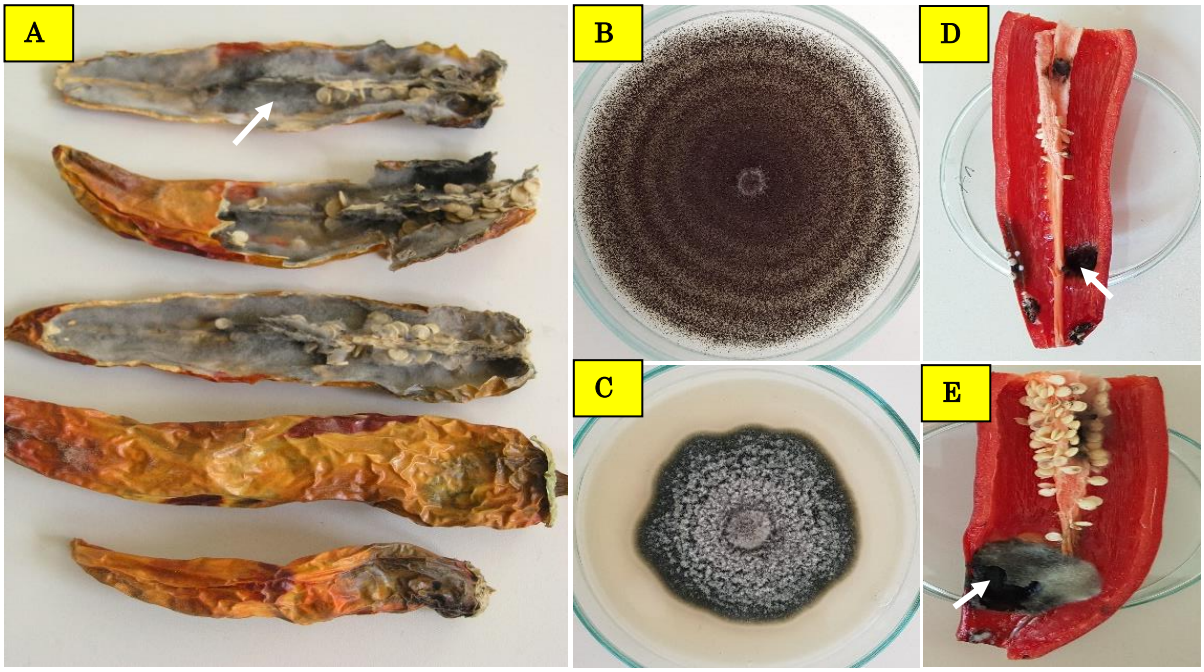
Kurutmalık biber meyvelerinde çürümelere neden olan (Şekil 1A) fungal etmenleri belirlemek amacıyla yapılan izolasyonlarda *Aspergillus niger, A. flavus, Fusarium incarnatum*, *Botrytis cinerea*, *Cladosporium* spp, *Penicillium italicum, P. digitatum ve Rhizopus stolonifer* gibi patojenik ve saprofitik karakterli fungal türler izole edilmiştir. Elde edilen izolatlar arasında bir çok bitkide hasat öncesi ve sonrası dönemde önemli derecede verim kayıplarına sebep olan fungal etmenlerden *Aspergillus niger* ve *Alternaria alternata* (Şekil 1B,C) en yaygın 2 tür olarak bulunmuştur. Morfolojik olarak ön teşhisleri yapılmış olan bu 2 türe ait izolatlara karşı uçucu yağların etkileri araştırılmadan önce sağlıklı biber meyvelerinde patojenisite testleri yapılmıştır. Yapılan patojenisite çalışmaları sonrası fungal izolatların, inokule edilmiş meyvelerin iç dokularında izole edildikleri doğal enfekteli meyvelerdeki belirtilere benzer belirtilere neden olduğu gözlenmiştir (Şekil 1D,E). Patojenisite testi yapılan sağlıklı meyvelerde gelişen fungal izolatlar (re-izolatlar) için, orijinal izolatlara uygulanmış olan benzer teşhis yöntemleri uygulanmış, yapılan teşhis çalışmaları sonrası re-izolatların orijinal izolatlarla aynı türler oldukları belirlenerek bunların biberde patojen oldukları teyit edilmiştir. Türlerin morfolojik tanıları yapılan MALDI-TOF (Şekil 2) analizi ile teyit edilmiştir. Uçucu yağların antifungal etkinlikleri, izolasyonlar sırasında yaygın türler olarak belirlenen *Aspergillus niger* ve *Alternaria alternata* etmenlerine karşı araştırılmıştır.

Bitki Uçucu Yağlarının, *Alternaria alternata*'ya olan *in vitro* Antifungal Etkileri

A. alternata'ya karşı denemedeki uçucu yağların MIC değerlerine bakıldığında en etkili uçucu yağların, 2.0 µl $petri^{-1}$ dozla *T. spicata* ve *T. vulgaris* olduğu, bunları sırasıyla 4.0 µl $petri^{-1}$ dozla *O. syriacum*, 8.0 µl $petri^{-1}$ dozla *F. vulgare*, 16 µl $petri^{-1}$ doz ile *L. nobilis* ve *E. camaldulensis* uçucu yağlarının izlediği belirlenmiştir (Şekil 3).

Yapılan istatistik analiz sonucunda *A. alternata*'ya karşı denenen farklı uçucu yağ konsantrasyonları arasında farkın önemli olduğu belirlenmiş olup (Çizelge 1) bu uçucu yağ konsantrasyonlarının fungal hastalık etmeninin misel gelişimini % engellenmesi üzerine olan antifungal etkinliği ise Çizelge 2'de verilmiştir. Yapılan probit analiz sonrası uçucu yağların *A. alternata*'nın misel gelişimini %50 oranında engelleyen etkili konsantrasyon

(EC_{50}) değerleri, *T. spicata* için 0.548 µl $petri^{-1}$, *O. syriacum* için 0.596 µl $petri^{-1}$, *T. vulgaris* için 0.682 µl $petri^{-1}$, *F. vulgare* için 1.305 µl $petri^{-1}$ ve *L. nobilis* için 3.351 µl $petri^{-1}$ olarak hesaplanırken, en yüksek EC_{50} değeri *E. camaldulensis* için 3.355 µl $petri^{-1}$ olarak belirlenmiştir (Çizelge 1). Uçucu yağların *A. alternata*'ya karşı fungisidal/fungistatik etkilerine bakıldığında *T. spicata*, *T. vulgaris* ve O. syriacum uçucu yağları MIC değerlerindeki antifungal etkinliğin **fungisidal**, *F. vulgare, L. nobilis* ve *E. camaldulensis* uçucu yağları ise **fungistatik** özellikte olduğu belirlenmiştir (Çizelge 2, Şekil 4).



Şekil 1. Hastalık belirtisi gösteren biber meyveleri (a) ve iç kısımlarda görülen fungal gelişim (ok). Hastalıklı biberlerin iç dokularından izole edilen *A. niger* (b) ve *A. alternata* (c) izolatlarının petrilerde tipik koloni gelişimi. Patojenite testleri sonucu biber meyvelerinde *A. niger* (d) ve *A. alternata* (e) izolatlarının inokulasyon noktalarında oluşturdukları iç çürüklüğü belirtileri (ok).

Figure 1. (a) Pepper fruits showing disease symptoms and fungal mycelial growth seen in the interior (arrow) parts. Typical colony development fungal disease agents A. niger (b) and A. alternata (c) isolates obtained from the internal tissues of diseased peppers. Typical internal rot symptoms (arrows) caused by A. niger (d) and A. alternata (e) isolates at the inoculation points in pepper fruits following pathogenicty test.

Analyte ID:	As2
Analyte Creation Date/Time:	2017-04-14T02:24:06.751
Applied MSP Library(ies):	
Applied Taxonomy Tree:	Projects, Bruker Taxonomy, Taxonomy

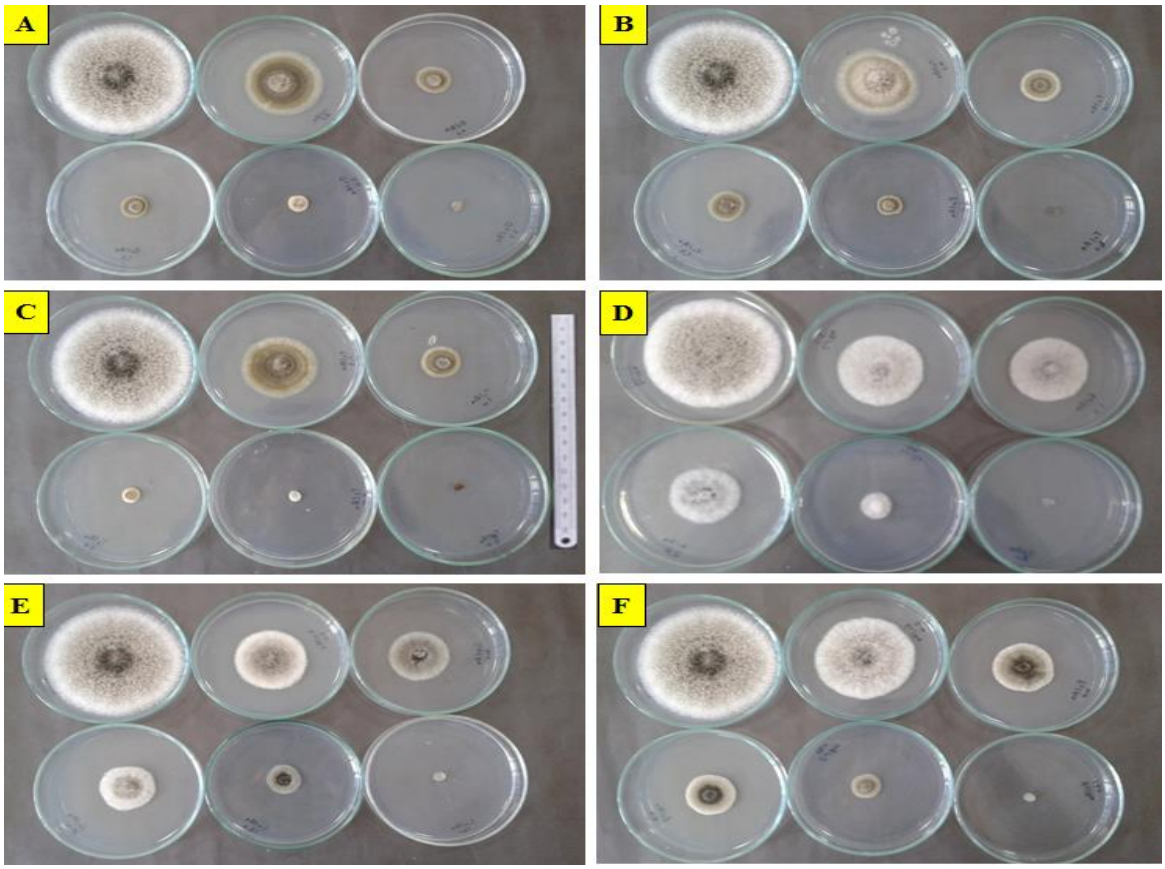
Rank (Quality)	Matched Pattern	Score Value	NCBI Identifier
1 (++)	Aspergillus niger e7158 LLH	2.264	126754417
2 (++)	Aspergillus niger D_16_256_7_3 LLH	2.22	126754417
3 (++)	Aspergillus niger 2008_146035 MUZ	2.18	126754417
4 (++)	Aspergillus niger 01 MPA_1261 MPA	2.128	126754417
5 (++)	Aspergillus niger DSM 22593 DSM	2.063	126754417

Analyte ID:	b5
Analyte Creation Date/Time:	2017-02-17T02:59:40.715
Applied MSP Library(ies):	
Applied Taxonomy Tree:	Projects, Bruker Taxonomy, Taxonomy

Rank (Quality)	Matched Pattern	Score Value	NCBI Identifier
1 (+++)	Alternaria alternata DSM 62010 DSM	2.344	126754417
2 (++)	Alternaria alternata DSM 62006 DSM	2.071	126754417
3 (+)	Alternaria alternata 111116_04 IMD	1.976	126754417
4 (+)	Alternaria alternata DSM 12633 DSM	1.91	126754417
5 (-)	Alternaria alternata DSM 1102 DSM	1.649	126754417

Şekil 2. Hastalık belirtisi gösteren biber meyvelerinden elde edilen *A. niger* ve *A. alternata* izolatlarının MALDI-TOF tanılama sonuçları.

Figure 2. MALDI-TOF identification results of A. niger and A. alternata isolates obtained from symptomatic pepper fruits



Şekil 3. *In vitro* koşullarda (A) *O. syriacum*, (B) *T. vulgaris*, (C) *T. spicata*, (D) *F. vulgare*, (E) *L. nobilis*, ve (F) *E. camaldulensis* uçucu yağlarının *A. alternata*'nın misel gelişiminin engellenmesi üzerine olan antifungal etkileri.

Figure 3. The antifungal effects of (A) O. syriacum, (B) T. vulgaris, (C) T. spicata, (D) F. vulgare, (E) L. nobilis, and (F) E. camaldulensis essential oils on inhibition of mycelial growth of the fungal agent A. alternata.

Çizelge 1. Farklı uçucu yağların uygulandığı petrilerde gelişen *A. alternata* koloni çapları (mm)

T*able 1. Colony diameters (mm) of A. alternata in different essential oil applied petri plates*

	Farklı uçucu yağların misel gelişiminin (mm) engellenmesi üzerine etkinliği					
Doz	***T. spicata***	***T. vulgaris***	***O. syriacum***	***L. nobilis***	***E. camaldulensis***	***F. vulgare***
0	84.3e	84.3f	84.3f	81.7f	81.7f	73.7h
1	43.3d	53.7e	48.3e	47.3e	55.0e	49.3g
2	20.7c	25.3d	21.3d	38.3d	34.3d	44.0f
3	11.7b	16.7c	15.3c	31.0c	27.7c	40.3e
4	0.0a	11.0b	9.7b	22.3b	17.0b	33.7d
5	0.0a	0.0a	0.0a	0.0a	0.0a	16.7c
6	nt	nt	nt	nt	nt	6.3b
7	nt	nt	nt	nt	nt	0.0a
EC_{50}	0.548	0.682	0.596	3.351	3.555	1.305

Ts, Tv ve Os Dozları (sırasıyla): 0, 0.5, 1.0, 1.5, **2.0, 4.0** µl petri^{-1}; Ln ve Ec Dozları: 0, 2.0, 4.0, 8.0, 12.0, **16.0** µl petri^{-1}; Fv Dozları (sırasıyla): 0, 0.5, 1.0, 1.5, 2.0, 4.0, 6.0, **8.0 µl** petri^{-1}

Sütun içerisinde yer alan ortalama değerlerin yanındaki farklı küçük harfler, Tukey HSD testine göre uygulamalar arasındaki farkın istatistiksel olarak önemli olduğunu gösterir ($P<0.05$).

nt: bu dozda test edilmedi

Bitki Uçucu Yağlarının *Aspergillus niger*'in Misel Gelişiminin Engellenmesi Üzerine olan *in vitro* Antifungal Etkisi

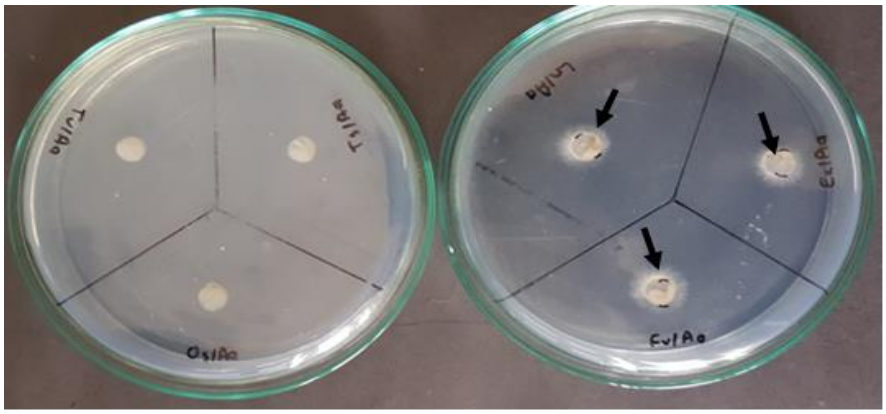
Farklı uçucu yağların buhar fazında *A. niger*'in misel gelişimini engellemesi üzerine olan antifungal etki sonuçları Çizelge 3 ve Şekil 5'de verilmiştir. *Aspergillus niger*'e karşı uçucu yağların MIC değerlerine incelendiğinde en etkili uçucu yağların 4.0 petri^{-1} µl dozla *T. spicata* ve *O. syriacum* olduğu, bunları sırasıyla 6.0 µl petri^{-1} dozla *T. vulgaris*, 8.0 µl

petri^{-1} dozla *F. vulgare*, 35.0 ve 40.0 µl petri^{-1} dozlar ile *L. nobilis* ve *E. camaldulensis* uçucu yağlarının izlediği görülmüştür. Yapılan istatistik analiz sonucunda *A. niger*'e karşı denenen farklı uçucu yağ konsantrasyonları arasında farkın önemli olduğu belirlenmiş olup (Çizelge 3) bu uçucu yağ konsantrasyonlarının fungal hastalık etmeninin misel gelişimini % engellenmesi üzerine olan antifungal etkinliği Çizelge 4'de verilmiştir.

Çizelge 2. Farklı uçucu yağların *A. alternata'nın* misel gelişimini engelleme (%) potensiyelleri
Table 2. Inhibitory potentials (%) of different essential oil on mycelial growth of A. alternata

	Farklı uçucu yağların misel gelişimini % engelleme potansiyelleri					
Doz	***T. spicata***	***T. vulgaris***	***O. syriacum***	***L. nobilis***	***E. camaldulensis***	***F. vulgare***
0	0.0	0.0	0.0	0.0	0.0	0.0
1	48.6	36.4	42.7	42.1	32.7	33.1
2	75.5	70.0	74.7	53.1	58.0	40.3
3	86.2	80.2	81.8	62.1	66.1	45.3
4	100.0*	87.0	88.5	72.7	79.2	54.3
5	100.0	100.0*	100.0*	100.0**	100.0**	77.4
6	nt	nt	nt	nt	nt	91.4
7	nt	nt	nt	nt	nt	100.0**

Ts, Tv ve Os Dozları (sırasıyla): 0, 0.5, 1.0, 1.5, **2.0, 4.0** µl petri^{-1}; Ln ve Ec Dozları: 0, 2.0, 4.0, 8.0, 12.0, **16.0** µl petri^{-1}; Fv Dozları (sırasıyla): 0, 0.5, 1.0, 1.5, 2.0, 4.0, 6.0, **8.0 µl** petri^{-1}
* ve **, bu dozlarda uçucu yağın etkinliğinin **fungisidal** veya **fungistatik** olduğunu göstermektedir. nt: bu dozda test edilmedi.

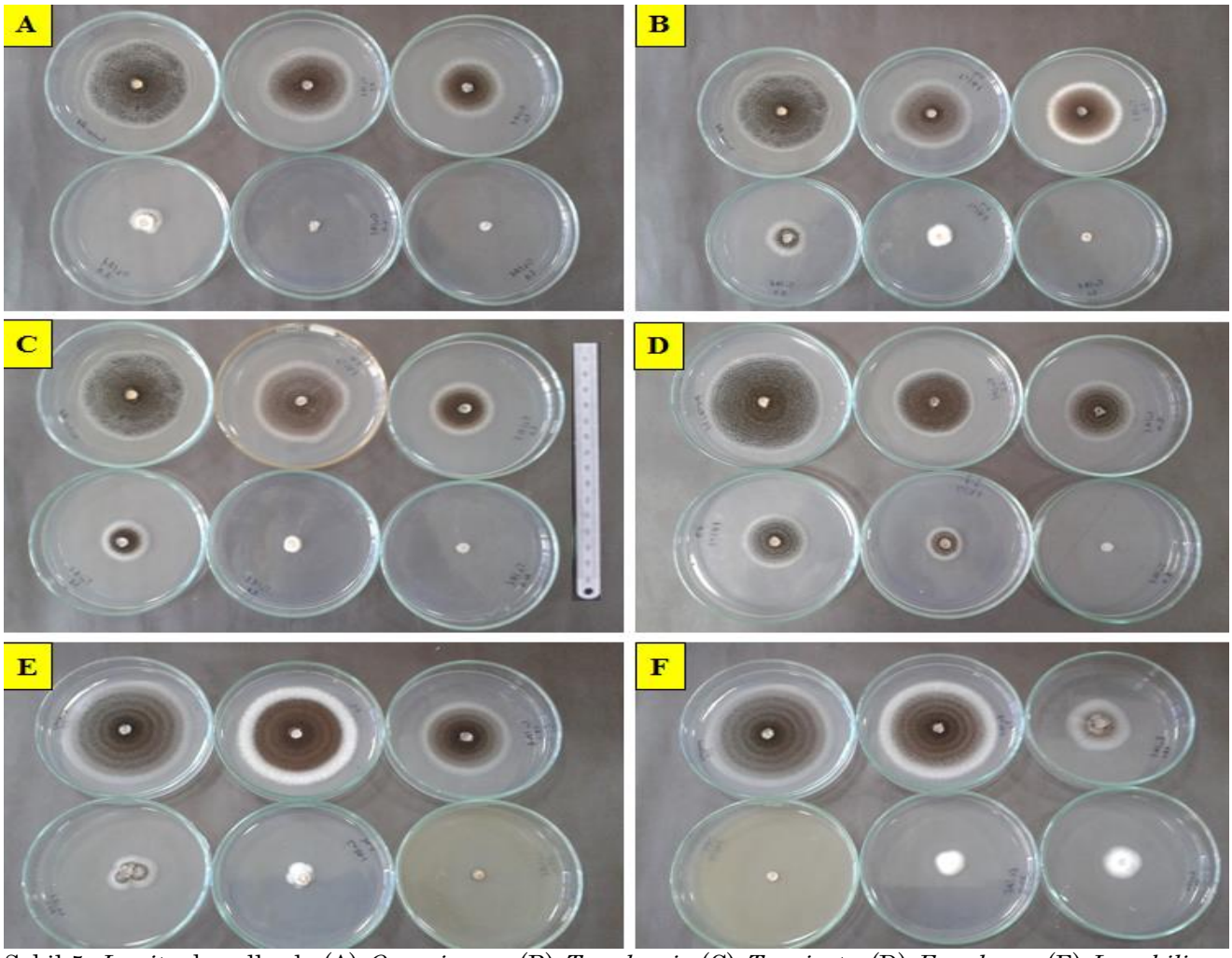


Şekil 4. Fungal etmen *A. alternata*'ya karşı minimum engellemenin görüldüğü dozlarda uçucu yağların fungisidal ve fungistatik (ok) etkisi.
Figure 4. Fungicidal and fungistatic (arrow) effects of essential oil at minimum inhibition concentrations against the fungal agent A. alternata.

Çizelge 3. Farklı uçucu yağların uygulandığı petrilerde gelişen *A. niger*'in koloni çapları (mm)
T*able 3. Colony diameters (mm) of A. niger in different essential oil applied petri plates*

	Farklı uçucu yağların misel gelişiminin (mm) engellenmesi üzerine etkinliği					
Doz	***T. spicata***	***T. vulgaris***	***O. syriacum***	***L. nobilis***	***E. camaldulensis***	***F. vulgare***
0	59.7f	59.3g	59.3f	69.7h	69.7h	65.3i
1	57.7e	58.3f	54.3e	67.3g	69.3h	55.7h
2	40.3d	50.3e	43.3d	52.7f	40.7g	51.7g
3	29.3c	35.3d	31.3c	38.3e	30.7f	44.3f
4	11.3b	24.3c	19.3b	25.7d	23.3e	41.0e
5	0.0a	15.3b	0.0a	17.3c	19.7d	39.3d
6	nt	0.0a	0.0a	11.3b	15.0c	33.3c
7	nt	nt	nt	0.0a	8.7b	19.3b
8	nt	nt	nt	nt	0.0a	0.0a
EC_{50}	1.324	1.910	1.410	15.599	14.559	2.144

Ts, Tv ve *Os* Dozları (sırasıyla): 0, 0.5, 1.0, 1.5, 2.0, **4.0, 6.0** µl petri^{-1}; Ln ve Ec Dozları (sırasıyla): 0, 5.0, 10.0, 15.0, 20.0, 25.0, 30.0, **35.0, 40.0** µl petri^{-1}; Fv Dozları (sırasıyla): 0, 0.25, 0.5, 1.0, 1.5, 2.0, 4.0, 6.0, **8.0** µl petri^{-1}
Sütun içerisinde yer alan ortalama değerlerin yanındaki farklı küçük harfler, Tukey HSD testine göre uygulamalar arasındaki farkın istatistiksel olarak önemli olduğunu gösterir ($P<0.05$). nt: bu dozda test edilmedi



Şekil 5. *In vitro* koşullarda (A) *O. syriacum*, (B) *T. vulgaris*, (C) *T. spicata*, (D) *F. vulgare*, (E) *L. nobilis*, ve (F) *E. camaldulensis* uçucu yağlarının *A. niger*'in misel gelişiminin engellenmesi üzerine olan antifungal etkileri.

Figure 5. The antifungal effects of (A) O. syriacum, (B) T. vulgaris, (C) T. spicata, (D) F. vulgare, (E) L. nobilis, and (F) E. camaldulensis essential oils on inhibition of mycelial growth of the fungal agent A. niger.

Çizelge 4. Farklı uçucu yağların *A. niger'in* misel gelişimini engelleme (%) potensiyelleri
Table 4. Inhibitory potentials (%) of different essential oils on mycelial growth of A. niger

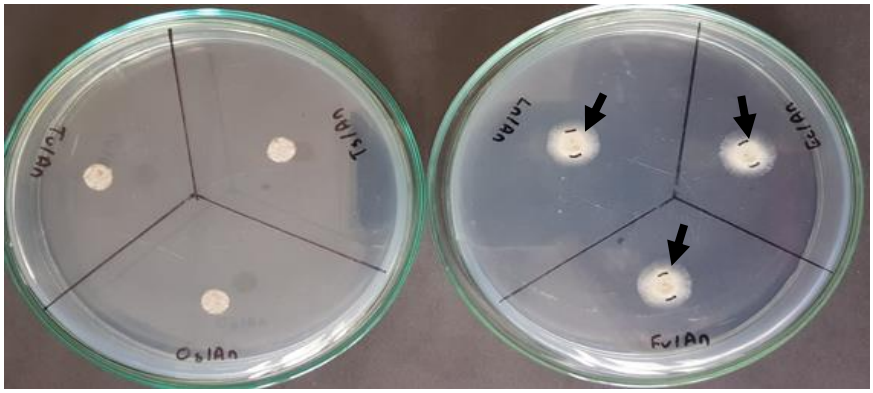
	Farklı uçucu yağların misel gelişimini % engelleme potansiyelleri					
Doz	***T. spicata***	***T. vulgaris***	***O. syriacum***	***L. nobilis***	***E. camaldulensis***	***F. vulgare***
0	0.0	0.0	0.0	0.0	0.0	0.0
1	3.4	1.7	8.4	3.3	0.5	14.8
2	32.4	15.2	27.0	24.4	41.6	20.9
3	50.8	40.4	47.2	45.0	56.0	32.1
4	81.0	59.0	67.4	63.1	66.5	37.2
5	100.0*	74.2	100.0	75.1	71.8	39.8
6	nt	100.0*	100.0*	83.7	78.5	49.0
7	nt	nt	nt	100.0**	87.6	70.4
8	nt	nt	nt	nt	100.0**	100.0**

Ts, Tv ve *Os* Dozları (sırasıyla): 0, 0.5, 1.0, 1.5, 2.0, **4.0, 6.0** µl petri^{-1}; Ln ve Ec Dozları (sırasıyla): 0, 5.0, 10.0, 15.0, 20.0, 25.0, 30.0, **35.0, 40.0** µl petri^{-1}; Fv Dozları (sırasıyla): 0, 0.25, 0.5, 1.0, 1.5, 2.0, 4.0, 6.0, **8.0** µl petri^{-1}

* ve **, bu dozlarda uçucu yağın etkinliğinin **fungisidal** veya **fungistatik** olduğunu göstermektedir. nt: bu dozda test edilmedi.

Yapılan probit analiz sonrası uçucu yağların *A. niger*'in misel gelişimini %50 oranında engelleyen etkili konsantrasyon (EC_{50}) değerleri, *T. spicata* için 1.324 µl petri^{-1}, *O. syriacum* için 1.410 µl petri^{-1}, T. vulgaris için 1.910 µl petri^{-1}, F. vulgare için 2.144 µl petri^{-1}, *E. camaldulensis* için 14.559 µl petri^{-1} olarak hesaplanırken, en yüksek EC50 değeri için *L. nobilis* 15.599 µl petri^{-1}olarak belirlenmiştir (Çizelge 3).

Uçucu yağların *A. niger*'e karşı fungisidal/fungistatik etkileri incelendiğinde *T. spicata, T. vulgaris ve O. syriacum* uçucu yağları **fungisidal,** *F. vulgare, L. nobilis* ve *E. camaldulensis* uçucu yağları ise **fungistatik** etki gösterdikleri belirlenmiştir (Çizelge 4, Şekil 6).



Şekil 6. Fungal etmen *A. niger*'e karşı minimum engellemenin görüldüğü dozlarda uçucu yağların fungisidal/fungistatik etkisi.

Figure 6. Fungicidal and fungistatic (arrow) effects of essential oil at minimum inhibition concentrations against the fungal agent A. niger.

Çalışmada kullanılan farklı uçucu yağların *Alternaria alternata* ve *Aspergillus niger*'in misel gelişimi üzerine olan etkilerinin araştırıldığı denemeler sonucunda elde edilen MIC değerleri, antifungal etki ve EC_{50} değerleri Çizelge 5'de özetlenmiştir. Uçucu yağlar, MIC değerleri ve EC_{50} sonuçları açısından değerlendirildiğinde fungal etmenlere karşı en yüksek antifungal etkinlik 3 farklı kekik türü olan *T. spicata, O.syriacum* ve *T. vulgaris* uçucu yağları tarafından gösterilmiş olup, bu yağları sırasıyla *F. vulgare* ve *L. nobilis* uçucu yağları takip etmiştir. Fungal etmenlere karşı en düşük etki ise *E. camaldulensis*'de görülmüştür.

Sonuçlar değerlendirildiğinde, test edilen her iki fungus türüne karşı MIC değerinde en güçlü antifungal etkiler (% 100 engelleme) sırasıyla *Thymbra spicata* (2.0-4.0 µl petri^{-1}), *Origanum syriacum* (4.0 µl petri^{-1}) ve *Thymus vulgaris* (4.0-6.0 µl petri^{-1}) uçucu yağları tarafından gösterilmiş olup, *Foeniculum vulgare* uçucu yağı 8.0 µl petri^{-1} ve *Laurus nobilis* uçucu yağı 16.0-35.0 µl petri^{-1} dozlarında antifungal etki göstermişlerdir. Test edilen her iki türe karşı en düşük antifungal etki ise *Eucalyptus camaldulensis* (16.0-40.0 µl petri^{-1}) uçucu yağı uygulanmış petrilerde kayıt edilmiştir (Çizelge 5) .

Çizelge 5. Çalışmalarda kullanılan farklı uçucu yağların *A. alternata* ve *A. niger*'in misel gelişimini engelleyen minimum engelleme konsantrasyonları (MIC) ve misel gelişimini %50 engelleyen etkili konsantrasyon değerleri (EC_{50})

Table 5. Minimum inhibitory concentrations (MIC) of different essential oils and effective concentration that inhibit mycelial growth by 50% (EC_{50})

	Fungal etmenlerinin misel gelişimini engelleyen Minimum Engelleme Konsantrasyonları (MIC) ve EC_{50} Değerleri (µl petri^{-1})			
	A. alternata		***A. niger***	
Uçucu Yağlar	**MIC**	**EC_{50}**	**MIC**	**EC_{50}**
T. spicata	2.0*	0.548	4.0*	1.324
O. syriacum	4.0*	0.596	4.0*	1.410
T. vulgaris	4.0*	0.682	6.0*	1.910
F. vulgare	8.0**	1.305	8.0**	2.144
L. nobilis	16.0**	3.351	35.0**	15.599
E. camaldulensis	16.0**	3.555	40.0**	14.559

* ve **, bu dozlarda uçucu yağın etkinliğinin **fungisidal** veya **fungistatik** olduğunu göstermektedir.

Alternaria alternata ve *Aspergillus niger* ile bunların bağlı oldukları cinslerde yer alan diğer birçok türün insan ve hayvan sağlığına zararlı kuvvetli mikotoksin üretme yeteneklerine sahip oldukları bildirildiğinden (Rheeder ve ark. 2002; Jens ve ark. 2007; Reddy ve ark., 2010) bu çalışmada bu türlerin mikotoksin üretme potansiyelleri ayrıca araştırılmamıştır. *Penicillium*, *Aspergillus*, *Alternaria* ve *Fusarium* cinsine ait türlerin önemli derecede mikotoksin üreticisi funguslar oldukları

(McKee, 1995; Kabak & Dobson, 2017), *Aspergillus* türlerinin daha çok Aflatoxin (Uylaşer ve ark., 2005), *Alternaria* türlerinin ise Alternariol (AOH) ve Alternariol monometil (Yiannikouris & Jouany, 2002) gibi mikotoksinleri ürettikleri önceden yapılmış çalışmalarla belirlenmiştir.

Daha önce yapılmış çalışmalar ele alındığında uçucu yağ ve ekstraktlarının antimikrobiyal etkinlikleri daha çok insan/hayvan/gıda patojenlerine karşı araştırılmış olup, özellikle hasat sonrası mikotoksin oluşturan bitki patojenlerine karşı etkinlikleri, oldukça kısıtlı sayıda çalışamada araştırılmıştır. Yapılan literatür araştırmasında doğrudan biber meyvelerinde çürümelere neden olan *A. alternata* ve *A. niger* fungal etmenlerine karşı bitki uçucu yağların antifungal etkilerinin araştırıldığı bir çalışmaya rastlanılmamış olup, biberden izole edilmiş bu fungal etmenlere karşı farklı bitki türlerinden elde edilmiş uçucu yağların etkinliği ilk kez bu çalışma ile ortaya koyulmuştur.

Depolanmış farklı tarımsal ürünlerde mikotoksin oluşturan *Aspergillus niger* (Sokolic-Mihalak ve ark., 2012; Ghaffar ve ark., 2015; Fitsiou ve ark., 2016; Hossain ve ark., 2016; Tsimogiannis ve ark., 2017) ve *Alternaria alternata*'ya karşı (Xu ve ark., 2014) çalışmalarda kullanılan bitkilerden farklı bitki uçucu yağ ve ekstraktlarının antifungal etkiler gösterdikleri bildirilmiştir. Bu çalışmadan elde edilen sonuçlar önceden yapılmış çalışma sonuçlarıyla karşılaştırıldığında sonuçların birbirlerini destekler nitelikte oldukları görülmektedir. Nitekim çalışmada kullanılan bitki uçucu yağların tamamı *A. alternata* ve *A. niger*'e karşı kullanıldıkları farklı konsantrasyonlara bağlı olarak değişen oranlarda antifungal etkiler göstermişlerdir. Uçucu yağların antifungal etkinliği, elde edildiği bitki türü, uygulandığı fungal tür ve konsantrasyonlarına bağlı olarak fungisidal ve fungisitatik etkiler gösterdiği yapılan *in vitro* çalışmalarla belirlenmiştir.

Çalışmalarda kullanılan bazı kekik türlerine ait uçucu yağlarının sebzelerde sorun olan toprak (*Rhizoctonia solani, Phytopthora capsici, Sclerotinia sclerotiorum*) ve yaprak kökenli (*Botrytis cinereae* ve *Phytopthora infestans*) birçok fungal hastalık etmenlerine karşı oldukça düşük konsantrasyonlarda antifungal etkilere sahip oldukları önceden yapılmış *in vitro* ve *in vivo* çalışmalarda da bildirilmiştir (Yeğen ve ark. 1992; Soylu ve ark., 2006; Soylu ve ark., 2007; Soylu ve ark., 2010). Bu çalışmada da, sözü geçen çalışmaları destekler nitelikte test edilen fungal etmenlere karşı *T. spicata, O. syriacum, T. vulgaris* uçucu yağları 2.0-4.0 petri^{-1} konsantrasyonlarıyla en yüksek antifungal etki gösteren uygulamalar olmuştur.

Uçucu yağların, çalışmadaki fungal etmenlere karşı sergiledikleri antifungal etkiye, uçucu yağın içerdiği bileşenlerin etkinliklerinden kaynaklandığı düşünülebilir. Nitekim uçucu yağların sahip oldukları fenolik bileşiklerin antimikrobiyal aktivitelerden sorumlu oldukları daha önce yapılan bir çok çalışmalarda bildirilmiştir (Tripathi ve ark., 2008). Bu çalışmada en yüksek antifungal etkiler sergileyen *T. spicata, O. syriacum, T. vulgaris* kekik türlerinin antifungal etkilerinin, uçucu yağının içeriğinde yer alan carvacrol ve thymol gibi fenolik bileşiklerden kaynaklandığı bildirilmiştir (Ravid & Putievsky, 1983; Nguefack ve ark., 2012; Mamadalieva ve ark., 2017; Khan ve ark., 2019; Lima ve ark., 2019; Karpinski, 2020; Souza ve ark., 2022). Yakın zamanda uçucu yağların antifungal etkilerinin araştırıldığı çalışmada (Kara ve ark., 2022), rezene uçucu yağı içeriğinde limonene, estragole ve *trans*-anethole, defne uçucu yağı içerisinde ise sabinene, eucalyptol, α-terpinyl acetate gibi uçucu bileşenlerin antifungal etkinlikten sorumlu oldukları bildirilmiştir. Bu çalışmaya benzer yapılmış başka çalışmalarda *trans*-anethole, terpinen-4-ol, eugenol, carvone, 1,8-cineole (eucalyptol) ve thymol gibi uçucu yağ ana bileşenlerinin *Fusarium* spp. *Aspergillus* spp. *Alternaria* sp. ve *Penicillium* sp. gibi fungal etmenlere karşı *in vitro* antimikrobiyal etkinliğe sahip oldukları bildirilmiştir (Mimica-Dukic ve ark., 2003; Morcia ve ark., 2012; Wang ve ark., 2018; Andrade-Ochoa ve ark., 2021). Çalışmalarda kullanılan bitki uçucu yağların bitkilerde ve gıdalarda sorun fungal ve bakteriyel hastalık etmenlere karşı oldukça yüksek düzeylerde antimikrobiyal etkilere sahip oldukları, kullanılan uçucu yağların antimikrobiyal dozlarında fungal ve bakteriyel etmenlerin hücrelerinde morfolojik bozulmalara neden oldukları daha önceden yapılan çalışmalarla ortaya konulmuştur (Soylu ve ark., 2009; Mengüllüoğlu & Soylu, 2012; Bozkurt ve ark., 2020; Kara ve ark., 2020). Yakın zamanda yapılmış olan çalışmada biber meyvelerinden izole edilen, iç çürüklüğe neden olan *Aspergillus niger, Alternaria alternata* ve *Fusarium incarnatum* gibi hastalık etmenlerine karşı farklı kimyasal yapıdaki isothiocyanate bileşiklerden methyl isothiocyanate (MITC), 2-propenyl (Allyl) isothiocyanate (AITC), benzyl isothiocyanate (BITC) ve ethyl isothiocyanate (EITC)'ın oldukça düşük konsantrasyonlarda antifungal etkinlik gösterdiği bildirilmiştir (Atay & Soylu, 2022).

Denemelerde kullanılan uçucu yağ ve ana bileşelerin farklı fungus türlerine karşı antifungal etkilerinin olduğu bildirilmiş olmakla beraber, mevcut çalışmada kullanılan kekik türleri, defne, rezene ve okaliptus uçucu yağlarının biber meyvelerinde belirlenen *A. niger* ve *A. alternata* etmenlerine karşı güçlü antifungal etkilerinin, söz konusu ana bileşlerden (özellikle oransal olarak fazla olduklarından dolayı carvacrol, *trans*-anethole ve eucalyptol vb.) kaynaklı olduğu düşünülebilir.

Kontrol petrilerindeki misel gelişimiyle kıyaslandığında uçucu yağların, fungus misel gelişmelerini doza bağlı bir şekilde *in vitro*'da engelleyebildikleri tespit edilmiştir. Bununla beraber, engelleme bölgesindeki misel gelişimi ışık mikroskobu yardımıyla incelendiğinde bu bölgelerde bir takım yapısal bozukluklar meydana geldiği belirlenmiştir. Nitekim uçucu yağ uygulanan petrilerdeki fungusların hifleri ve konidilerinde yapılan mikroskobik gözlemler sonucunda hiflerde deformasyonlar, hif çapının incelmesi ve parçalanması, sitoplazmik pıhtılaşma, konidilerde şekil bozukları (özellikle misel gelişimini %100 engelleyen konsantrasyonlarda) gibi bazı morfolojik yapısal değişimlere sebep olduğu görülmüştür. Özellikle kekik türlerinin meydana getirdiği yapısal değişimlerin, diğer uçucu yağların oluşturduğundan çok daha fazla olduğuda da gözlemlenmiştir. Daha önce yapılmış bazı çalışmalarda da uçucu yağların fungusların misel yapısında benzer deformasyonlara neden olduğu, yapısal bozulmalara ise uçucu yağ içeriğinde bulunan ana bileşenlerin mikroorganizmanın hücre zarına zarar vermesi dolayısıyla hücre duvarı sentezini düzenleyen enzimatik reaksiyonların olumsuz etkilenebileceğinden kaynaklı olabileceği bildirilmiştir (Soylu ve ark., 2006; Soylu ve ark., 2007; Soylu ve ark., 2010; Lucas ve ark., 2012; Yong ve ark., 2015; Kachur & Suntres, 2020).

SONUÇ ve ÖNERİLER

Sonuç olarak, bitkisel kökenli uçucu yağların hasat öncesi ve sonrası biber meyvelerinde çürümelere neden olan fungal hastalık etmenlerine karşı *in vitro* şartlarında antifungal etki gösterdikleri belirlenmiştir. Özellikle kekik uçucu yağları en etkili antifungal etkiyi gösterirken *E. camaldulensis* uçucu yağı en düşük antifungal etkiyi göstermiştir. Bu çalışmada, *in vitro* koşullarda fungal patojenlere karşı yüksek düzeyde etkileri saptanan uçucu yağların (farklı kekik türlerinden elde edilen uçucu yağlar), hastalık etmenleriyle mücadelede pestisitlere alternatif olabilecek çevre dostu doğal preparatlar oldukları değerlendirilmiştir.

Bitki uçucu yağlarının uçucu özellikleri göz önüne alındığında bunlar fumigant olarak teksel ve/veya karışım halinde preparatları yapılarak depolanmış ürünlerde sorun olan fungal hastalık etmenlerine karşı uygulanabileceği düşünülmektedir. Bunun yanı sıra, farklı bitki türlerinden elde edilecek uçucu yağların *in vitro* ve *in vivo* koşullarda araştırılması ve bitki patojenleriyle mücadelede pratikte kullanılmalarına imkan sağlayacak yöntemlerin geliştirilmesi de önem arz edecektir.

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Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan eder.

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Merzifon Karası Üzüm Çeşidinin (*Vitis vinifera* L.) Fenolik Madde, Flavonoid ve Antioksidan Aktivitesi

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ÖZET
Bu çalışmada, Merzifon Karası üzüm çeşidinin (*Vitis vinifera* L.) fenolik madde, flavonoid ve antioksidan aktivitesinin belirlenmesi amaçlanmıştır. Bu üzümün oda sıcaklığındaki meyvenin tamamı ile oda sıcaklığındaki kabuk kısmı ve 50 °C' de kabuk kısmı ile 50 °C' de meyvenin tamamından elde edilen ekstraklardaki antioksidan aktivite, toplam fenolik madde miktarı ve toplam flavonoid madde miktarı incelenmiştir. Antioksidan aktivite DPPH (2.2-difenil1- pikrilhidrazil) yöntemiyle ölçülmüştür. En yüksek antioksidan aktivite, oda sıcaklığındaki meyvenin tamamından tespit edilmiştir. En yüksek fenolik (102.46 mg 100 g) ve flavonoid (44.95 mg 100 g) madde miktarları oda sıcaklığındaki meyvenin tamamından elde edilmiş olup, bunu oda sıcaklığındaki kabuk kısmı izlemektedir.

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Anahtar Kelimeler
Vitis vinifera
Merzifon karası
Fenolik madde
Flavonoid
Antioksidan aktivite

Phenolic Compound, Flavonoid and Antioxidant Activity of Merzifon Black Grape Variety (*Vitis vinifera* L.)

ABSTRACT
In this study, it was aimed to determine the phenolic compound, flavonoid and antioxidant activity of the Merzifon Black grape variety (*Vitis vinifera* L.). The antioxidant activity, total phenolic compound and total flavonoid content of the whole fruit of this grape at room temperature, the shell part at room temperature and the skin part at 50 °C and the extracts obtained from the whole fruit at 50 °C were examined. Antioxidant activity was measured by the DPPH (2.2-diphenyl-1- picryhydrazyl) method. The highest antioxidant activity was detected from the whole fruit at room temperature. The highest amounts of phenolic (102.46 mg 100 g) and flavonoid (44.95 mg 100 g) compounds were obtained from the whole fruit at room temperature, followed by the peel at room temperature.

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GİRİŞ
Antik çağın derinliklerinden bu yana günümüze gelen üzüm, Dünya genelinde fazla miktarda üretilmekte ve tüketilmekte olan meyvelerden biridir. Anavatanı Anadolu ve Kafkaslar olan üzüm, daha sonraları, Avrupa ve diğer ülkelerde tanınmış olup işlem görmüştür ve kültürel yapı içerisine girmiştir (Kağa, 2007).

Dünyada'ki 7.449.000 hektarlık bağ alanının % 6' lık bölümünün ülkemizde olduğu, 2019 yılı istatiksel sonuçlarda görülmektedir. Bu verilere göre Türkiye bağcılık alanı yönünden İspanya, Çin, Fransa, İtalya'dan sonra 5. sırada yer almaktadır. Dünya toplam üzüm üretim miktarı 2018 yılında 77.8 milyon olup, bu miktarın 3.9 milyonluk tonu Türkiye'de üretilmiştir (Oıv, 2001).

Türkiye'de Öküzgözü, Narince, Shraz, Boğazkere, Kalecik Karası, Sauvignon Blanch, Cabernet

Sauvignon, Sergi Karası, Emir, Papaz Karası, Boğazkere, Çalkarası, Sultaniye ve Merzifon Karası gibi üzüm çeşitleri yetiştirilmektedir (Çiçek, 2018). Türkiye'de yetiştirilen bu üzümlerin kullanım alanlarında da çeşitlilik gözlenmektedir. Türkiye'de üzüm, taze ve kuru tüketim yanında meyve suyu yapımında, fermentasyon yöntemiyle şarap ve sirke üretiminde, reçel veya pekmez yapımında kullanılmaktadır. Bu kullanım farklılıklar ile Türkiye tarımına ve ekonomisine büyük ölçüde katkı sağlamaktadır (Göktürk ve ark., 1997).

Üzümün en yüksek fenolik bileşik içeriklerine sahip meyve türlerinden biri olduğu belirtilmiştir (Manach ve ark., 2005). Genel itibariyle üzümlerin içerdiği bileşenlerde su, karbonhidrat, protein ve yağ bulunmaktadır (Şen, 2019). Aynı zamanda bol miktarda antioksidan içeren üzümün, önemli aktif bileşenleri fenolik bileşenlerdir (Nassiri-Asl & Hosseinzadeh, 2016). Gündelik yaşamdaki beslenmemizin vazgeçilmez bir parçası olan fenolik bileşenler, bitkisel gıdalarda büyük ölçüde bulunan ve antioksidanların büyük bir grubunu oluşturan organik maddelerdir (Pehlivan & Uzun, 2015). Bol miktarda fenolik bileşenler bulunduran üzümün sağlık açısından oldukça önemli bir rolü vardır. Yapılan çalışmalar, üzüm bitkisinden elde edilen özütlerde, kateşinler, epikateşin ve epikateşin-3-O-gallat, dimerik, trimerik ve tetramerik prosiyanidinlerin bulunduğunu göstermiştir. İçeriğinde bulunan bu prosiyanidinler monomerik fenolik bileşiklerdir (Saito ve ark., 1998).

Bu çalışmanın bitkisel materyalini oluşturan Merzifon Karası üzüm çeşidi, Amasya ilinin Merzifon ilçesinde antik çağlardan kalan bir üzüm çeşitidir. Türkiye coğrafyasında kaybolmaya yüz tutmuş bu üzüm çeşidi, geç olgunlaşmaktadır ve bağ bozumu genellikle ekim ayının ilk haftasıdır. Bu üzüm çeşidinin üzümleri orta büyüklükte olup koyu mor renklidir, ince kabuklu ve şeker oranı yüksektir (Kurt & Öztürk Çalı, 2022). Ayrıca sıkı salkımlara sahiptir. Bu çalışmada, Türkiye coğrafyasında yetiştirilen ancak yetiştirildiği Amasya ilinin dışında pek tanınmayan, yok olmaya yüz tutmuş olan Merzifon Karası üzüm çeşidinin antioksidan aktivitesinin belirlenmesi ve diğer üzüm çeşitleriyle karşılaştırılması amaçlanmıştır.

MATERYAL ve METOD

Bu çalışmanın bitkisel materyalini oluşturan Merzifon Karası üzüm çeşidinin (*Vitis vinifera* L.) olgun meyveleri, Amasya ilinin Merzifon ilçesindeki Sarıköy'den 650 adet Merzifon Karası üzüm çeşidi teveği (onca) içeren 3000 m²'lik bir bağ alanından 10 Ekim 2018 tarihinde toplanmıştır.

Örnekleme Yöntemi

Merzifon Karası üzüm çeşidinin antioksidan aktivitesini belirlemek için, üzümden özütler elde edilmiştir. Bağdan rastgele olarak sağlıklı 3 adet tevekten birer salkım ve her bir salkımlardan olgun, aynı irilikte, sağlıklı 15 adet üzüm tanesi alınmış ve toplamda 45 adet üzümün tamamının ve sadece kabuk kısmının ayrı ayrı darası alınarak oluşturulmuş özütlerinden 300 gr olacak şekilde erlenmayere eklenmiştir. Her bir erlenmayerlerin içine kabuk kısmını ve üzümün kendisini geçecek kadar % 96' lık etil alkol eklenmiştir. Hazırlanan bu karışımlar ayrı ayrı önce oda sıcaklığında daha sonra 50 °C' de manyetik karıştırıcıda üçer gün bekletildikten sonra alkol miktarını uçurarak üzümün özütü elde edilmiş ve numaralandırılmıştır (Çizelge 1). Daha sonra numaralandırılan özütler DPPH Radikal Söndürücü Kapasite Yöntemi, Metal Şelatlama Aktivitesi Tayini, Toplam Fenolik ve Toplam Flavonoid Tayini yöntemlerinde kullanılmıştır.

Laboratuvar analizleri

DPPH Radikal Söndürücü Kapasite Yöntemi

İlk olarak Brand-Williams ve ark. (1995) tarafından bulunan bu yöntem 1998 yılında kullanılmaya başlanmıştır (Okan ve ark., 2013). Yöntem doğal antioksidanların, DPPH radikalini süpürücü etkisini ölçmeye dayalı bir yöntemdir. Radikal söndürücü yöntemi tepkime ortamındaki konsantrasyonu 50-250 µg ml olacak şekilde metanolde hazırlanan örnek çözeltilerinin 3 ml' lik çözeltisine 1 ml 1x10-3 M DPPH çözeltisi (metanolde) ilave edilir. Vortekste 30 saniye karıştırılarak oda sıcaklığında ve karanlıkta 30 dakika bekletilir. Süre sonunda UV Spektrofotometresinde 517 nm de absorbans okunur. Pozitif kontrol olarak BHT (Butillendirilmiş Hidroksi Toluen) ve BHA (Butillendirilmiş Hidroksi Anisol) kullanılır. DPPH radikalini süpürme etkisi aşağıdaki formül kullanılarak hesaplanır (Bondet ve ark., 1997). Mor menekşe renginde olan bu radikal, 517 nm' de ölçüldüğünde maksimimum absorbansı verir (Bayram ve ark., 2019).

Çizelge 1. Ekstraksiyonda kullanılan üzüm kısımlarının numaraları

Table 1. Numbers of grape parts used in extraction

Ekstraksiyonda Kullanılan Üzüm Kısımları *(Grape Parts of Used in Extraction)*	Numara *(Number)*
50 °C' de üzümün tamamı	1
50 °C' de üzümün kabuk kısmı	2
Oda sıcaklığında üzümün kabuk kısmı	3
Oda sıcaklığında üzümün tamamı	4

Metal Şelatlama Aktivitesi Tayini

Metal şelatlama aktivitesi ekstratların Fe iyonlarını şelatlama özelliği bakılarak değerlendirilmiştir. Metal şelatlama özelliği olan antioksidanlar, serbest

demiri bağlayarak onu etkisiz hale getirmektedir. Böylece serbest radikal oluşumu önlenmektedir (Arora ve ark., 1998). Antioksidan maddeler tarafından şelatlanan demir iyonları ferrozin tarafından bağlanamayacağı için oluşacak olan mor renk şiddeti daha düşük olacak ve absorbans daha düşük okunacaktır. Düşük absorbans değeri yüksek şelatlama aktivitesini göstermektedir. Standart olarak iyi bir metal şelatlayıcı olan EDTA (Etilendiamin tetra asetik asit) kullanılmıştır. Çıkan sonuçlar EDTA standartıyla karşılaştırılmıştır.

Toplam Fenolik Bileşen Tayini

Bu analiz yönteminde folin-ciocalteu (FCR) reaktifi kullanılarak gallik asit eş değeri üzerinden hesaplama yapılmıştır (Singleton & Rossi, 1965) Toplam fenolik bileşen tayinini, suda ve diğer organik çözücülerde çözünmüş olan fenolik bileşiklerin Folin reaktifi ile alkali ortamda renkli kompleks oluşturması esasına dayanır. Gallik asit farklı konsantrasyonlarda hazırlanan standartları ile folin çözeltisinde korelasyon grafiği çizilmiş ve R2 değeri 0.9987 bulunmuştur. Grafik denklemi y=0.01460x+0.04 olarak tespit edilmiştir. Bu grafikten yararlanılarak elde edilen ekstrenin 1 gramında bulunan gallik asit eş değeri total fenolik bileşen miktarını vermektedir.

Toplam Flavonoid Tayini

Bu yöntemde total flavonoid miktarı Kuarsetin eş değeri olarak verilmektedir. Kuarsetin farklı konsantrasyonlarda hazırlanan standartları üzerinden korelasyon grafiği çizildiğinde y=0.022x+0.0176 denklemi elde edilmiş, R2 =0.9917 değeri bulunmuştur.

İstatistik Analizler

Elde edilen ölçümlerin istatistiksel analizleri SPSS 20 for Windows istatistik programında, varyans analizi de Ki Kare (Chi square) testi ile yapılmıştır.

BULGULAR ve TARTIŞMA

Bu çalışmada Merzifon Karası üzümün kabuk ve meyvesinin tamamının oda sıcaklığı ve 50 °C 'deki ekstraksiyonu gerçekleştirilerek antioksidan aktiviteleri ölçülmeye çalışılmıştır. DPPH analizi bulguları Çizelge 2' de verilmiştir. Buna göre, en düşük IC(50) değeri oda sıcaklığındaki üzümün tamamından elde edilmiştir. Bunu sırasıyla, 50 °C' deki üzümün kabuk kısmı, oda sıcaklığındaki üzümün kabuk kısmı ile 50 °C'deki üzümün tamamı izlemektedir. Alicante Bouschet, Cabernet Sauvignon, Kalecik Karası, Öküzgözü, Alphonse Lavallee, Hafızali ve Trakya İlkeren ile 5 farklı yabani asma tipi olmak üzere toplamda 12 çeşit üzümün tane eti, tane kabuğu, bütün tane ve çekirdeğinin DPPH yöntemiyle antioksidan aktivitesinin belirlendiği bir çalışmada çekirdeklerin diğer bitki kısımlarına göre daha yüksek antioksidan etki gösterdiği belirtilmiştir (Yeğin & Uzun, 2018). Çekirdek için Hafızali çeşidi, üzüm kabuğu için Kalecik Karası ve Yabani 4 genotipi, üzüm meyvesinin tamamı için Öküzgözü çeşidi, üzümün tane eti için de Cabernet Sauvignon çeşidi antioksidan aktivite bakımından öne çıkan üzüm çeşitleri olmuştur. Aynı çalışmada bütün tane ile kabuk kısmı kıyaslandığında; Cabernet Sauvignon, Öküzgözü, Hafizali ve Yabani-1 asma çeşitlerinin bütün tanenin antioksidan aktivitesinin kabuktan daha yüksek olduğu tespit edilmiştir. Merzifon Karasında oda sıcaklığındaki meyvenin tamamının en yüksek antioksidan aktiviteyi göstermesi ile bunu kabuğun izlemesine dair bulgular; Cabernet Sauvignon, Öküzgözü, Hafizali ve Yabani-1 asma çeşitleri için Yeğin ve Uzun (2018)' un bulgularıyla uyumludur. Merzifon Karası üzüm çeşidinde en yüksek antioksidan aktivitenin oda sıcaklığındaki üzümün tamamında tespit edilmesinin nedeninin, çekirdekteki antioksidan aktivitenin yüksekliğinden kaynaklanabileceği düşünülmektedir. Nitekim; Cabernet Sauvignon, Öküzgözü, Hafizali ve Yabani-1 asma çeşitlerinde çekirdekteki antioksidan aktivitenin yüksek oluşunun bütün meyvenin de antioksidan etkisini yükselttiği Yeğin ve Uzun (2018)'un yaptıkları çalışmada da görülmektedir. Üzüm çekirdeklerinin yüksek antioksidan içermesi Narince üzümü üzerine çalışmalar yapan Göktürk Baydar ve ark. (2007), Andjelkovic ve ark. (2013) ile Muscadine grubu üzüm çeşitlerinde de Pastrana-Bonilla ve ark. (2003) tarafından bildirilmiştir.

Çizelge 2. DPPH analizi bulguları
Table 2 DPPH analysis findings

Numara *(Number)*	*25 µg ml*	*50 µg ml*	*100 µg ml*	*200 µg ml*	*400 µg ml*	*IC50*
1	51.09	53.21 ^{f}	57.53	60.04 ^{e}	61.26 ^{ef}	163.01^{bc}
2	42.71	60.65	68.08	71.08	72.05	41.92 ^{ac}
3	40.11	50.39 ^{f}	58.91	60.24e ^{f}	65.56 ^{ef}	125.40 ^{ab}
4	61.10	62.93	74.43	77.87	81.90	25<
BHT	61.84	74.57^{a}	78.36	85.62 ^{a}	94.69 ^{ac}	
TROLOX	59.59	70.52 ^{ac}	72.54	81.36 ^{c}	90.57 ^{ac}	

"a" ve 1 grubu, "b" ve 2 grubu, "c" ve 3 grubu, "e" ve BHT grubu, "f" ve TROLOX grubu istatistiki açıdan p<0.05 düzeyinde anlamlıdır.
"a" and 1 group, "b" and 2 group, "c" and 3 group, "e" and BHT group, "f" and TROLOX group are statistically significant at the p<0.05 level.

Organizmamız için gerekli olan temel elementlerden biri de demirdir. Fakat organizmamızda bulunan demir lipit, protein gibi bileşenlerle, istenmeyen oksidatif reaksiyonlarına girerek serbest radikal oluşumuna sebebiyet vermektedir. Bu sebeple antioksidan maddelerinin demiri indirgeme yeteneği oldukça önemlidir (Rival ve ark., 2001). Çizelge 3' de metal şelatlama tayini bulguları görülmektedir. EDTA standartına yakın en yüksek metal şelatlama kapasitesi 4 numaralı ekstre olan oda sıcaklığındaki üzümün tamamıdır. Bunu sırasıyla oda sıcaklığındaki üzümün kabuk kısmı ve 50 °C' de üzümün kabuk kısmı ile 50 °C' de üzümün tamamı izlemektedir.

Çizelge 3. Metal şelatlama tayini bulguları
Table 3 Metal chelating determination findings

Numara *(Number)*	*25 µg ml*	*50 µg ml*	*100 µg ml*	*200 µg ml*	*400 µg ml*
1	20.93 ^{e}	24.45 ^{e}	27.20 ^{e}	29.60d ^{de}	39.45 ^{de}
2	12.98 ^{e}	28.48 ^{e}	29.71 ^{e}	36.87 ^{e}	42.87 ^{e}
3	23.09 ^{e}	24.45 ^{e}	28.64 ^{e}	34.71 ^{e}	46.84 ^{e}
4	19.59 ^{e}	24.45 ^{e}	37.12 ^{e}	51.89 ^{ae}	59.42 ^{ae}
EDTA	70.85 ^{abcd}	72.32 ^{abcd}	79.47 ^{abcd}	81.09 ^{abcd}	97.06 ^{abcd}

"a" ve 1 grubu, "b" ve 2 grubu, "c" ve 3 grubu, "d" ve 4 grubu, "e" ve EDTA grubu istatistiki açıdan $p<0.05$ düzeyinde anlamlıdır.
"a" and 1 group, "b" and 2 group, "c" and 3 group, "e" and EDTA group are statistically significant at the $p<0.05$ level.

Bu çalışmada, Merzifon Karası üzüm çeşidinin en yüksek fenolik madde miktarı oda sıcaklığındaki üzümün tamamında tespit edilirken, bunu sırasıyla oda sıcaklığındaki üzümün kabuk kısmı, 50 °C' de üzümün kabuk kısmı ve 50 °C' de üzümün tamamı izlemektedir (Çizelge 4). Merzifon Karası üzüm çeşidinin en yüksek fenolik madde miktarı oda sıcaklığındaki üzümün tamamında tespit edilmesinin temelinde bu üzümün çekirdeğinden kaynaklandığı düşünülmektedir. Üzüm çekirdeklerinin tane etine göre yaklaşık dört kat, kabuk ve bütün taneye göre de yaklaşık iki ya da üç kat daha fazla fenolik madde içerdiği bildirilmiştir (Yeğin & Uzun, 2018). Yapılan diğer çalışmalarda da üzümdeki fenolik madde miktarının daha çok çekirdekte ve kabukta bulunduğu gösterilmiştir (Sulc ve ark., 2005; Mozetic ve ark., 2006; Göktürk Baydar ve ark., 2007). Kalecik Karası üzüm çeşidinde toplam fenolik madde miktarı üzümün tamamında 562 mg GAE 100 g YA-1 ile üzümün kabuk kısmında 686 mg GAE 100 g YA-1, Cabernet Sauvignon üzüm çeşidinde üzümün tamamında 626 mg GAE 100 g YA-1 iken üzümün kabuk kısmında 653 mg GAE 100 g, Alicante Bouschet üzüm çeşidinde ise üzümün tamamında 587 mg GAE 100 g YA-1 iken üzümün kabuk kısmında 687 mg GAE 100 g YA-1 tespit edilmiştir (Yeğin & Uzun, 2018). Aynı çalışmada koyu renkli üzüm çeşitlerinin kabuktaki toplam fenolik madde miktarı değişim oranı en düşük 653 mg GAE 100 g YA-1 ile Cabernet Sauvignon ve en yüksek 726 mg GAE 100 g YA-1 ile Öküzgözü üzüm çeşidi olduğu bildirilmiştir. Bütün tanedeki toplam fenolik madde miktarı değişim oranı ise en düşük 484 mg GAE 100 g YA-1 ile Trakya İlkeren, en yüksek 626 mg GAE 100 g YA-1 Cabernet Sauvignon'dur. Aynı çalışmada koyu renkli üzüm çeşitleri arasındaki fenolik madde miktarı arasındaki farklılığın üzümün içerdiği fenolik madde gruplarından biri olan antosiyaninlerden kaynaklandığı belirtilmiştir. Daha önce yapılan çalışmalarda da, koyu renkli üzüm çeşitleri arasındaki farklılığının tanenin su içeriğinden iriliğe kadar çok değişik nedenlerden kaynaklanabileceği belirtilmiştir (Kanner ve ark., 1994). Merzifon Karası üzüm çeşidinde bu oranlar en yüksek oda sıcaklığındaki üzümün tamamında, bunu takiben oda sıcaklığındaki üzümün kabuk kısmında tespit edilmiştir (Çizelge 4). Üzüm tiplerinin birbirlerinden farklı fenolik içeriğine sahip olmasının nedeninin üzüm çeşitlerinin genotipinden, iklim ve toprak koşulları ile bağcılıkta kullanılan farklı kültürel işlemlerden kaynaklanabileceği bildirilmiştir (Revilla ve ark., 1997; Montealegre ve ark., 2006). Ayrıca, Yeğin ve Uzun (2018), incelemiş oldukları üzüm çeşitlerindeki en yüksek fenolik madde miktarlarının üzüm çekirdeklerinden elde edildiğini, bunu sırasıyla üzüm kabuğu, üzüm tanesi ve tane etinin izlediğini belirtmişlerdir.

Çizelge 4. Toplam fenolik bileşen tayini bulguları
Table 4 Total phenolic component determination findings

Numara *(Number)*	Gallic Asit Eş Değeri (mg GAE 100 g YA $^{-1}$) *Gallic Acid Eguivalent (mg GAE 100 g YA^{-1})*
1	43.14 ^{cd}
2	58.87 ^{d}
3	80.18 ^{a}
4	102.46 ^{ab}

"a" ve 1 grubu, "b" ve 2 grubu, "c" ve 3 grubu, "d" ve 4 grubu istatistiki açıdan $p<0.05$ düzeyinde anlamlıdır.
"a" and 1 group, "b" and 2 group, "c" and 3 group, "d" and 4 group are statistically significant at the $p<0.05$ level.

Merzifon Karası üzüm çeşidinin flavonoid tayini bulguları çizelge 5'te verilmiştir. Buna göre, en

yüksek toplam flavonoid miktarı oda sıcaklığındaki meyvenin tamamında tespit edilmiş olup, bunu sırasıyla oda sıcaklığındaki kabuk, 50 °C' de üzümün kabuk kısmı ile 50 °C' de üzümün tamamı izlemektedir. Yang ve ark. (2009), şaraplık üzümlerde en yüksek flavonoid miktarının 301.8 mg 100 g YA-1 olarak tespit edildiğini bildirmişlerdir. Beyaz renkli sofralık bir çeşit olan Müşküle üzüm çeşidinde ise toplam flavonid madde miktarının 1069 mg 100 g YA-1 olarak tespit edildiği bildirilmiştir (Karadeniz ve ark., 2005). Üzüm çeşitlerinin flavonoid miktarlarındaki farklılığın, genotipik yapıdaki farklılıktan kaynaklanabileceği gibi, ekolojik koşulların farklılığından, bağcılıkta kullanılan farklı kültürel işlemlerden ya da ekstraksiyon yöntemlerinin farklılığından da kaynaklanabileceği düşünülmektedir. Merzifon Karası üzüm çeşidinin en yüksek flavonoid miktarının oda sıcaklığındaki üzümün tamamında bulunması ve bu değerin oda sıcaklığındaki üzümün kabuk kısmından yüksek olmasına dair bulgular Yeğin ve Uzun (2018)'nun bulgularıyla uyumludur. Bu çalışmada, Cabernet Sauvignon, Kalecik Kararası, Hafizali, Yabani-1, Yabani-3 ve Yabani-4 üzüm çeşitlerinin de bütün tanedeki toplam flavonoid miktarı tane kabuğundan yüksek bulunmuştur. Toplam flavonoid madde miktarının en yüksek olarak üzüm çekirdeğinde saptandığı Kustova ve ark. (2015) tarafından bildirilmiştir. Merzifon Karası üzüm çeşidinin en yüksek flavonoid miktarının oda sıcaklığındaki üzümün tamamında bulunması, fenolik madde miktarında olduğu gibi çekirdekten kaynaklandığı düşünülmektedir.

Çizelge 5. Toplam flavonoid tayini bulguları
Table 5. Total flavonoid determination findings

Numara *Number*	*Toplam Flavonoid (mg 100 g YA^{-1})* *Total Flavonoid (mg 100 g YA^{-1})*
1	17.78 ^{d}
2	19.67 ^{d}
3	23.31 ^{d}
4	44.95 ^{abc}

"a" ve 1 grubu, "b" ve 2 grubu, "c" ve 3 grubu, "d" ve 4 grubu istatistiki açıdan $p<0.05$ düzeyinde anlamlıdır.
"a" and 1 group, "b" and 2 group, "c" and 3 group, "d" and 4 group are statistically significant at the $p<0.05$ level.

SONUÇ ve ÖNERİLER

Bu çalışma sonucunda, en yüksek antioksidan aktivite oranı Merzifon Karası üzüm çeşidinin oda sıcaklığındaki meyvenin tamamından elde edilmiştir. Bunu sırasıyla 50 °C' de üzümün kabuk kısmı, oda sıcaklığındaki üzümün kabuk kısmı ve 50 °C' deki üzümün tamamı izlemektedir. Merzifon Karası üzüm çeşidinde, en yüksek antioksidan aktivitenin oda sıcaklığındaki üzümün tamamında tespit edilmesinin nedeninin, çekirdekteki antioksidan aktivitenin yüksekliğinden kaynaklanabileceği düşünülmektedir. Merzifon Karası üzüm çeşidinde en yüksek fenolik ve flavonoid madde miktarları oda sıcaklığındaki meyvenin tamamında tespit edilirken, bunu sırasıyla oda sıcaklığındaki kabuk, 50 °C' de üzümün kabuk kısmı ve 50 °C' deki üzümün tamamı izlemektedir. Sıcaklığın, üzümün tamamı ile kabuk kısımlarındaki fenolik ve flavonoid miktarlarında olumsuzluğa neden olduğu düşünülmektedir. Merzifon Karası üzüm çeşidinin fenolik ve flavonoid madde miktarlarının oda sıcaklığındaki kabuğa nazaran oda sıcaklığındaki meyvenin tamamında yüksek çıkmasının nedeninin çekirdekten kaynaklandığı düşünülmektedir. Üzüm çeşitleri arasında fenolik ve flavonoid madde miktarlarının farklılık göstermesinin nedeninin üzüm çeşitlerinin genotipik yapısından, bu üzüm çeşidinin yetiştirildiği bölgenin iklim ve toprak yapısından, üzümün yetiştirildiği bağda kullanılan tekniklerin farklılığından, ekstraksiyon yöntemi farklılığından, ekstraksiyon yönteminde kullanılan çözücülerin farklılığından ya da ekstrasyonu yapılacak olan üzümün konsantrasyonundan kaynaklanabileceği düşünülmektedir.

Merzifon Karası, Anadolu coğrafyasında unutulmaya yüz tutmuş olup, Amasya ilinin Merzifon ilçesinde antik çağlardan kalan bir üzüm çeşididir. Köklü bir bağcılık kültürüne sahip ülkemizde, Merzifon Karası üzüm çeşidinin, Türkiye bağcılığına katacağı zenginlik göz önüne alındığında önemi çok büyüktür. Merzifon Karası'nın, bu çalışma ile tespit edilmiş olan gerek üzümün tamamının gerekse kabuğunun antioksidan etki göstermesi, gene bu üzüm çeşidinin fenolik ve flavonoid madde içeriğine sahip olmasıyla da insan sağlığına olumlu etki göstereceği açıktır.

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Araştırmacıların Katkı Oranı Beyan Özet

Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan eder.

Çıkar Çatışması Beyanı

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

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Bozcaada Çavuşu ve Mevcut Tozlayıcı Çeşitleri ile Bazı Muhtemel Tozlayıcı Çeşitlerin Polen Canlılık ve Çimlenme Oranlarının Belirlenmesi

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ÖZET

Bu araştırmada, Bozcaada Çavuşu ve mevcut tozlayıcı çeşitleri ['Kuntra' ('Karasakız') ve 'Vasilâki'] ile bazı muhtemel tozlayıcı çeşitlerin ('Alphonse Lavallée', 'Amasya Beyazı', 'Atasarısı', 'Italia', 'Müşküle', 'Trakya İlkeren', 'Yalova Çekirdeksizi' ve 'Yalova İncisi') polen canlılık ve çimlenme oranlarının belirlenmesi amaçlanmıştır. Üzüm çeşitlerinin polen canlılık testi %1'lik TTC ile belirlenirken, polen çimlenme testi Agar–petri yöntemiyle gerçekleştirilmiştir. İki yıllık araştırma bulgularına göre; bütün üzüm çeşitleri arasında en yüksek canlı polen oranları sırasıyla 'Yalova Çekirdeksizi' (%41.30) ve 'Yalova İncisi' (%37.6), en yüksek yarı canlı polen oranları 'Yalova İncisi' (%53.33), 'Yalova Çekirdeksizi' (%42.93) ve 'Kuntra' (%42.08) üzüm çeşitlerinden elde edilmiştir. Bütün üzüm çeşitleri arasında en yüksek polen çimlenme oranları sırasıyla 'Trakya İlkeren' (%16.19), 'Kuntra' (%14.69) ve 'Alphonse Lavallée' (13.08), en düşük polen çimlenme oranı ise 'Bozcaada Çavuşu' (%0.00) üzüm çeşidinden alınmıştır. Bu araştırmada, mevcut tozlayıcılar olan 'Kuntra' ve 'Vasilâki' üzüm çeşitlerinin yanısıra, 'Italia', 'Müşküle' ve 'Atasarısı' üzüm çeşitlerinin de, ümitvar sofralık tozlayıcı çeşitler olarak yapılacak olan keseleme denemelerine dâhil edilmelerinden olumlu sonuçlar alınabileceği belirlenmiştir.

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Anahtar Kelimeler
Bozcaada Çavuşu
Kuntra
Vasilâki
Polen canlılığı
Polen çimlenmesi

Determination of Pollen Viability and Pollen Germination Rates of Bozcaada Cavusu and Current Pollinator Varieties with Some Probable Pollinator Varieties

ABSTRACT

In this research, it was aimed to determine the pollen viability and pollen germination rates of Bozcaada Cavusu and current pollinator varieties ['Kuntra' ('Karasakız') and 'Vasilâki'] with some probable pollinator varieties ('Alphonse Lavallée', 'Amasya Beyazi', 'Atasarisi', 'Italia', 'Müsküle', 'Trakya İlkeren', 'Yalova Cekirdeksizi' ve 'Yalova Incisi'). While pollen viability test of grape varieties was determined with 1% TTC, pollen germination test was carried out by Agar–petri method. According to the two–year research findings, among all grape varieties, the highest viable pollen rates were determined in 'Yalova Cekirdeksizi' (41.30%) and 'Yalova Incisi' (37.16%) grape varieties, respectively. The highest semi–viable pollen rates were obtained from 'Yalova Incisi' (53.33%), 'Yalova Cekirdeksizi' (42.93%) ve 'Kuntra' (42.08%) grape varieties, respectively. Among all grape varieties, the highest pollen germination rates were determined in 'Trakya Ilkeren' (16.19%), 'Kuntra' (14.69%) and 'Alphonse Lavallée' (13.08%) grape varieties, respectively; the lowest pollen germination rate was obtained from in 'Bozcaada Cavusu' (0.00%) grape variety. In this research, in addition to 'Kuntra' and 'Vasilâki' which are current pollinator, it was thought that 'Italia', 'Müsküle' and 'Atasarisi' grape varieties can be as a promising table pollinator varieties and they included in the cross pollination studies to be carried out from now on.

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GİRİŞ

Bağcılık dünyada, 30°–50° Kuzey ile 30°–40° Güney enlem derecelerinde yapılmaktadır. Bu enlem derecelerinde bulunan Türkiye, asmanın (*Vitis vinifera* L.) anavatanı olan ülkeler arasında olup, kadim ve köklü bir bağcılık kültürüne sahiptir (Ağaoğlu, 1999). Standart sofralık bir üzüm çeşidi olan Çavuş üzüm çeşidi, Türkiye'nin Bozcaada/Çanakkale, Marmara ve Ege Bölgeleri, İç Batı Karadeniz ve İç Anadolu Bölgesi'ndeki belirli bazı bağ yöreleri olmak üzere hemen her yerinde yetiştirilmektedir (Tangolar ve ark., 1996; Anonim, 1997; Uslu & Samancı, 1997a; Kiracı ve ark., 2002; Dardeniz, 2002; Dardeniz ve ark., 2011). Ancak Bozcaada Çavuşu üzüm çeşidinin sinonimlerinin mevcut olması nedeniyle bazen isim karmaşası ortaya çıkabilmekte, Bozcaada Çavuşu çeşit isminin yanı sıra, Beyaz Çavuş ve Çavuş isimleri en fazla kullanılan sinonimleri oluşturmaktadır (Uslu & Samancı, 1997a; Çalışkan & Ağaoğlu 1998; Dardeniz ve ark., 2011). Türkiye'nin birçok bölgesinde yetiştirilmekle birlikte, Çanakkale ilinin Bozcaada ilçesindeki yoğun yetiştiriciliğinden dolayı çoğu kez Bozcaada Çavuşu üzüm çeşidi olarak tanınmaktadır (Uslu & Samancı, 1997a; Uslu & Samancı, 1997b; Dardeniz ve ark., 2011). Bozcaada Çavuşu üzüm çeşidinin Bozcaada'ya özgü olduğu, ada iklimi ve toprak koşullarında daha kaliteli bir ürün alındığı, bununla birlikte diğer Çavuş üzüm çeşitleriyle kıyaslandığında üstün özelliklere sahip olduğunun ortaya konulmasıyla, bu üzüm çeşidine 2020 yılı içerisinde "Bozcaada Çavuşu Üzümü" olarak Coğrafi İşaret Tescil Belgesi alınmıştır.

Bozcaada Çavuşu üzüm çeşidi Morfolojik erdişi fizyolojik dişi çiçek yapısına sahip olmasından dolayı kendi çiçek tozları ile döllenme yeteneğine sahip değildir. Bu nedenle, Bozcaada Çavuşu üzüm çeşidinin döllenip tane tutabilmesi için tozlayıcı (babalık/dölleyici) çeşitlere ihtiyaç duyulmaktadır (Oraman, 1965; Oraman, 1972; Ağaoğlu, 1999; Dardeniz ve ark., 2011). Bozcaada Çavuşu üzüm çeşidinde tozlayıcı (babalık) çeşit olarak Bozcaada'da Kuntra (Karasakız, Mavrupalya, Makbule) ve Vasilâki (Anadolu Yapıncağı, Altıntaş) üzüm çeşitleri (Anonim, 1997; Dardeniz, 2002; Çelik, 2006; Dardeniz ve ark., 2011) tozlayıcı çeşitler olarak kullanılmaktadır (Dardeniz ve ark., 2011).

Üzüm yetiştiriciliğinde en temel unsur bağlardan yüksek miktarda verim ile birlikte kaliteli üzüm elde etmektir. Asmalardan optimum düzeyde verim alabilmenin en önemli unsuru tane tutumudur. Tane tutumu olabilmesi için tozlanma ve döllenmenin uygun şekilde gerçekleşmesi gerekmektedir. Tozlanma ve döllenme, dolayısıyla tane tutumunun optimum seviyede gerçekleşmesinde, çiçek yapısı ve polen kalitesinin önemli bir etkisi bulunmaktadır. Polen kalitesini polen canlılığı, polen çimlenmesi ve polen tüpü oluşturmaktadır (Sharafi & Bahmani, 2011). Polen verimliliği olarak nitelendirilen polen kalitesi, olgun polenin canlılığı, in vitro koşullarda polenin çimlenmesi ve bunun sonucunda polen tüpünün oluşumu ile büyümesi gibi farklı özelliklerin kombinasyonunun bir sonucudur (Stanley & Linskens, 1974; Pereira ve ark., 2018). Başarılı bir tozlanma için, polen miktarının bol ve yüksek kaliteli olması gereklidir (Sharafi, 2011; Kara ve ark. 2017). Tozlanma ve döllenmenin en temel unsurlarından olan polen canlılık seviyesi ve polen çimlenme oranı, beslenme koşulları ve farklı çevresel faktörlerin uygunluğu ile tozlayıcı çeşitle tozlanan dişi çiçekli çeşidin karşılıklı uyumlarına bağlıdır (Eti, 1991; Dantas ve ark., 2005; Sharafi ve ark., 2010; Perveen & Ali, 2010). Polen kalitesi, verim elde etmenin yanı sıra kontrollü tozlaşma için polenlerin depolama potansiyelinin araştırılmasında, çeşitlerin kendi içlerinde ve çeşitler arasında uyumsuzluğun değerlendirilmesinde ve klonal seçim ile genetik ıslah çalışmalarında da önem taşımaktadır (Dafni & Firmage, 2000; Pereira ve ark., 2018). Konuyla ilgili birçok meyve türünde in vivo koşullarda yapay tozlanma çalışmaları, in vitro koşullarda farklı besin ortamı ve çimlendirme yöntemleriyle çimlenme testleri ve bununla birlikte farklı boyama yöntemiyle canlılık testleri gerçekleştirilmiştir (Eti ve ark., 1990; Eti ve ark., 1996; Koyuncu ve ark., 2000; Korkutal ve ark., 2004; Ilgın ve ark., 2007; Kara, 2012; Engin ve ark., 2015). In vitro polen çimlenme testleri, polenin tane tutum performansını tahmin etmede pratik ve güvenilir bir yöntemdir (Nagarajan ve ark., 1965).

Bu araştırmada, Bozcaada Çavuşu ve mevcut tozlayıcı çeşitleri ile bazı muhtemel tozlayıcı çeşitlerin polen canlılık ve çimlenme oranlarının belirlenmesi amaçlanmıştır.

MATERYAL ve METOD

Bozcaada Çavuşu ve mevcut tozlayıcı çeşitleri [Kuntra (Karasakız) ve Vasilâki] ile bazı muhtemel tozlayıcı çeşitlerin (Alphonse Lavallée, Amasya Beyazı, Atasarısı, Italia, Müşküle, Trakya İlkeren, Yalova Çekirdeksizi ve Yalova İncisi) polen canlılık ve çimlenme oranlarının belirlenmesinin amaçlandığı bu araştırma, 2018 ve 2019 yıllarında yürütülmüştür. Çanakkale ili Bozcaada ilçesi Papazbahçe mevkiinde 39°49′14′′–39°49′15′′ enlem ile 26°01′41′′–26°01′43′′ boylam koordinatlarındaki bağlardan Bozcaada Çavuşu, Kuntra (Karasakız) ve Vasilâki üzüm çeşitlerine ait çiçek salkımları (somak); 40°04′26′′–26°21′43′′ enlem ve boylam koordinatlarındaki

'ÇOMÜ Dardanos Yerleşkesi Ziraat Fakültesi Bitkisel Üretim Araştırma ve Uygulama Birimi'nde yer alan 'Sofralık Üzüm Çeşitleri Uygulama ve Araştırma Bağı'ndan ise Alphonse Lavallée, Amasya Beyazı, Atasarısı, Italia, Müşküle, Trakya İlkeren, Yalova Çekirdeksizi ve Yalova İncisi üzüm çeşitlerinin çiçek salkımları materyal olarak kullanılmıştır. Bozcaada Çavuşu, Kuntra ve Vasilâki bağ plantasyonları 5BB Amerikan asma anacı üzerinde ve 140 cm x 140 cm aralık ve mesafede olup, orta yüksek gövdeli goble terbiye sistemi ile tesis edilmiştir. Bağ plantasyonlarına 4–5 yılda bir 2 ton da^{-1} ihtimar ettirilmiş ahır gübresi verilmektedir. Amasya Beyazı, Atasarısı, Italia, Müşküle ve Yalova Çekirdeksizi üzüm çeşitleri 5BB, Yalova İncisi üzüm çeşidi 41B Amerikan asma anacı üzerine 3.0 m x 1.5 m aralık ve mesafede tek kollu sabit kordon terbiye sistemi ile kurulmuştur. Alphonse Lavallée ve Trakya İlkeren üzüm çeşitleri 5BB Amerikan asma anacı üzerine, orta yüksek gövdeli goble terbiye sistemi ile oluşturulmuştur. Mevcut bağ plantasyonuna 3 yılda bir 50 kg da^{-1} NPK (15:15:15) ticari gübre uygulanmaktadır.

Polen canlılık ve çimlenme testleri kapsamında çeşitlere ait henüz açılmamış haldeki çiçek salkımları (somak) bağ makası yardımıyla kesilip, kilitli torbalara alınmıştır. Alınan çiçek salkımları soğuk hava sağlanmış kutular ile 'ÇOMÜ Ziraat Fakültesi Bahçe Bitkileri Bölümü Pomoloji Laboratuvarı'na getirilmiştir. Bozcaada Çavuşu üzüm çeşidi ve tozlayıcı çeşitlerin çiçek salkımları 2018 yılında 10 Mayıs tarihinde, 2019 yılında ise 21 Mayıs tarihinde alınmıştır. Alphonse Lavallée, Amasya Beyazı, Atasarısı, Italia, Müşküle, Trakya İlkeren, Yalova Çekirdeksizi ve Yalova İncisi üzüm çeşitlerinin çiçek salkımları, Bozcaada'ya kıyasla uyanma ve çiçek açma tarihi 1 hafta kadar daha geç olduğundan 2018 yılında 18 Mayıs, 2019 yılında ise 29 Mayıs tarihinde toplanmıştır. Temin edilen çiçek salkımlarından alınan anterler ışıklı bir ortamda tutularak polenlerin meydana çıkması sağlanmış, elde edilen bu polenlerde polen canlılığı ve polen çimlenme testleri gerçekleştirilmiştir.

Polen Canlılık Testi

Polen canlılık düzeyi in vitro ortamda, farklı boyama yöntemleri ile canlılığı devam eden polenlerin boyanması sonucunda belirlenmektedir (Kahraman, 2014). Polen canlılık testi için %1'lik TTC (2, 3, 5 Triphenyl tetrazolium chloride) boyama yöntemi uygulanmıştır. Polen canlılık testi 3 tekerrür şeklinde planlanmış olup, her bir tekerrür için 1 lam kullanılmış ve 100 adet polen ekimi gerçekleştirilmiştir. Hazırlanan TTC çözeltisinden lam üzerine 1 damla damlatılmış, üzerine fırça yardımıyla polenler serpilmiş ve lamel ile kapatılmıştır. Birkaç saat bekletilmesinin ardından Olympus CX41 mikroskobunda polenlerin canlılık sayımları yapılmıştır. %1'lik TTC çözeltisi ile kırmızı renge boyanan polenler canlı, pembe renge boyananlar yarı canlı ve boyanmayıp renksiz kalan polenler ise cansız olarak kabul edilmiştir (Eti, 1991).

Polen Çimlenme Testi

Sakkaroz agar–petri (%1 agar+%15 sakkaroz) yöntemi ile yapılan polen çimlenme testi 3 tekerrür şeklinde planlanmış olup, her bir tekerrür için 1 petri kabı kullanılmış ve 100 adet polen ekimi gerçekleştirilmiştir. 1 g agar agar ve 15 g sakkaroz 100 ml saf su ile tamamlanıp, ısıtıcı tablalı manyetik karıştırıcıda hazırlanmıştır. Elde edilen ortam petri kaplarına 2 mm kalınlığında olacak şekilde dökülüp soğumaya bırakılmıştır. Ortam yarı katı halde iken fırça yardımıyla homojen bir şekilde polen ekimi yapılmıştır. Petri kaplarına, çimlendirme sırasında gerekli nemin muhafaza edilmesi amacıyla saf su ile nemlendirilmiş filtre kâğıtları yerleştirilmiştir. Petri kapları 25°C'de 2 gün boyunca tutulmuş, daha sonra Olympus CX41 mikroskop altında sayımlar yapılarak polen çimlenme oranları tespit edilmiştir (Parfitt & Ganeshan, 1989).

İstatistik Analizler

Yapılan bu araştırma, tesadüf parselleri deneme desenine göre polen ve çimlenme testlerinde 3 tekerrürlü her tekerrürde 1'er adet lam ve petri kabı yer alacak şekilde planlanmıştır. Elde edilen bulgular 'SAS 9.1.3. Portable' istatistik paket programı kapsamında varyans analizi ile belirlenmiş, incelenen parametrelerde çeşitler arasındaki farklılık LSD çoklu karşılaştırma testiyle $p<0.05$ düzeyinde değerlendirilmiştir.

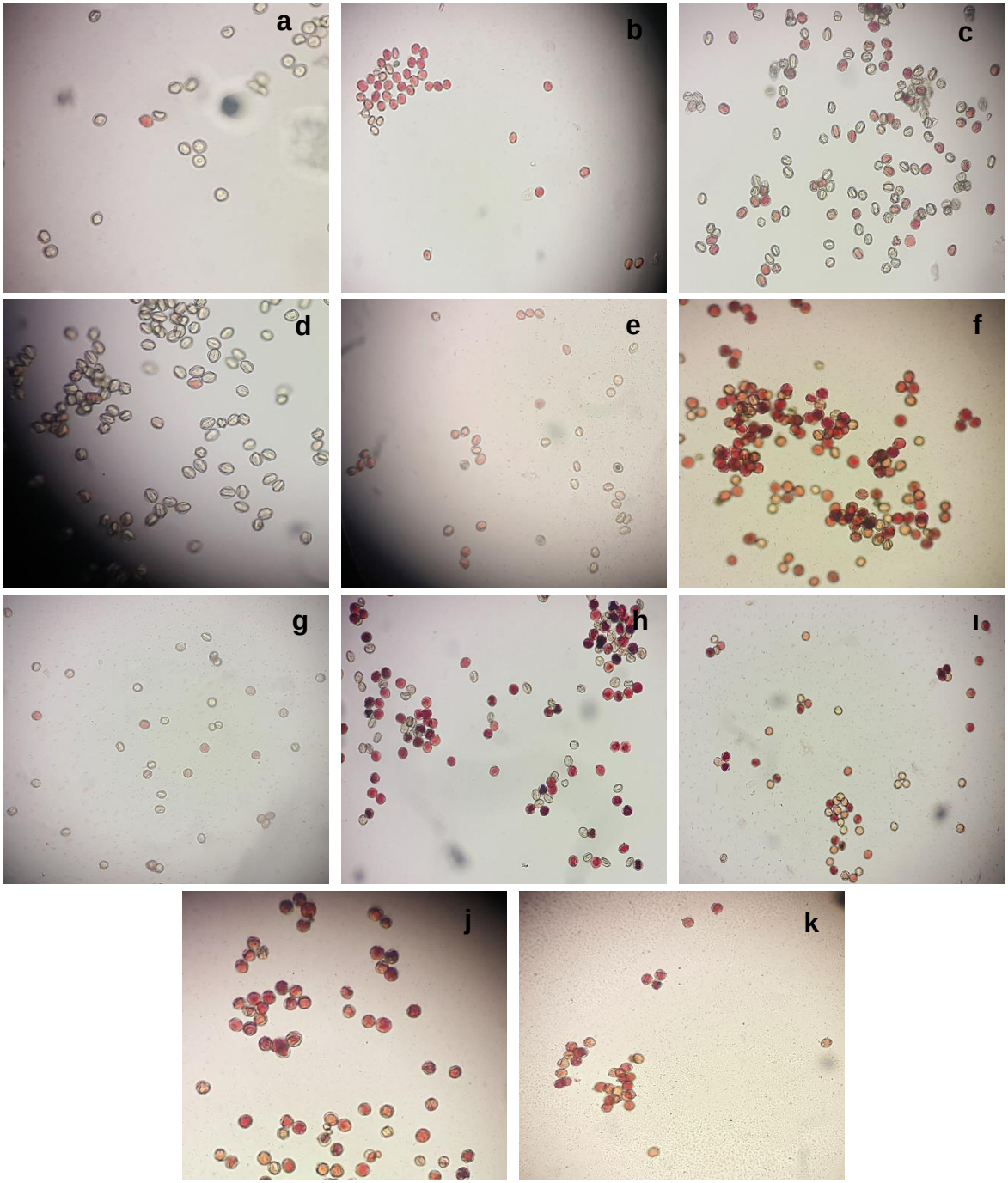
BULGULAR ve TARTIŞMA

Polen Canlılık ve Çimlenme Oranları

Bozcaada Çavuşu ve mevcut tozlayıcı çeşitleri ile bazı muhtemel tozlayıcı çeşitlerin polen canlılık oranları Çizelge 1, Çizelge 2 ve Çizelge 3'te, çimlenme oranları Çizelge 4'te sunulmuştur. Polen canlılık testinde %1'lik TTC ile boyanma sonucunda kırmızı renkte olan polenler canlı, pembe renkte olan polenler yarı canlı ve renk almayan polenler cansız olarak kabul edilmiş ve farklı üzüm çeşitlerine ait polenlerin canlılık durumları Şekil 1'de gösterilmiştir.

Canlı polen oranlarına ait 2018 yılı bulgularında; sırasıyla %36.40, %34.95, %34.47 ve %32.68 ile Atasarısı, Yalova Çekirdeksizi, Müşküle ve Yalova İncisi üzüm çeşitleri en yüksek canlı polen oranlarına sahipken, %1.29 ile Alphonse Lavallée üzüm çeşidi en düşük canlı polen oranına sahip üzüm çeşidi olmuştur (Çizelge 1). Bozcaada Çavuşu üzüm çeşidi ve mevcut tozlayıcı çeşitler olan Kuntra (Karasakız) ve Vasilâki üzüm çeşitlerine ait canlı polen oranlarına kendi

aralarında bakıldığında; en yüksek canlı polen oranının %25.57 ile Vasilâki üzüm çeşidinden, en düşük canlı polen oranının %2.92 ile Bozcaada Çavuşu üzüm çeşidinden elde edildiği, Kuntra üzüm çeşidinin ise %13.72 ile ara grubu oluşturduğu görülmektedir (Çizelge 1).



Şekil 1. Farklı üzüm çeşitlerine ait polenlerin canlılık durumları (a–Bozcaada Çavuşu, b–Kuntra, c–Vasilâki, d–Alphonse Lavallée, e–Amasya Beyazı, f–Atasarısı, g–Italia, h–Müşküle, ı–Trakya İlkeren, j–Yalova Çekirdeksizi, k–Yalova İncisi) (Şahin, 2019; özgün fotoğraf).

Figure 1. Viability status of pollen of different grape varieties (a–Bozcaada Çavuşu, b–Kuntra, c–Vasilâki, d–Alphonse Lavallée, e–Amasya Beyazı, f–Atasarısı, g–Italia, h–Müşküle, ı–Trakya İlkeren, j–Yalova Çekirdeksizi, k–Yalova İncisi) (Şahin, 2019; original photo).

Canlı polen oranlarının 2019 yılı bulguları incelendiğinde; sırasıyla %47.65, %41.64, %35.45 ve %33.84 ile Yalova Çekirdeksizi, Yalova İncisi, Vasilâki ve Kuntra üzüm çeşitleri en yüksek, sırasıyla %1.13, %4.46 ve %7.10 ile Alphonse Lavallée, Trakya İlkeren ve Bozcaada Çavuşu üzüm çeşitleri en düşük canlı polen oranlarına sahip çeşitler olmuştur. Bozcaada Çavuşu üzüm çeşidi ve

mevcut tozlayıcı çeşitler olan Kuntra ve Vasilâki üzüm çeşitlerine ait canlı polen oranlarına kendi aralarında bakıldığında; en yüksek canlı polen oranlarının %35.45 ve %33.84 ile Vasilâki ve Kuntra üzüm çeşitlerinden, en düşük canlı polen oranının %7.10 ile Bozcaada Çavuşu üzüm çeşidinden elde edildiği görülmektedir (Çizelge 1).

Çizelge 1. Farklı üzüm çeşitlerindeki canlı polen canlılık oranları (%)
Table 1 Viable pollen rates of different grape varieties (%)

Üzüm çeşitleri	Canlı (%)		
	2018	2019	Ort.
Bozcaada Çavuşu	2.92 ±3.16 ef* B**	7.10 ±2.93 e B	5.01 ±2.71 g B
Kuntra (Karasakız)	13.72 ± 2.34 d AB	33.84 ±6.57 abc A	23.78 ±3.14 de A
Vasilâki	25.57 ± 10.64 bc A	35.45 ±10.63 abc A	30.51 ±4.85 bcd A
Alphonse Lavallée	1.29 ± 2.23 f	1.13 ±1.04 e	1.21 ±1.55 g
Amasya Beyazı	3.09 ± 4.75 ef	24.00 ±18.20 cd	13.55 ±6.80 f
Atasarısı	36.40 ± 4.31 a	28.57 ±12.37 bcd	32.49 ±5.64 bc
Italia	9.86 ±0.32 de	25.18 ±1.24 cd	17.52 ±0.54 ef
Müşküle	34.47 ±2.63 a	16.15 ±5.65 de	25.31 ±3.71 cde
Trakya İlkeren	24.28 ±4.01 c	4.46 ±2.59 e	14.37 ±3.25 f
Yalova Çekirdeksizi	34.95 ±2.48 a	47.65 ±11.99 a	41.30 ±6.99 a
Yalova İncisi	32.68 ±5.96 ab	41.64 ±12.16 ab	37.16 ±8.37 ab
LSD (0.05)*	7.9018	15.998	8.3088
LSD (0.05)**	13.086	14.799	7.3669

Ort.: Ortalama. LSD (0.05): 0.05 düzeyinde önemli. *: Farklı üzüm çeşitleri arasındaki polen canlılık oranları. **: Bozcaada Çavuşu üzüm çeşidi ve Bozcaada'daki mevcut tozlayıcı çeşitleri arasındaki polen canlılık oranları.

İki yılın ortalama canlı polen oranları incelendiğinde; sırasıyla %41.30 ve %37.16 ile Yalova Çekirdeksizi ve Yalova İncisi üzüm çeşitleri en yüksek canlı polen oranına sahipken, sırasıyla %1.21 ve %5.01 ile Alphonse Lavallée ve Bozcaada Çavuşu üzüm çeşitleri en düşük canlı polen oranlarına sahip olmuştur (Çizelge 1). Bozcaada Çavuşu üzüm çeşidi ve mevcut tozlayıcı çeşitler olan Kuntra ve Vasilâki üzüm çeşitlerine ait canlı polen oranları kendi aralarında değerlendirildiğinde; en yüksek canlı polen oranı sırasıyla %30.51 ile Vasilâki ve %23.78 ile Kuntra üzüm çeşitlerinden, en düşük canlı polen oranı ise %5.01 ile Bozcaada Çavuşu üzüm çeşidinden elde edilmiştir (Çizelge 1). Burada yıllara göre farklı canlı polen oranlarının elde edilme sebebinin, çiçek tozlarının yıllar bazında iklime bağlı farklı olgunlaşma düzeylerinden kaynaklanmış olabileceği düşünülmektedir.

Üzüm çeşitlerine ait 2018 yılı yarı canlı polen oranları incelendiğinde; sırasıyla %57.66 ve %45.01 ile Yalova İncisi ve Yalova Çekirdeksizi üzüm çeşitleri en yüksek yarı canlı polen oranına sahipken, %5.70 ile Bozcaada Çavuşu üzüm çeşidinin en düşük yarı canlı polen oranına sahip olduğu belirlenmiştir. Bozcaada Çavuşu üzüm çeşidi ve mevcut tozlayıcı çeşitler olan Kuntra ve Vasilâki üzüm çeşitlerine ait yarı canlı polen oranlarına kendi aralarında bakıldığında; en yüksek yarı canlı polen oranları sırasıyla %35.12 ve %30.70 ile Kuntra ve Vasilâki üzüm çeşitlerinden elde edilirken, en düşük yarı canlı polen oranı ise %5.70 ile Bozcaada Çavuşu üzüm çeşidinde belirlenmiştir (Çizelge 2).

Yarı canlı polen oranlarının 2019 yılı bulguları incelendiğinde; sırasıyla %49.04, %49.00, %43.35, %41.18, %40.84, %40.50, %37.88 ve %34.53 ile Kuntra, Yalova İncisi, Amasya Beyazı, Vasilâki, Yalova Çekirdeksizi, Italia, Alphonse Lavallée ve Atasarısı üzüm çeşitleri en yüksek yarı canlı polen oranına sahipken, %17.66 ile Trakya İlkeren üzüm çeşidinin en düşük yarı canlı polen oranına sahip olduğu belirlenmiştir. Bozcaada Çavuşu üzüm çeşidi ve mevcut tozlayıcı çeşitler olan Kuntra ve Vasilâki üzüm çeşitlerine ait yarı canlı polen oranlarına kendi aralarında bakıldığında; en yüksek yarı canlı polen oranı %49.04 ile Kuntra üzüm çeşidinden, en düşük yarı canlı polen oranı %26.86 ile Bozcaada Çavuşu üzüm çeşidinden elde edilmiş, %41.18 ile Vasilâki üzüm çeşidi ara grubu oluşturmuştur (Çizelge 2).

İki yılın ortalama yarı canlı polen oranları incelendiğinde; sırasıyla %53.33, %42.93 ve %42.08 ile Yalova İncisi, Yalova Çekirdeksizi ve Kuntra üzüm çeşitlerinin en yüksek, %16.28 ile Bozcaada Çavuşu üzüm çeşidinin en düşük yarı canlı polen oranına sahip çeşit olduğu belirlenmiştir (Çizelge 2). Bozcaada Çavuşu üzüm çeşidi ve mevcut tozlayıcı çeşitler olan Kuntra ve Vasilâki üzüm çeşitlerine ait yarı canlı polen oranları kendi aralarında değerlendirildiğinde; en yüksek yarı canlı polen oranları sırasıyla %42.08 ve %35.94 ile Kuntra ve Vasilâki üzüm çeşitlerinden, en düşük yarı canlı polen oranı ise %16.28 ile Bozcaada Çavuşu üzüm çeşidinden alınmıştır (Çizelge 2).

Üzüm çeşitlerinin 2018 yılı cansız polen oranları incelendiğinde; sırasıyla %91.38, %82.10 ve %77.91 ile Bozcaada Çavuşu, Alphonse Lavallée ve Amasya Beyazı üzüm çeşitlerinin en yüksek cansız polen oranlarına sahip çeşitler, %9.66 ile Yalova İncisi üzüm çeşidinin ise en düşük cansız polen oranına

sahip üzüm çeşidi olduğu tespit edilmiştir. Bozcaada Çavuşu üzüm çeşidi ve mevcut tozlayıcı çeşitler olan Kuntra ve Vasilâki üzüm çeşitlerine ait cansız polen oranlarına kendi aralarında bakıldığında; en yüksek cansız polen oranı %91.38 ile Bozcaada Çavuşu üzüm çeşidinden, en düşük cansız polen oranları sırasıyla %43.72 ve %51.15 ile Vasilâki ve Kuntra üzüm çeşidinden elde edilmiştir (Çizelge 3).

Çizelge 2. Farklı üzüm çeşitlerindeki yarı canlı polen oranları (%)
Table 2 Semi–viable pollen rates of different grape varieties (%)

Üzüm çeşitleri	Yarı canlı (%)		
	2018	2019	Ort.
Bozcaada Çavuşu	5.70 ±3.07 g B	26.86 ±7.39 bc B	16.28 ± 3.40 f B
Kuntra (Karasakız)	35.12 ±2.39 bcd A	49.04 ±14.67 a A	42.08 ±6.25 abc A
Vasilâki	30.70 ±6.86 cde A	41.18 ±6.59 ab AB	35.94 ±4.79 bcd A
Alphonse Lavallée	16.61 ±8.11 fg	37.88 ±15.83 ab	27.25 ± 9.49 def
Amasya Beyazı	19.00 ±21.21 efg	43.35 ±2.63 ab	31.18 ±11.65 cde
Atasarısı	39.54 ±5.82 bc	34.53 ±18.33 abc	37.04 ±11.21 bcd
Italia	29.07 ±0.32 cdef	40.50 ±16.57 ab	34.79 ±5.90 bcd
Müşküle	33.24 ±2.63 bcd	29.40 ±2.62 bc	31.32 ±2.45 cde
Trakya İlkeren	24.20 ±4.97 efd	17.66 ±3.18 c	20.93 ±4.00 ef
Yalova Çekirdeksizi	45.01 ±1.84 ab	40.84 ±7.44 ab	42.93 ±3.49 ab
Yalova İncisi	57.66 ±2.77 a	49.00 ±6.64 a	53.33 ±4.62 a
LSD (0.05)*	13.720	18.392	11.593
LSD (0.05)**	9.0906	20.421	9.898

Ort.: Ortalama. LSD (0.05): 0.05 düzeyinde önemli. *: Farklı üzüm çeşitleri arasındaki polen canlılık oranları. **: Bozcaada Çavuşu üzüm çeşidi ve Bozcaada'daki mevcut tozlayıcı çeşitleri arasındaki polen canlılık oranları.

Çizelge 3. Farklı üzüm çeşitlerindeki cansız polen oranları (%)
Table 3 Non–viable pollen rates of different grape varieties (%)

Üzüm çeşitleri	Cansız (%)		
	2018	2019	Ort.
Bozcaada Çavuşu	91.38 ±4.76 a A	66.04 ±6.82 ab A	78.71 ±1.04 a A
Kuntra (Karasakız)	51.15 ±3.54 bc B	17.13 ±8.11 ef B	34.14 ±3.62 fg B
Vasilâki	43.72 ±9.50 cd B	23.45 ±13.28 def B	33.59 ±6.30 fg B
Alphonse Lavallée	82.10 ±9.22 a	60.98 ±16.48 ab	71.54 ±11.04 ab
Amasya Beyazı	77.91 ±25.80 a	32.66 ±17.41 de	55.29 ±7.51 cd
Atasarısı	24.07 ±6.63 ef	36.91 ±7.44 cd	30.49 ± 6.60 g
Italia	61.07 ±8.12 b	34.33 ±16.16 de	47.70 ± 5.61 de
Müşküle	32.29 ±0.32 de	54.45 ±6.15 bc	43.37 ±3.20 ef
Trakya İlkeren	51.52 ±7.99 bc	77.87 ±3.85 a	64.70 ±5.92 bc
Yalova Çekirdeksizi	20.03 ±2.74 ef	11.51 ±4.64 f	15.77 ±3.68 h
Yalova İncisi	9.66 ±4.24 f	9.36 ±6.02 f	9.51 ±4.54 h
LSD (0.05)*	16.743	18.340	10.039
LSD (0.05)**	12.914	19.598	8.467

Ort.: Ortalama. LSD (0.05): 0.05 düzeyinde önemli. *: Farklı üzüm çeşitleri arasındaki polen canlılık oranları. **: Bozcaada Çavuşu üzüm çeşidi ve Bozcaada'daki mevcut tozlayıcı çeşitleri arasındaki polen canlılık oranları.

Cansız polen oranlarının 2019 yılı bulguları incelendiğinde; sırasıyla %77.87, %66.04 ve %60.98 ile Trakya İlkeren, Bozcaada Çavuşu ve Alphonse Lavallée üzüm çeşitlerinin en yüksek cansız polen oranlarına, %9.36 ve %11.51 ile Yalova İncisi ve Yalova Çekirdeksizi üzüm çeşitlerinin ise en düşük cansız polen oranlarına sahip çeşitler oldukları saptanmıştır. Bozcaada Çavuşu üzüm çeşidi ve mevcut tozlayıcı çeşitler olan Kuntra ve Vasilâki üzüm çeşitlerine ait cansız polen oranlarına kendi aralarında bakıldığında; en yüksek cansız polen oranını %66.04 ile Bozcaada Çavuşu üzüm çeşidi, en düşük cansız polen oranını ise sırasıyla %17.13 ve %23.45 ile Kuntra ve Vasilâki üzüm çeşitlerinin oluşturduğu görülmektedir (Çizelge 3).

İki yılın ortalama cansız polen oranları incelendiğinde; sırasıyla %78.71 ve %71.54 ile Bozcaada Çavuşu ve Alphonse Lavallée üzüm çeşitleri en yüksek cansız polen oranlarını verirken, sırasıyla %9.51 ve %15.77 ile Yalova İncisi ve Yalova Çekirdeksizi üzüm çeşitleri en düşük cansız polen oranına sahip çeşitler olmuştur. Bozcaada Çavuşu üzüm çeşidi ve mevcut tozlayıcı çeşitler olan Kuntra ve Vasilâki üzüm çeşitlerine ait cansız polen oranları kendi aralarında değerlendirildiğinde; en yüksek cansız polen oranının %78.71 ile Bozcaada Çavuşu

üzüm çeşidinden, en düşük cansız polen oranının ise sırasıyla %33.59 ve %34.14 ile Vasilâki ve Kuntra üzüm çeşidinden elde edildiği görülmektedir (Çizelge 3).

Farklı üzüm çeşitlerindeki polen çimlenme oranlarının 2019 yılı bulguları incelendiğinde; sırasıyla %24.00, %21.63 ve %16.94 ile Kuntra, Trakya İlkeren ve Alphonse Lavallée üzüm çeşitlerinin en yüksek, %0.00 ile Bozcaada Çavuşu üzüm çeşidinin en düşük polen çimlenme oranına sahip çeşit olduğu tespit edilmiştir. Bozcaada Çavuşu üzüm çeşidi ve mevcut tozlayıcı çeşitler olan Kuntra ve Vasilâki üzüm çeşitlerine ait polen çimlenme oranlarına kendi aralarında bakıldığında; en yüksek polen çimlenme oranı %24.00 ile Kuntra (Karasakız) üzüm çeşidinden, en düşük polen çimlenme oranı %0.00 ile Bozcaada Çavuşu üzüm çeşidinden elde edilmiş, Vasilâki üzüm çeşidi %7.84 ile ara grubu oluşturmuştur (Çizelge 4).

Çizelge 4. Farklı üzüm çeşitlerindeki polen çimlenme oranları
Table 4 Pollen germination rates of different grape varieties (%)

Üzüm çeşitleri	Polen çimlenme oranı (%)		
	2018	2019	Ort.
Bozcaada Çavuşu	0.00 ±0.00 d* C**	0.00 ±0.00 e C	0.00 ±0.00 f C
Kuntra (Karasakız)	5.37 ±1.43 bcd A	24.00 ±5.27 a A	14.69 ±2.85 ab A
Vasilâki	2.95 ±0.49 cd B	7.84 ±0.99 cd B	5.40 ±0.47 de B
Alphonse Lavallée	9.22 ±1.41 ab	16.94 ±2.21 ab	13.08 ±0.93 abc
Amasya Beyazı	5.74 ±0.55 bc	11.77 ±1.00 bc	8.76 ±0.75 cde
Atasarısı	7.39 ±1.66 abc	8.59 ±0.56 cd	7.99 ±0.80 cde
Italia	5.87 ±2.04 bc	6.71 ±2.69 cde	6.29 ±1.31 de
Müşküle	11.96 ±5.75 a	3.76 ±2.22 de	7.86 ±2.19 de
Trakya İlkeren	10.75 ±6.32 ab	21.63 ±11.91 a	16.19 ±8.16 a
Yalova Çekirdeksizi	9.06 ±5.46 ab	11.38 ±0.98 bc	10.22 ±2.87 bcd
Yalova İncisi	3.33 ±2.89 cd	5.13 ±3.71 cde	4.23 ±3.22 ef
LSD (0.05)*	5.6519	7.2856	5.1600
LSD (0.05)**	1.7448	6.1907	3.3372

Ort.: Ortalama. LSD (0.05): 0.05 düzeyinde önemli. *: Farklı üzüm çeşitleri arasındaki polen çimlenme oranları. **: Bozcaada Çavuşu üzüm çeşidi ve Bozcaada'daki mevcut tozlayıcı çeşitleri arasındaki polen çimlenme oranları.

İki yılın ortalama verileri incelendiğinde; sırasıyla %16.19, %14.69 ve %13.08 ile Trakya İlkeren, Kuntra ve Alphonse Lavallée üzüm çeşitleri en yüksek polen çimlenme oranına sahipken, %0.00 ile Bozcaada Çavuşu üzüm çeşidi en düşük polen çimlenme oranına sahip olmuştur. Bozcaada Çavuşu üzüm çeşidi ve mevcut tozlayıcı çeşitler olan Kuntra ve Vasilâki üzüm çeşitlerine ait polen çimlenme oranları kendi aralarında değerlendirildiğinde; en yüksek polen çimlenme oranının %14.69 ile Kuntra üzüm çeşidinden, en düşük polen çimlenme oranının %0.00 ile Bozcaada Çavuşu üzüm çeşidinden elde edildiği belirlenmiş, Vasilâki üzüm çeşidi %5.40 ile ara grubu teşkil etmiştir (Çizelge 4).

Üzüm çeşitlerine ait 2018 yılı polen çimlenme oranları incelendiğinde; sırasıyla %11.96, %10.75, %9.22, %9.06 ve %7.39 ile Müşküle, Trakya İlkeren, Alphonse Lavallée, Yalova Çekirdeksizi ve Atasarısı üzüm çeşitlerinin en yüksek, %0.00 ile Bozcaada Çavuşu üzüm çeşidinin ise en düşük polen çimlenme oranına sahip çeşitler olduğu tespit edilmiştir (Çizelge 4).

Bozcaada Çavuşu üzüm çeşidi ve mevcut tozlayıcı çeşitler olan Kuntra (Karasakız) ve Vasilâki üzüm çeşitlerine ait polen çimlenme oranlarına kendi aralarında bakıldığında; en yüksek polen çimlenme oranı %5.37 ile Kuntra üzüm çeşidinden, en düşük polen çimlenme oranı %0.00 ile Bozcaada Çavuşu üzüm çeşidinden elde edilmiş, Vasilâki üzüm çeşidi ise %2.95 ile ara grubu oluşturmuştur (Çizelge 4).

Jovanovi–Cvetkovic ve ark. (2016) ile Kara ve ark. (2017)'nın yapmış oldukları çalışmalarda, dişi çiçek yapısına sahip üzüm çeşitlerinin (Bilatina ve Ekşi Kara) polen tanelerinde in vitro çimlenmenin meydana gelmediği, bununla birlikte başarılı bir çimlenme ve tane tutumu için canlı polenlere ve yüksek polen çimlenme oranına sahip üzüm çeşitlerine ihtiyaç duyulduğu belirtilmektedir. Bozcaada Çavuşu üzüm çeşidinin polenlerinde in vitro çimlenmenin meydana gelmemesi, Bilatina ve Ekşi Kara üzüm çeşitlerinin polen çimlenme sonuçlarıyla benzerlik taşımaktadır.

Biasi ve Conner (2016), hermafrodit ve dişi çiçekli farklı üzüm çeşitlerinin polenlerinde canlılık ve çimlenme testleri uygulamışlardır. Hermafrodit ve dişi çiçekli üzüm çeşitlerinin polen canlılık oranlarının (%79.50–%97.50) yüksek olduğu ve çimlenme testlerinin (%0.00–%18.20) araştırma bulguları ile benzerlik gösterdiği tespit edilmiştir. Bununla birlikte dişi çiçekli Fry ve Supreme üzüm çeşitlerinde Bozcaada Çavuşu üzüm çeşidinde olduğu gibi polenlerin canlı, ancak çimlenmenin görülmediği

belirlenmiştir.

Bir üzüm çeşidinin tozlayıcı bir çeşit olarak önerilebilmesi için sabit bir polen çimlenme oranı bulunmamaktadır. Bu durum dişi çiçek yapısına sahip üzüm çeşidinin yetiştirildiği ekolojiyle yakından ilişkilidir. Örneğin; tozlayıcı çeşidin çiçek tozu canlılığının zayıf olduğu, dişi çiçekli çeşit ve tozlayıcı çeşidin çiçek açma tarihlerinin tam olarak çakışmadığı, ancak çiçeklenme tarihindeki iklim koşullarının (sıcaklık ve yağış) uygun olduğu yıllarda seyrek ama çok iri taneli salkımlar elde edilebilir. Bununla birlikte, çiçeklenme tarihindeki iklim koşullarının (sıcaklık ve yağış) olumsuz olduğu bazı yıllarda ise dişi çiçekli çeşitlerde tane tutumunun azalmasıyla salkımlarda boncuklanma (millerandage) sonucunda büyük ölçüde partenokarpi meydana gelmesi muhtemeldir. Bunun aksine tozlayıcı çeşidin çiçek tozu canlılığının yüksek olduğu, dişi çiçekli çeşit ve tozlayıcı çeşidin çiçek açma tarihlerinin tam olarak çakıştığı ve çiçeklenme tarihindeki iklim koşullarının (sıcaklık ve yağış) da uygun olduğu yıllarda ise sık ve küçük taneli salkımlar oluşması beklenebilir. Yani babalık çeşitlerde istenen randımanın alınması o yılın iklim koşullarıyla yakından ilişkili olup, farklı bağlarda farklı babalık çeşitlerin bulundurulması bir sigorta görevi teşkil edecektir.

İşçi (2021)'nin yapmış olduğu çalışmada Alicante Boushet (%75.75), Cardinal (%84.25) ve Syrah (%74.25) üzüm çeşitlerinin polen canlılık oranlarının yüksek ancak polen çimlenme oranlarının (%53.75–%69.25–%67.00) daha düşük bulunduğu yönündeki bulgular, bu araştırma sonucunda Yalova Çekirdeksizi ve Yalova İncisi üzüm çeşitlerinden elde edilmiş olan bulgular ile benzerlik göstermektedir.

Kelen ve Demirtaş (2003), Karataş ve ark. (2005) ve Khaleel (2017)'in morfolojik erdişi fizyolojik dişi çiçek yapısına sahip üzüm çeşitlerinde yapmış oldukları polen canlılık ve çimlenme testlerinde, çeşitlerin polen canlılıklarının düşük ve bununla birlikte polenlerde çimlenmenin tespit edilemediği belirtilmektedir. Morfolojik erdişi fizyolojik dişi çiçek yapısına sahip Bozcaada Çavuşu üzüm çeşidinden elde edilmiş olan bulgular, araştırmacıların bu yöndeki bulgularını destekler niteliktedir.

İşçi (2021)'nin Alphonse Lavallée ve Italia üzüm çeşitlerinin polen özellikleri üzerine yapmış olduğu bir çalışmadaki polen canlılık oranları (%24.25–%75.00) ile yürütmüş olduğumuz araştırma sonucunda yine aynı üzüm çeşitlerinden elde ettiğimiz canlı polen ve yarı canlı polen toplamlarının benzer olduğu sonucuna varılmıştır. Elde edilmiş olan polen çimlenme oranlarının (%38.25–%79.50) genel olarak bulgularımıza kıyasla daha yüksek olmasının, çiçek salkımlarının çiçek gelişim evresinin daha ileriki bir aşamasında alınmış olmasından kaynaklanabileceği düşünülmektedir.

SONUÇ ve ÖNERİLER

Canlı, yarı canlı ve cansız polen canlılık oranlarına bakıldığında; Kuntra ve Vasilâki üzüm çeşitlerine en yakın değerleri veren Italia, Müşküle ve Atasarısı üzüm çeşitleri, Bozcaada Çavuşu üzüm çeşidine ümitvar tozlayıcı çeşitler olarak ön plana çıkmaktadır. Polen çimlenme oranları dikkate alındığında ise; Kuntra ve Vasilâki üzüm çeşitlerinin ortalama değerleri arasında polen çimlenme oranlarını veren sırasıyla Italia, Müşküle, Atasarısı, Amasya Beyazı, Yalova Çekirdeksizi ve Alphonse Lavallée üzüm çeşitleri, yine ümitvar tozlayıcı çeşitler olarak görülmektedir. Ancak bu gibi araştırmalarda ara çalışma olarak keseleme denemelerinin yapılması, uygun tozlayıcı çeşitleri belirlemede daha doğru bir yaklaşımdır. Yapılan çalışmada keseleme denemeleri planlanmamış olmakla birlikte, üzüm çeşitlerinin polen canlılık ve polen çimlenme oranları bir arada değerlendirildiğinde incelenmiş olan üzüm çeşitleri arasında Italia, Müşküle ve Atasarısı üzüm çeşitlerinin, Bozcaada Çavuşu üzüm çeşidi için muhtemel ümitvar sofralık tozlayıcı çeşitler olarak kullanılabilecekleri düşünülmektedir.

TEŞEKKÜR

Bu araştırma makalesi, 'Bozcaada Çavuşu Üzüm Çeşidinin Bozcaada'daki Mevcut Tozlayıcı Çeşitleri ile Bazı Muhtemel Tozlayıcı Çeşitlerin Polen Canlılığı ve Çimlenme Oranlarının Belirlenmesi' başlıklı yüksek lisans tez çalışmasının bulguları ile hazırlanmıştır.

Araştırmacıların Katkı Oranı Beyan Özeti

Yazarlar makaleye eşit oranda katkı sağlamıştır.

Çıkar Çatışması Beyanı

Makale yazarları aralarında herhangi bir çıkar çatışması yoktur.

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Bazı Patates (*Solanum tuberosum* L.) Çeşitlerinin in vitro Şartlarda Tuzluluğa Toleransının Belirlenmesi

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ÖZET

Patates (*Solanum tuberosum* L.) in vitro ve in vivo şartlarda tuz stresine orta derecede hassasiyet gösteren tarla bitkilerinden biridir. Tuzluluk stresi patates yumru üretiminde önemli ve tahrip edici etkilere sahiptir. Bu nedenle, patates genotiplerinin tuz stresine karşı in vitro şartlarda denenmesi, tarla denemelerine alternatif yararlı bir araç olarak ortaya çıkmaktadır. Bu araştırmanın temel amacı, in vitro mikroçoğaltım tekniğini kullanarak bazı patates çeşitlerinin tuzluluk stres toleransına tepkisini ortaya koymaktır. Çalışmada Van Gogh ve Granola patates çeşitlerinin tek boğum kesimi eksplantları kullanılmış ve MS ortamına 0.0, 250, 500, 750, 1000, 1500 ve 2000 mg L^{-1} konsantrasyonlarında NaCl ilave edilmiştir. Bitkicikler 6 hafta süre ile uzun gün fotoperiyot (16 saat aydınlık, 8 saat karanlık) şartlarında tutulmuşlardır. Hasat edilen bitkiciklerde eksplant rejenerasyon oranı (%), bitkicik boyu (cm), boğum, yaprak ve kökçük sayıları, yaprak boyu ve eni (mm), sap kalınlığı (mm), kökçük uzunluğu (mm) ile bitki yaş ve kuru ağırlıkları (g) ölçümleri yapılmıştır. Elde edilen sonuçlardan en bitki rejenerasyın oranının 250 mg L^{-1} ve 750 mg L^{-1} NaCl içeren ortamlardan (%90.63) elde edilmiştir. Mikroçoğaltım yönünden önemli olan en uzun bitki boyunun kontrol (28.71 cm) ve 250 mg L^{-1} NaCl içeren ortamdan (27.99 cm) elde edildiği, Van Gogh çeşidinin (25.09 cm) Granola çeşidine (16.67 cm) göre daha uzun bitkicikler verdiği belirlenmiştir. En fazla kökçük sayıları 250 mg L^{-1} NaCl ortamından (73.38 adet) ve Van Gogh çeşidinden (76.18 adet) elde edilmiştir. En uzun kökçükler 2000 mg L^{-1} NaCl içeren ortamdan (11.36 cm) ve Van Gogh çeşidinden (9.45 cm) elde edilmiştir. Bütün bu verilere göre artan tuz konsantrasyonuna bağlı olarak bitkilerde vejetatif gelişmenin büyük oranda etkilendiği, ancak Van Gogh çeşidinin Granola çeşidine göre tuzlu ortamlara daha fazla tolerans gösterdiği belirlenmiştir.

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Anahtar Kelimeler
Patates
Solanum tuberosum L.
Doku kültürü
In vitro
Tuzluluğa tolerans

Determination of the Salinity Tolerance of Some Potato (*Solanum tuberosum* L.) Varieties Under in vitro Conditions

ABSTRACT

Potato (*Solanum tuberosum* L.) is one of the moderately sensitive field crops tos alt stress under in vitro and in vivo conditions. The salinity stress has significant and destructive effects on potato tuber production. Therefore, in vitro screening of potato genotypes for salt stress indicates a beneficial instrument as an alternative to field trials. The principal goal of this research was to reveal reaction in salinity stress tolerance of some potato cultivars using in vitro micropropagation technique. Single node explants of Van Gogh and Granola potato cultivars together with the 0.0, 250, 500, 750, 1000, 1500 and 2000 mg L^{-1} NaCl concentrations added MS media were used in the study. Explants were cultured under long-day photoperiod conditions (16 h light, 8 h dark) for 6 weeks. Observations such as days to the shoot initiation, explant regeneration rate, the length of shoots and radicles, the number of nodes and radicles, the length and width of leaves, stem tickness, fresh and dry weight of plantlets were recorded in the research. The highest explant

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regeneration rate was obtained from both 250 mg L^{-1} ve 750 mg L^{-1} NaCl including MS medium (90.63%). The results revealed that the longest plantlets was obtained on control media with no NaCl (28.71 cm), and Van Gogh gave longer plantlets (25.09 cm) compared to Granola (16.67 cm). The highest radicle number obtained on 250 mg L^{-1} NaCl including medium (73.38) and cultivar Van Gogh (76.18), however the longest radicles were determined on 2000 mg L^{-1} NaCl including medium (11.36 cm) and Van Gogh (9.45 cm). Findings presented here clearly indicated that although morphological characteristics of in vitro grown potato plantlets were affected by increasing NaCl concentrations, Van Gogh showed more tolerance to salinity environments.

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GİRİŞ

Solanaceae familyası içerisinde yer alan patates (*Solanum tuberosum* L.) dünyanın 150'den fazla ülkesinde üretilmekte olan, dünyada buğday, mısır ve çeltikten sonra en fazla üretilen, insan beslenmesinde önemli yeri olan bir endüstri bitkisidir. Klasik patates ıslahının değişik aşamalarında uygulanan biyoteknolojik metotlar ve doku kültürü yöntemleri ile hem hızlı çoğaltım sağlanabilmekte, hem de gen kaynaklarının muhafaza edilmesi ve hastalıklardan ari bitkiler elde edilmesi mümkün olabilmektedir (Kumlay, 2014; Mohapatra & Batra, 2017; Tazeb, 2017). Doku kültürü ortamlarında meristem, sürgün ucu, sap ince hücre tabakası, sap kesimi, kotiledon, hipokotil, yaprak diskleri, kökler ve boğumlardan hızlı çoğaltım yapılabilmektedir (Kumlay ve ark., 2014; de Morais ve ark., 2018; Kumar ve ark., 2019).

Dünyada sulanabilir tarım arazilerinin yaklaşık beşte birinin toprak tuzluluğundan olumsuz etkilendiği, artan bu problemden dolayı, gelecek 25 yıl içerisinde tarıma elverişli alanların %30 civarında azalacağı ve bu oranın 2050 yılına kadar %50'ye ulaşacağı (Wang ve ark., 2003), bu nedenle gıda üretiminde sürdürebilirliğin sağlanabilmesi için, tuzluluğa tolerant yeni bitki tür ve çeşitlerinin geliştirilmesinin çok önemli olduğu (Chinnusamy ve ark., 2005; Sobhanian ve ark., 2011) vurgulanmıştır. Son zamanlarda geliştirilen in vitro teknikler ile geleneksel yöntemlerle ıslah edilen patates melez hatlarının ya da genotiplerinin kısa sürede ve düşük maliyetle tuzluluğa dayanımlarının test edilebileceği metotlar geliştirilmiştir. (Kumlay ve ark., 2014; Kumlay ve ark., 2015; Campos ve ark., 2016; Kumlay, 2016).

Yapılan çalışmalarda in vitro şartlarda tuzluluk stresine maruz bırakılan farklı patates eksplantlarından geliştirilen bitkiciklerde büyümenin yavaşladığı ve mikroyumru veriminin düştüğü (Zhang & Donnelly, 1997), bitkiciklerin kök ve gövdelerinde K+ içeriğinin azaldığı, Na+ içeriğinin arttığı (Aghaei ve ark., 2009), çimlenme, kanopi genişlemesi ve yaşlanmanın teşvik edildiği (Jefferies, 1996), 50 mM üzerindeki konsantrasyonlarda çeşitlerin çoğunda çimlenmenin engellendiği (Zakaria ve ark., 2008), yumru gelişimi ve veriminin olumsuz yönde etkilendiği (Zhang ve ark., 2005) belirlenmiştir. Ayrıca, artan tuz stresine bağlı olarak, patates bitkiciklerinin fizyolojik özelliklerinde değişimler meydana geldiği, fotosentetik pigmentasyonun, çözünür şekerler, protein ve bitki yaş ağırlığının azaldığı, buna karşın prolin miktarının arttığı (Mosavi ve ark., 2018), bitkicik ve kök uzunluğu, dal, boğum, yaprak ve kök sayıları ile, yaprak boyu ve eni, bitkicik yaş ve kuru ağırlığı gibi karakterlerin olumsuz etkilendiği (Mansoor ve ark., 2010; Gowayed ve ark., 2017; Ibrahim ve ark., 2017; Ahmed ve ark., 2020; Rashid ve ark., 2020) ortaya konulmuştur. Başka bir çalışmada ise, bütün çeşitlerde 60 mM NaCl dozuna kadar bitki gelişiminin normal seyrinde devam ettiği, ancak Challisha çeşidinin 90 mM konsantrasyona kadar sürgün ve kök uzunluğu, boğum sayısı ile bitkicik yaş ve kuru ağırlıkları yönünden iyi performans gösterdiği (Biswas ve ark., 2017) rapor edilmiştir.

Vejetatif gelişim parametreleri dikkate alındığında patates genotiplerinin tuz stresine tolerans yönünden farklılıklar gösterdiği (Khierallah & Jawad, 2017; Jawad & Khierallah, 2018), dünyada yaygın olarak üretimi yapılan Desiree ve Kennebec patates çeşitlerinin orta derecede tuza dayanıklı çeşitler olduğu (Sasikala & Prasad, 1994), Cardinal çeşidinin bitkiciklerinde %4 tuz konsantrasyonu üzerinde hiçbir canlılık belirtisi görülmediği (Farhatullah & Farhatullah, 2002) kayda geçmiştir. Turan (2000) doktora tez çalışmasında tuzluluğa en toleranslı çeşidin Obelix olduğunu, Homayoun ve ark. (2011) Agria çeşidinin tuz stresine daha dayanıklı olduğunu, Sudhersan ve ark. (2012) çalışılan patates çeşitlerinden yedisinin tuza toleranslı, altısının tuza

duyarlı ve geriye kalan oniki çeşidin ise tuza yüksek duyarlı olduğunu, Munira ve ark. (2015) Sagita ve Felsina çeşitlerinin tuz stresine karşı daha dayanıklı olduğunu, buna karşın Shilbilati ve Lalpakri çeşitlerinin ise tuz stresine karşı hassas olduklarını, Murshed ve ark. (2015) ise Taurus ve Sultana çeşitlerinin tuz stresine toleranslı, Toscana, Soraya ve Kenita çeşitlerinin ise hassas olduklarını kayda geçmişlerdir.

Bu araştırmanın temel amacı, in vitro mikroçoğaltım tekniği kullanılarak bazı patates çeşitlerinin tuzluluk stres toleransına tepkisini ortaya koymaktır. Bu amaçla, yaygın olarak dikimi yapılan Granola ve Van Gogh patates (*Solanum tuberosum* L.) çeşitlerinden alınan tek boğum kesimi eksplantlarının mikroçoğaltımında değişik NaCl konsantrasyonlarının etkisi belirlenmeye çalışılmıştır.

MATERYAL ve METOD

Materyal

Yaygın olarak dikimi yapılan orta geçci, yüksek verimli ve hastalıklara dayanıklı olan Granola çeşidi ile bu özelliklerin yanında endüstriyel özellikleri uygun olan Van Gogh çeşidi araştırmanın materyalini oluşturmuştur.

Bitki Rejenerasyonu İçin Doku Kültürü Ortamlarının Hazırlanması ve Sterilizasyonu

Patates bitkisinin mikroçoğaltımında yaygın olarak kullanılan bitki büyüme düzenleyicileri (BBD) ve bunların kombinasyonları hücre bölünmesine ve genişlemesine, büyümeye, hücre uzamasına, doku gelişimine, kök oluşumu ve sürgün gelişimine neden olan maddelerdir (Kumlay & Eryiğit, 2011; Kumlay, 2014). Ortama BBD ilavesiyle sürgün gelişiminin hızlandığı (Sarder, 2006; Nuwagira ve ark., 2015), yanal sürgün gelişimi ve boğum sayısının arttığı ve iyi bir sürgün gelişimi için oksin + sitokinin hormonlarının kombine edilmesi gerektiği ortaya konulmuştur (Daneshmand ve ark., 2010; Xhulaj & Gixhari, 2018).

Patates bitkisinin doku kültürü ortamlarında tuzluluğa toleransının belirlenmesinde kullanılan MS besi ortamı için gerekli olan makro ve mikro besin elementleri ile organik bileşiklerin stok çözeltileri hazırlanmış ve bunlardan gerekli miktarlar alınarak ortam hazırlanmıştır. Sürgün gelişimi için % 5 sukroz ilave edilerek iyice çözünmesi sağlanmış, ortamın pH'ı 5.6-5.8'e ayarlanarak steril çift distile su ile hacmi 1 litreye tamamlanmış, pH ayarlandıktan sonra % 8 agar ilave edilerek, çözelti kaynama noktasına yakın bir değere kadar ısıtılıp agarın ortamda tortu ve kalıntı bırakmayacak şekilde çözünmesi sağlanmıştır. Cam balon içerisinde bulunan besi ortamları otoklavda 121˚C'da 15 dakika tutulduktan sonra hafifçe soğutulmuş, balon dışarıdan dokunulacak bir sıcaklığa düştüğünde (yaklaşık 40-50˚C) sıcaklığa karşı hassas olan BBD 0.22 µm miliporlardan geçirilerek ortama ilave edilmiştir. Hormon ilavesinden sonra cam balonda bulunan besi yerleri donmadan her bir kavanoza 20-25 ml besi ortamı konularak kavanozlarda katılaşmaları beklenmiştir.

Hazırlanan Eksplantların Besi Ortamlarına Aktarılması ve İnkübasyon

Bu araştırmada, Granola ve Van Gogh patates çeşitlerinden meristem kültürü yoluyla elde edilen bitkiciklerin tek boğum kesimleri ve içerisinde besi ortamı olan kavanozların her birinin içerisine 3 ya da 4 eksplant olacak şekilde aktarılmıştır. Ortamlar şu şekilde ayarlanmıştır: %5 sukroz içeren 0.0 mM mg L^{-1} NaCl (Kontrol), 250 mg L^{-1} NaCl; 500 mg L^{-1} NaCl; 750 mg L^{-1} NaCl; 1000 mg L^{-1} NaCl; 1500 mg L^{-1} NaCl, 2000 mg L^{-1} NaCl. Eksplantlar 16 saat aydınlık 8 saat karanlıkta (24 ± 2˚C), 2000 lüks ışık yoğunluğundaki fotoperiyot şartlarında 6 hafta süreyle kültüre alınmış ve gerekli gözlemler kaydedilmiştir.

Alınan gözlemler, ölçümler ve verilerin değerlendirilmesi

Altı haftalık kültür sürecinden sonra bitkiciklerde; eksplant rejenerasyon (%), sürgün uzunluğu (cm), boğum sayısı (adet), sap kalınlığı (mm), yaprak sayısı (adet), yaprak boyu ve eni (mm), kökçük sayısı (adet), kökçük uzunluğu (mm), bitkicik yaş ve kuru ağırlığı (mg) gözlemleri kaydedilmiştir.

Verilerin Değerlendirilmesi

Araştırmada Granola ve Van Gogh patates çeşitleri ve kontrol ile birlikte 6 farklı NaCl uygulaması içeren besi ortamları kullanılmıştır. Sonuçlar "Tesadüf Parsellerinde Faktöriyel Deneme Deseni"ne göre 4 tekerrür üzerinden SPSS istatistik programında değerlendirmeye tabi tutulmuş, ortalamaların karşılaştırılmasında Duncan çoklu karşılaştırma testi uygulanmıştır.

BULGULAR ve TARTIŞMA

Çalışma sonucunda değerlendirmeye tabi tutulan çeşit ve NaCl uygulamalarının doku kültürü ortamında elde edilen bitkiciklerin morfolojik ve diğer özelliklerine olan etkileri ayrı alt başlıklar altında değerlendirilmiştir.

Eksplant rejenerasyon oranları

Eksplant rejenerasyon oranları üzerine çeşitler arasındaki farkın istatistiki olarak önemsiz olduğu ($P > 0.05$), buna karşın NaCl konsantrasyonları ile çeşit × NaCl interaksiyonu çok önemli olduğu ($P < 0.01$)

tespit edilmiştir (Çizelge 1).

Çeşitler arasındaki fark önemli bulunmamış, ortamlardan en yüksek oranlar %90.63 ile 250 mg L^{-1} NaCl ve 750 mg L^{-1} NaCl dozundan, en düşük oran ise %50 ile 2000 mg L^{-1} NaCl dozundan elde edilmiş, interaksiyon incelendiğinde; en yüksek oran konrol ortamında Granola'dan ve 750 mg L^{-1} NaCl ortamda Van Gogh'dan (her ikisi de % 100) elde edilmiş, en düşük oran ise 2000 mg L^{-1} NaCl içeren ortamda Granola ve Van Gogh çeşitlerinden (% 50) alınmıştır (Çizelge 1).

Çizelge 1. Eksplant rejenerasyon oranları ortalamaları ve istatistiki analiz sonuçları
Table 1. Explant regeneration rates averages and statistical analysis results.

NaCl Kons. (mg L^{-1})	Çeşitler				NaCl Ort. (mg L^{-1})		Varyasyon Kaynakları	S.D	F
	Granola		Van Gogh						
0	100.0±0.00	A	75.00±0.00	D	**87.50 ± 4.72**	**A**	Tekerrür	3	0.61
250	93.75±6.25	AB	87.50±7.22	BC	**90.63 ± 4.57**	**A**	Çeşitler	1	1.50
500	75.00±0.00	D	75.00±0.00	D	**75.00 ± 0.00**	**B**	NaCl Kons.	6	24.78**
750	81.25±6.25	CD	100.0±0.00	A	**90.63 ± 4.57**	**A**	Çeşitler×NaCl	6	9.67**
1000	75.00±0.00	D	75.00±0.00	D	**75.00 ± 0.00**	**B**	Hata	36	
1500	56.25±6.25	E	88.50±7.22	BC	**71.88 ± 7.38**	**B**	Genel	55	
2000	50.00±0.00	E	50.00±0.00	E	**50.00 ± 0.00**	**C**	** : % 1 seviyesinde önemli		
Çeşit Ort.	**75.89 ± 3.52**		**78.57 ± 3.07**		**77.23 ± 2.32**				

Artan tuz konsantrasyonlarına bağlı olarak her iki çeşitte rejenerasyon oranlarının olumsuz etkilendiği görülmektedir. Sasikala ve Prasad (1993) % 0.8 oranı üzerindeki tuz dozlarının, rejenerasyon oranlarında % 1.4'e kadar azalmalara sebep olduğunu not etmişlerdir. Sonuçlar; Turan (2000), Kaya ve İpek (2003), Karakullukçu ve Adak (2008) tarafından yapılan çalışmalarla benzerlik arz etmektedir.

Bitkicik boyu (cm)

Çeşitler, NaCl uygulamaları ve çeşitler × NaCl uygulamaları arasındaki interaksiyonun bitkicik boyu üzerine etkisi çok önemli ($p<0.01$) olmuştur. Çeşitlerden en uzun bitkicikler Van Gogh'tan (25.09 cm), tuz uygulamalarında en uzun bitkicikler kontrol (28.71 cm) ve 250 mg L^{-1} NaCl ortamından (27.99 cm), en kısa bitkicikler ise 1500 mg L^{-1} NaCl ortamından (14.51 cm) elde edilmiştir. İnteraksiyonlara bakıldığında en uzun bitkicikler Van Gogh çeşidinden 250 mg L^{-1} NaCl ortamından (34.55 cm) ve Granola çeşidinden kontrol ortamında (30.05 cm), en kısa bitkicikler ise Granola çeşidinden (16.67 cm) 1500 mg L^{-1} NaCl ortamında (14.51 cm) gözlenmiştir (Çizelge 2).

Araştırma sonucunda elde edilen bulgular Kaya ve İpek (2003), Karakullukçu ve Adak (2008), Rahman ve ark. (2008), Zakaria ve ark. (2008), Aghaei ve ark. (2009), Sudhersan ve ark. (2012) ve Zaman ve ark. (2015) tarafından takdim edilen sonuçlarla uyum içerisindedir.

Çizelge 2. Bitkicik boyu (cm) ortalamaları ve istatistiki analiz sonuçları
Table 2. Plantlet length (cm) averages and statistical analysis results.

NaCl Kons. (mg L^{-1})	Çeşitler				NaCl Ort. (mg L^{-1})		Varyasyon Kaynakları	S. D	F
	Granola		Van Gogh						
0	30.05±0.41	B	27.28±0.99	C	**28.71 ± 0.71**	**A**	Tekerrür	3	0.93
250	21.43±0.61	EF	34.55±0.95	A	**27.99 ± 2.53**	**A**	Çeşitler	1	889.5**
500	17.60±0.45	H	26.73±0.24	C	**22.16 ± 1.74**	**B**	NaCl Kons.	6	226.1**
750	13.43±0.56	I	20.83±0.31	FG	**17.13 ± 1.43**	**D**	Çeşitler ×NaCl	6	49.53**
1000	14.35±0.19	I	23.60±0.18	D	**18.98 ± 1.75**	**C**	Hata	36	
1500	9.20±0.20	J	19.82±0.34	G	**14.51 ± 2.02**	**E**	Genel	55	
2000	10.65±0.07	J	22.75±0.66	DE	**16.50 ± 2.31**	**D**	** : % 1 seviyesinde önemli		
Çeşit Ort.	**16.67 ± 1.29**	**B**	**25.09 ± 0.92**	**A**	**20.88 ± 0.97**				

Bitkicik başına boğum sayısı (adet)

Çeşitler, NaCl uygulamaları ve çeşitler × NaCl uygulamaları arasındaki interaksiyonun bitkicik boyu başına boğum sayısı üzerine etkisi çok önemli ($p<0.01$) olarak belirlenmiştir. Çeşitlerden Van Gogh'un 5.43 adet boğum sayısı ile ön plana çıktığı, ortamlardan en yüksek boğum sayısının kontrol ve 250 mg L^{-1} NaCl ortamlarından (6.13 adet), en düşük boğum sayısının ise 2000 mg L^{-1} dozundan (3.63 adet) elde edildiği ortaya konulmuştur. İnteraksiyonlara bakıldığında; en fazla sayıda boğumların kontrol ortamında Van Gogh'tan elde edildiği (8.50 adet), en düşük boğum sayısı ise 500 mg L^{-1} ve 2000 mg L^{-1} dozlarını içeren MS ortamında kültüre alınmış Granola çeşidinden (3.25 adet) sağlandığı tespit edilmiştir (Çizelge 3).

Sonuçlardan, artan NaCl dozu ile boğum sayısının 500 mg L^{-1} konsantrasyonuna kadar belirgin bir şekilde düştüğü, ancak 500 mg L^{-1} uygulamasından itibaren boğum sayılarının birbirine yakın değerlerde

seyrettiği ya da çok az düşüş olduğu görülmektedir. Elde edilen bulgular; artan NaCl dozları ile tuz stresinin bitkiciklerde fizyolojik potansiyelin ve boğum sayılarının azaldığını vurgulayan Turan (2000), Aazami ve ark. (2010), Mansoor ve ark. (2010), Khenifi ve ark. (2011) ve Zaman ve ark. (2015)'nın sonuçlarıyla benzerlik göstermektedir.

Çizelge 3. Bitkicik başına boğum sayısı (adet) ortalamaları ve istatistiki analiz sonuçları.
Table 3. Averages of the number of nodes per plantlet (number) and statistical analysis results.

NaCl Kons. (mg L^{-1})	Çeşitler				NaCl Ort. (mg L^{-1})		Varyasyon Kaynakları	S.D	F
	Granola		Van Gogh						
0	6.00±0.00	B	6.25±0.25	B	**6.13 ± 0.13**	**A**	Tekerrür	3	0.80
250	4.00±0.41	E-G	8.50±0.29	A	**6.25 ± 0.88**	**A**	Çeşitler	1	96.27**
500	3.25±0.48	G	5.50±0.29	BC	**4.38 ± 0.50**	**B**	NaCl Kons.	6	31.58**
750	4.25±0.25	D-F	4.00±0.00	E-G	**4.13 ± 0.13**	**B**	Çeşitler×NaCl	6	18.96**
1000	4.25±0.25	D-F	5.00±0.41	CD	**4.63 ± 0.26**	**BC**	Hata	36	
1500	3.50±0.29	FG	4.75±0.25	C-E	**4.13 ± 0.30**	**BC**	Genel	55	
2000	3.25±0.25	G	4.00±0.00	E-G	**3.63 ± 0.18**	**C**	** : % 1 seviyesinde önemli		
Çeşit Ort.	**4.07 ± 0.20**	**B**	**5.43 ± 0.29**	**A**	**4.75 ± 0.20**				

Sap kalınlığı (mm)

Çeşitler, NaCl uygulamaları ve çeşitler × NaCl uygulamaları arasındaki interaksiyonun çok önemli ($p<0.01$) olarak belirlenmiştir. Çeşitlerden Van Gogh'tan (1.21 mm), ortamlardan 2000 mg L^{-1} tuz ortamından (1.23 mm) en yüksek sap kalınlığı sağlanmıştır. İnteraksiyonlara bakıldığında en yüksek değerin 1500 mg L^{-1} NaCl ortamında bekletilen Van Gogh'tan elde edildiği (1.38 mm), bunu 750 mg L^{-1} NaCl × Van Gogh interaksiyonunun takip ettiği (1.33 mm), en ince sap kalınlığı ise Granola çeşidinin 750 mg L^{-1} NaCl ortamında (0.43 mm) belirlendiği kayıt altına alınmıştır (Çizelge 4).

Artan tuz konsantrasyonuna bağlı olarak sap kalınlığının artması, stres şartlarında bitkilerin boyundan ziyade enine büyümeye meyilli olduğunu ve bunun sap kalınlığını artırdığını belirten Kumlay (2014), Kumlay ve ark. (2014) ve Kumlay (2016)'ın çalışmaları ile benzerlik arz etmektedir.

Çizelge 4. Sap kalınlığı (mm) ortalamaları ve istatistiki analiz sonuçları
Table 4. Stem thickness (mm) averages and statistical analysis results

NaCl Kons. (mg L^{-1})	Çeşitler				NaCl Ort. (mg L^{-1})		Varyasyon Kaynakları	S.D	F
	Granola		Van Gogh						
0	0.70±0.00	G	1.15±0.03	C	**0.93 ± 0.09**	**C**	Tekerrür	3	2.21
250	0.50±0.04	HI	1.15±0.03	C	**0.83 ± 0.13**	**D**	Çeşitler	1	1260**
500	0.45±0.03	I	1.13±0.03	CD	**0.78 ± 0.13**	**D**	NaCl Kons.	6	71,32**
750	0.58±0.03	H	1.33±0.03	AB	**0.95 ± 0.14**	**C**	Çeşitler×NaCl	6	30.68**
1000	0.98±0.03	E	1.28±0.03	B	**1.13 ± 0.06**	**B**	Hata	36	
1500	0.83±0.03	F	1.10±0.00	CD	**0.96 ± 0.05**	**C**	Genel	55	
2000	1.05±0.05	DE	1.38±0.03	A	**1.21 ± 0.07**	**A**	** : % 1 seviyesinde önemli		
Çeşit Ort.	**0.72 ± 0.04**	**B**	**1.21 ± 0.02**	**A**	**0.97 ± 0.04**				

Yaprak sayısı (adet)

Çeşitler ve NaCl uygulamalarının bitkicik başına yaprak sayısı üzerine etkisi çok önemli ($p<0.01$) olarak belirlenirken, çeşitler × NaCl interaksiyonunun ise önemli olmadığı ($p>0.05$) tespit edilmiştir (Çizelge 5). Van Gogh çeşidi birçok karakter yönünden ön planda olduğu halde, yaprak sayısı yönünden farklı bir durum meydana gelmiştir. Granola çeşidinde 12.89 adet yaprak sayısı elde edilirken, Van Gogh çeşidinde (10.54 adet) yaprak elde edilmiştir. Tuz konsantrasyonlarından en fazla yaprak sayısı kontrol ortamında (17.75 adet) belirlenmiş, en az yaprak sayısı ise 2000 mg L^{-1} NaCl içeren ortamda (8.50 adet) elde edilmiştir (Çizelge 5).

Elde edilen veriler; artan NaCl dozları ile oluşan tuz stresinin fizyolojik potansiyeli düşürdüğünü ve yaprak sayısının azaldığını belirten Turan (2000), Aazami ve ark. (2010), Mansoor ve ark. (2010) ve Khenifi ve ark. (2011)'nın çalışmalarıyla benzerlik göstermektedir.

Yaprak boyu (mm)

Çeşitler, NaCl uygulamaları ve çeşitler × NaCl uygulamaları arasındaki interaksiyon çok önemli ($p<0.01$) olarak belirlenmiştir. Çeşitlerden Van Gogh'tan 0.610 mm ile en uzun yaprak boyu elde edilirken, tuz uygulamalarında en uzun yaprak boyları 250 mg L^{-1} NaCl içeren ortamda (0.723 mm), en kısa yaprak boyları ise 2000 mg L^{-1} NaCl içeren ortamda (0.202 mm) gözlenmiştir. İnteraksyon incelendiğinde; en uzun yaprak boylarının Van Gogh'tan 250 mg L^{-1} NaCl içeren ortamda (0.990 mm)

belirlendiği, bunu yine aynı çeşidin 750 mg L^{-1} NaCl içeren ortamının (0.870 mm) takip ettiği ve en kısa yaprak boylarının da Granola'dan 500 mg L^{-1} NaCl içeren ortamda görüldüğü (0.100 mm) tespit edilmiştir (Çizelge 6).

Çizelge 5. Yaprak sayısı (adet) ortalamaları ve istatistiki analiz sonuçları
Table 5. Number of leaves (number) averages and statistical analysis results

NaCl Kons. (mg L^{-1})	Çeşitler				NaCl Ort. (mg L^{-1})		Varyasyon Kaynakları	S.D	F
	Granola		Van Gogh						
0	19.50±0.87		16.00±0.41		17.75 ± 0.80	A	Tekerrür	3	1.02
250	14.75±0.25		12.50±0.29		13.63 ± 0.46	B	Çeşitler	1	111.1**
500	12.75±0.63		11.50±0.50		12.13 ± 0.44	C	NaCl Kons.	6	116.5**
750	11.75±0.25		10.25±0.25		11.00 ± 0.33	D	Çeşitler×NaCl	6	1.95
1000	11.50±0.29		8.25±0.48		9.86 ± 0.67	E	Hata	36	
1500	10.25±0.25		8.00±0.00		9.13 ± 0.44	EF	Genel	55	
2000	9.75±0.25		7.25±0.25		8.50 ± 0.50	F	** : % 1 seviyesinde önemli		
Çeşit Ort.	12.89 ± 0.61	A	10.54 ± 0.56	B	11.71 ± 0.44				

Çizelge 6. Yaprak boyu (mm) ortalamaları ve istatistiki analiz sonuçları
Table 6. Leaf length (mm) averages and statistical analysis results

NaCl Kons. (mg L^{-1})	Çeşitler				NaCl Ort. (mg L^{-1})		Varyasyon Kaynakları	S.D	F
	Granola		Van Gogh						
0	0.483±0.01	E	0.783±0.01	C	0.633 ± 0.06	B	Tekerrür	3	0.30
250	0.455±0.02	EF	0.990±0.03	A	0.723 ± 0.10	A	Çeşitler	1	956.9**
500	0.100±0.00	K	0.715±0.01	A	0.408 ± 0.12	D	NaCl Kons.	6	203.96**
750	0.215±0.02	IJ	0.870±0.01	B	0.543 ± 0.12	C	Çeşitler×NaCl	6	142.42**
1000	0.420±0.01	FG	0.403±0.01	GH	0.411 ± 0.01	D	Hata	36	
1500	0.200±0.01	J	0.373±0.02	H	0.286 ± 0.03	E	Genel	55	
2000	0.265±0.01	I	0,138±0.04	A	0.201 ± 0.02	A	** : % 1 seviyesinde önemli		
Çeşit Ort.	0.305 ± 0.026	B	0.610± 0.051	A	0.458 ± 0.02				

Artan NaCl konsantrasyonlarına bağlı olarak, yaprak boyunun doğrusala yakın bir şekilde azalma gösterdiği ve bu sonuçların Cano ve ark. (1998) ve Turan (2000)'ın çalışmalarıyla paralellik arz ettiği görülmektedir.

Yaprak eni (mm)

Çeşitler, NaCl uygulamaları ve çeşitler × NaCl uygulamaları arasındaki interaksiyonun çok önemli ($p<0.01$) olarak belirlenmiştir. Çeşitler arasında Van Gogh'tan 0.457 mm ile en geniş yaprak eni elde edilirken, tuz uygulamalarında en geniş yapraklar 250 mg L^{-1} NaCl ortamında (0.440 mm), en dar yapraklar ise 1500 mg L^{-1} NaCl ortamında (0.256 mm) gözlenmiştir. İnteraksyon incelendiğinde; en geniş yaprakların Van Gogh'tan 250 mg L^{-1} NaCl ortamında (0.588 mm) belirlendiği, bunu yine aynı çeşidin kontrol ortamının (0.411 mm) takip ettiği ve en dar yaprakların ise Granola'nın çeşidinin 500 mg L^{-1} NaCl ortamında görüldüğü (0.100 mm) tespit edilmiştir (Çizelge 7).

Çizelge 7. Yaprak eni (mm) ortalamaları ve istatistiki analiz sonuçları
Table 7. Leaf width (mm) averages and statistical analysis results

NaCl Kons. (mg L^{-1})	Çeşitler				NaCl Ort. (mg L^{-1})		Varyasyon Kaynakları	S.D	F
	Granola		Van Gogh						
0	0.318±0.01	E	0.505±0.01	B	0.411 ± 0.04	B	Tekerrür	3	0.32
250	0.293±0.01	EF	0.588±0.02	A	0.440 ± 0.06	A	Çeşitler	1	1661**
500	0.100±0.00	I	0.438±0.02	C	0.268 ± 0.06	EF	NaCl Kons.	6	81.5**
750	0.150±0.01	H	0.510±0.01	B	0.330 ± 0.07	D	Çeşitler×NaCl	6	55.1**
1000	0.269±0.01	F	0.296±0.02	EF	0.283 ± 0.01	E	Hata	36	
1500	0.163±0.01	H	0.350±0.01	D	0.256 ± 0.04	F	Genel	55	
2000	0.200±0.00	G	0.513±0.01	B	0.356 ± 0.06	C	** : % 1 seviyesinde önemli		
Çeşit Ort.	0.213 ± 0.014	B	0.457 ± 0.019	A	0.335 ± 0.021				

Çeşit ve NaCl uygulaması ortalamalarının incelenmesinden, yaprak eninin belli bir NaCl konsantrasyonuna kadar azaldığı, sonra tekrar arttığı, yani doğrusal olmayan zikzaklar şeklinde artış ve azalışların olduğu görülmektedir. Bu sonuçların Cano ve ark. (1998) ve Turan (2000)'ın

çalışmalarıyla benzerliklerinin olduğu tespit edilmiştir.

Kökçük sayısı (adet)

Çeşitler, NaCl uygulamaları ve çeşitler × NaCl uygulamaları arasındaki interaksiyonun kökçük sayısı üzerine etkisi çok önemli ($p<0.01$) olarak belirlenmiştir (Çizelge 8).

Çeşitler arasında Van Gogh'tan 76.18 adet kökçük sayısı ile en fazla sayıda kökçükler elde edilirken, tuz uygulamalarında en fazla kökçükler 250 mg L^{-1} NaCl dozundan (73.88 adet) elde edilmiş, bunu 1000 mg L^{-1} NaCl (56.63 adet) ve 750 mg L^{-1} NaCl (54.63 adet) dozları takip etmiş, minimum sayıda kökçükler ise 2000 mg L^{-1} NaCl ortamında (0.256 mm) gözlenmiştir. İnteraksyon incelendiğinde; en fazla kökçüklerin Van Gogh'tan 250 mg L^{-1} NaCl içeren ortamdan (98.25 adet) sağlandığı, bunu yine aynı çeşidin 500 mg L^{-1} NaCl ortamının (81.75 adet) takip ettiği ve en az sayıda kökçüklerin ise Granola'nın 500 mg L^{-1} NaCl ortamında görüldüğü (16.25 adet) tespit edilmiştir (Çizelge 8).

Çizelge 8. Kökçük sayısı (adet) ortalamaları ve istatistiki analiz sonuçları
Table 8. Number of radicle (pieces) averages and statistical analysis results

NaCl Kons. (mg L^{-1})	Çeşitler				NaCl Ort. (mg L^{-1})		Varyasyon Kaynakları	S.D	F
	Granola		Van Gogh						
0	31.00±0.91	H	74.75±1.25	CD	**52.88 ± 8.30**	**C**	Tekerrür	3	0.11
250	48.50±0.65	F	98.25±1.49	A	**73.38 ± 9.43**	**A**	Çeşitler	1	3920**
500	16.25±0.48	I	81.75±1.65	B	**49.00 ± 12.40**	**D**	NaCl Kons.	6	106.8**
750	32.50±1.56	H	76.75±2.32	C	**54.63 ± 8.46**	**BC**	Çeşitler×NaCl	6	49.19**
1000	39.50±0.66	G	73.75±0.86	CD	**56.63 ± 6.50**	**B**	Hata	36	
1500	33.50±0.65	H	72.75±1.25	D	**53.13 ± 7.45**	**C**	Genel	55	
2000	30.50±0.65	H	55.25±0.85	E	**42.88 ± 4.70**	**E**	** : % 1 seviyesinde önemli		
Çeşit Ort.	**33.11 ± 1.77**	**B**	**76.18 ± 2.32**	**A**	**54.64 ± 3.24**				

Artan tuz konsantrasyonlarına bağlı olarak kökçük sayısının azaldığını rapor eden Kaya ve İpek (2003) ile Karakullukçu & Adak (2008)'ın çalışmalarının aksine, bu çalışmada elde edilen sonuçlar kökçük sayısında azalış olmasına rağmen, düşüşlerin

çok fazla olmadığını göstermektedir. Patates bitkisi ile yapılan diğer bazı çalışmalarda ise artan NaCl konsantrasyonu ile bitkilerde tuza toleransın gittikçe arttığı ya da belli bir seviyeye kadar tuz stresini tolere edebildiği ve bu toleransın neticesinde de kökçük sayılarında da artış olduğu gösterilmiştir (Zaman ve ark., 2015).

Kökçük uzunluğu (cm)

Çeşitler, NaCl uygulamaları ve çeşitler × NaCl uygulamaları arasındaki interaksiyonun kökçük uzunluğu üzerine etkisi çok önemli ($p<0.01$) olarak belirlenmiştir. Çeşitler arasında Van Gogh çeşidinden 9.45 cm kökçük uzunluğu ile en uzun kökçükler elde edilirken, tuz uygulamalarında ilginç olarak en uzun kökçükler en yüksek tuz konsantrasyonu olan 2000 mg L^{-1} NaCl içeren ortamdan (11.36 cm) elde edilmiş, bunu 500 mg L^{-1} NaCl (8.23 cm) içeren ortam takip etmiş, diğer NaCl içeren ortamların birbirine yakın kökçük uzunluğu değerleri verdiği gözlenmiş, minimum sayıda kökçükler ise 2000 mg L^{-1} NaCl içeren ortamda (0.256 mm) elde edilmiştir. İnteraksyon incelendiğinde; en uzun kökçüklerin Van Gogh'un 2000 mg L^{-1} NaCl (13.17 cm) ve aynı çeşidin 500 mg L^{-1} NaCl (12.70 cm) dozlarında belirlenmiş, bunu Granola'nın 500 mg L^{-1} NaCl dozu (9.54 cm) takip etmiş, en kısa kökçüklerin ise Granola'nın 2000 mg L^{-1} NaCl ortamında görüldüğü (6.29 adet) kayda geçmiştir (Çizelge 9).

Çizelge 9. Kökçük uzunluğu (cm) ortalamaları ve istatistiki analiz sonuçları
Table 9. Radicle length (cm) averages and statistical analysis results

NaCl Kons. (mg L^{-1})	Çeşitler				NaCl Ort. (mg L^{-1})		Varyasyon Kaynakları	S.D	F
	Granola		Van Gogh						
0	5.72±0.40	G	8.21±0.30	CD	**6.97 ± 0.52**	**CD**	Tekerrür	3	0.49
250	7.02±0.61	EF	7.66±0.13	DE	**7.34 ± 0.31**	**C**	Çeşitler	1	274.8**
500	3.77±0.12	H	12.70±0.23	A	**8.23 ± 1.69**	**B**	NaCl Kons.	6	41.82**
750	5.44±0.32	G	7.46±0.65	DE	**6.45 ± 0.51**	**D**	Çeşitler×NaCl	6	28.56**
1000	6.28±0.36	FG	8.21±0.22	CD	**7.25 ± 0.41**	**C**	Hata	36	
1500	6.26±0.33	FG	8.73±0.15	BC	**7.50 ± 0.50**	**C**	Genel	55	
2000	9.54±0.26	B	13.17±0.21	A	**11.36 ± 0.70**	**A**	** : % 1 seviyesinde önemli		
Çeşit Ort.	**6.29 ±0.33**	**B**	**9.45 ± 0.44**	**A**	**7.87 ± 0.35**				

Elde edilen bulgular, tuz dozu artışı ile kökçük uzunluğunun azaldığını bildiren Kaya ve İpek (2003), Karakullukçu ve Adak (2008)'ın çalışmalarıyla farklılıklar, Rahman ve ark. (2008) ve Zaman ve ark. (2015), tuza en çok toleranslı olan Kroda çeşidinin en uzun kökçükleri meydana getirdiğini, Rahman ve ark. (2018)' nın çalışmalarıyla benzerlikler göstermektedir.

Bitki yaş ağırlığı (g)

Çeşitler, NaCl uygulamaları ve çeşitler × NaCl uygulamaları arasındaki interaksiyonun bitki yaş ağırlığı üzerine etkisi çok önemli ($p<0.01$) olarak belirlenmiştir. Çeşitler arasında Van Gogh'tan 2.826 g ile en fazla bitki yaş ağırlığı tartılırken, tuz uygulamalarında en fazla yaş ağırlık en düşük tuz konsantrasyonu olan 250 mg L^{-1} NaCl dozundan (2.332 g) elde edilmiş, minimum yaş ağırlıktaki bitkicikler ise 750 mg L^{-1} NaCl dozunda (1.451 g) gözlenmiştir. İnteraksyon incelendiğinde; en fazla yaş ağırlık Van Gogh'tan 250 mg L^{-1} NaCl dozunda elde edilmiş, en az bitki yaş ağırlığı ise Granola'nın 500 mg L^{-1} NaCl dozunda (0.343 g) gözlenmiştir (Çizelge 10).

Çizelge 10. Bitki yaş ağırlığı (g) ortalamaları ve istatistiki analiz sonuçları
Table 10. Plant wet weight (g) averages and statistical analysis results

NaCl Kons. (mg L^{-1})	Çeşitler				NaCl Ort. (mg L^{-1})	
	Granola		Van Gogh			
0	0.665±0.070	F	2.703±0.053	C	1.684 ± 0.387	C
250	0.793±0.038	F	3.873±0.098	A	2.332 ± 0.584	A
500	0.343±0.011	G	3.015±0.047	B	1.679 ± 0.506	C
750	0.645±0.044	F	2.258±0.143	D	1.451 ± 0.313	D
1000	0.810±0.013	F	2.663±0.030	C	1.736 ± 0.350	C
1500	0.820±0.012	F	2.728±0.102	C	1.774 ± 0.364	C
2000	1.315±0.050	E	2.548±0.071	C	1.931 ± 0.236	B
Çeşit Ort.	**0.770 ± 0.054**	**B**	**2.826± 0.096**	**A**	**1.798 ± 0.149**	

Varyasyon Kaynakları	S.D	F
Tekerrür	3	2.01
Çeşitler	1	3426**
NaCl Kons.	6	35.14**
Çeşitler×NaCl	6	45.67**
Hata	36	
Genel	55	

** : % 1 seviyesinde önemli

Çizelge 10'un incelenmesinden de görülebileceği gibi, artan NaCl konsantrasyonlarına bağlı olarak, çalışılan her iki çeşitte de bitki yaş ağırlığının kontrole göre arttığı görülmektedir. Elde edilen bulgular daha önce kayda geçen araştırma sonuçlarıyla benzerlikler göstermektedir. Sasikala ve Prasad (1993) %0.2'lik düşük tuz dozunun bitki yaş ağırlığını önemli oranda artırdığını, Ochatt ve ark. 1998) en yüksek bitkicik yaş ve kuru ağırlığının 90 mM NaCl dozundan elde edildiğini, Rahman ve ark. (2008) Shepody ve Atlanta çeşitlerinin ise sürgün yaş ağırlığı yönünden daha olumlu sonuçlar verdiğini, Zaman ve ark. (2015) ise Kroda çeşidinin en fazla bitki yaş ağırlığı verdiğini kayıt altına almışlardır.

Bitki kuru ağırlığı (g)

Çeşitler, NaCl uygulamaları ve çeşitler × NaCl uygulamaları arasındaki interaksiyonun bitki kuru ağırlığı üzerine etkisi çok önemli ($p<0.01$) olarak belirlenmiştir. Çeşitler arasında Van Gogh'tan en fazla bitki kuru ağırlığı tartılırken (0.181 g), tuz uygulamalarında en fazla bitki kuru ağırlığı 1000 mg L^{-1} NaCl ortamından (0.144 g) elde edilmiş, bunu 250 mg L^{-1} NaCl ortamı takip etmiş (0.138 g), minimum kuru ağırlıktaki bitkicikler ise kontrol ortamından (0.096 g) elde edilmiştir. İnteraksyon incelendiğinde; en fazla bitki kuru ağırlıkları Van Gogh'tan 1000 mg L^{-1} NaCl dozunda (0.240 g) ve aynı çeşidin 250 mg L^{-1} NaCl dozunda (0.200 g) elde edilmiş, en az bitki kuru ağırlığı ise Granola'nın 1000 mg L^{-1} NaCl dozu (0.048 g) ile kontrol ortamında (0.045 g) not edilmiştir (Çizelge 11).

Çizelge 11'in incelenmesinden de görülebileceği gibi, artan NaCl konsantrasyonlarına bağlı olarak, çalışılan her iki çeşitte de bitki kuru ağırlığının kontrole göre arttığı görülmektedir. Elde edilen bu bulgular Karakullukçu ve Adak (2008), Rahman ve ark. (2008), Aghaei ve ark. (2009), Sudhersan ve ark. (2012) ve Zaman ve ark. (2015) tarafından kayda geçen araştırma sonuçlarıyla benzerlikler göstermektedir.

SONUÇ ve ÖNERİLER

Elde edilen sonuçlardan Van Gogh çeşidinin %78.57 rejenerasyon oranı ile Granola çeşidine göre (%75.89) tuzlu ortamlara daha toleranslı olduğu, mikroçoğaltım yönünden önemli olan en uzun bitki boyunun (25.09 cm), en fazla kök sayısı (76.18 adet) ve kök uzunluğunun (9.45 cm) Van Gogh çeşidinden elde edildiği görülmektedir.

NaCl konsantrasyonlarına bakıldığında; en yüksek rejenerasyon oranının 250 mg L^{-1} ve 750 mg L^{-1} NaCl dozlarında belirlendiği (%90.63), en uzun bitkiciklerin kontrol (28.71 cm) ve 250 mg L^{-1} NaCl dozlarında görüldüğü (27.99 cm), en fazla kök sayısının 250 mg L^{-1} NaCl ortamından (73.38 adet) ve en uzun köklerin 2000 mg L^{-1} NaCl içeren ortamdan sağlandığı (11.36 cm) ortaya konulmuştur.

İnteraksiyonlar incelendiğinde; en yüksek eksplant rejenerasyon oranı Granola'dan konrol ortamında ve Van Gogh'dan 750 mg L^{-1}NaCl ortamında (her ikisi de

% 100) elde edilmiş, en uzun bitkicikler Van Gogh'dan 250 mg L^{-1} NaCl ortamında (34.55 cm) gözlenmiş, en fazla kökleri Van Gogh'tan 250 mg L^{-1} NaCl içeren ortamdan (98.25 adet) sağlanmış, en uzun kökler Van Gogh'un 2000 mg L^{-1} NaCl dozunda (13.17 cm) belirlenmiştir.

Çizelge 11. Bitki kuru ağırlığı (g) ortalamaları ve istatistiki analiz sonuçları
Table 11. Plant dry weight (g) averages and statistical analysis results

NaCl Kons. (mg L^{-1})	Çeşitler				NaCl Ort. (mg L^{-1})	
	Granola		Van Gogh			
0	0.045±0.003	I	0.148±0.003	E	**0.096 ± 0.019**	**E**
250	0.075±0.003	F	0.200±0.008	B	**0.138 ± 0.024**	**A**
500	0.028±0.003	J	0.188±0.005	C	**0.106 ± 0.030**	**CD**
750	0.060±0.004	GH	0.165±0.003	D	**0.113 ± 0.020**	**C**
1000	0.048±0.003	I	0.240±0.003	A	**0.144 ± 0.036**	**A**
1500	0.055±0.003	HI	0.150±0.004	E	**0.103 ± 0.018**	**DE**
2000	0.068±0.003	FG	0.178±0.003	C	**0.123 ± 0.021**	**B**
Çeşit Ort.	**0.053± 0.003**	**B**	**0.181± 0.006**	**A**	**0.118 ± 0.009**	

Varyasyon Kaynakları	S.D	F
Tekerrür	3	1.13
Çeşitler	1	3703**
NaCl Kons.	6	41.80**
Çeşitler×NaCl	6	42.44**
Hata	36	
Genel	55	

** : % 1 seviyesinde önemli

Yaprakların fotosentezde en önemli organlardan biri olmasından ve Granola'nın Van Gogh'a göre tuzlu ortamlardan daha az oranda etkilenmesinden dolayı, daha sonraki mikroyumru çalışmalarında Granola'nın kullanılmasının uygun olacağı düşünülmektedir. Kök sayısı ve uzunluğu, mikroçoğaltımdan elde edilen bitkiciklerin önce saksılarda daha sonra da serada toprağa alıştırılmasında önemli olduğundan, kök sayısı ve uzunluğunun artırılması ya da tuz ortamında köklerin en az etkilenmesi arzu edilen bir durumdur. Kök sayısında olduğu gibi kök uzunluğunda da tuz stresine bağlı olarak bitkiciklerde bir tolerasyon mekanizmasının geliştiği, buna bağlı olarak kök uzunluğunun arttığı, ilginç bir şekilde en uzun köklerin en yüksek tuz dozu olan 2000 mg L^{-1} NaCl ortamından elde edildiği, diğer konsantrasyonlardaki kök uzunluklarının birbirine yakın değerler arasında seyrettiği kayıt altına alınmıştır.

Bütün bu değerlendirmelerden, artan tuz konsantrasyonuna bağlı olarak bitkilerde vejetatif gelişmenin büyük oranda etkilendiği, Van Gogh çeşidinin Granola çeşidine göre tuzlu ortamlara daha fazla tolerans göstermesine rağmen, yine de in vitro geliştirilen bitkiciklerde incelenen bazı özelliklerde düşüşler görüldüğü belirlenmiştir. Daha sonra yapılacak in vitro çalışmalarda ortama ilave edilecek asetil salisilik asit, askorbik asit ve jasmonik asit gibi bazı bitki büyüme düzenleyicileri ile patates bitkisinin tuzluluk stresine dayanıklılığının ve rejenerasyon yeteneğinin artırılabileceği düşünülmektedir. Ayrıca, elde edilen bu sonuçların daha sonraki aşamalarda sera ve tarlada test edilmesinin de yeni ıslah edilen patates hatlarında seleksiyon etkinliğini artırabileceği, bu sayede yeni projelerin üretilmesine temel oluşturabileceği ve devamında pek çok bilimsel çalışmanın yapılmasına imkân sağlayabileceği ümit edilmektedir.

TEŞEKKÜR

Bu çalışma Iğdır Üniversitesi Bilimsel Araştırma Projeleri (BAP) birimi -2013-FBE-L13 kodu ile desteklenen Tuba SÜRMEN'in **"Bazı Patates (*Solanum tuberosum* L.) Çeşitlerinin in vitro Şartlarda Tuzluluğa Toleransının Belirlenmesi"** adlı Yüksek Lisans Tez Projesi olup, tezin gerçekleştirilmesinde maddi olarak destekleyen Iğdır Üniversitesi BAP birimine katkılarından dolayı teşekkür ederiz.

Araştırmacıların Katkı Oranı Beyan Özeti

Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan eder.

Çıkar Çatışması Beyanı

Tüm yazarlar makalelerinde, sonuçları veya yorumları etkileyebilecek herhangi bir maddi veya diğer asli çıkar çatışması olmadığını beyan ederler.

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The Investigation of Effects of Pre-Harvest Rainfall on Lint Color Grade and Seed Germination Rate in Cotton (*Gossypium hirsutum* L.)

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ABSTRACT
Unexpected rainfall in harvest season has a detrimental effect on cotton lint color grade and germination rate and cause economic losses for farmers, seed producers and manufacturers. This study was conducted to elucidate the effects of different rainfall amounts on cotton technological characteristics and to evaluate the response of different cultivars in the harvest season of 2014 and 2015. Cotton bolls were exposed to 0 (control), 20, 35, 65, 95 and 125 mm of precipitation. The effects of rainfall were determined in GSN-24, Claudia, Gloria, ST-373, Flash, Carisma and ST-468 cotton cultivars (*Gossypium hirsutum* L.). The responses of cultivars to different cumulative rainfall were significant for lint color grade and seed germination rate. The linear curve in the decreasing direction according to the highest R^2 value was evaluated for germination rate (%), reflectance (Rd), trash count and trash area (%) whereas the polynomial curve was more likely for yellowness (+b). The effects of increased rainfall amounts on all observed characters were unfavorable. The different responses of cultivars indicated that the cultivars with the least loss for lint color grade and germination rate in seed production could be recommended in terms of being highly profitable.

Field Crops

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Pamukta (*Gossypium hirsutum* L.) Hasat Öncesi Yağışların Çimlenme ve Elyaf Renk Değerleri Üzerine Etkisi

ÖZET
Hasat döneminde gerçekleşen yağışlar pamukta elyaf renk derecesi ve tohum çimlenme oranını olumsuz etkilemekte ve çiftçi, tohumluk üreticisi ve tekstilci için ekonomik kayıplara neden olmaktadır. Bu çalışma hasat öncesi farklı miktarlarda oluşan yağışların pamukta lif teknolojik özelliklerine olan etkisini ve farklı çeşitlerin yağışlara olan tepkilerinin belirlenmesi amacıyla 2014 ve 2015 yıllarında yürütülmüştür. Kozalar kontrol (0 mm) ile birlikte 20, 35, 65, 95 ve 125 mm yağışa maruz bırakılmıştır. Yağışın GSN-24, Claudia, Gloria, ST-373, Flash, Carisma ve ST-468 çeşitlerine etkisi belirlenmiştir. Çeşitlerin elyaf renk değeri ve çimlenme oranı bakımından toplam yağışa olan tepkileri önemli bulunmuştur. En yüksek belirleme katsayısına (R^2) göre çimlenme oranı (%), yansıma (Rd), yabancı madde miktarı ve alanı (%) yönünden azalan düzeyde doğrusal regresyon eğrisi, buna karşın sarılık (+b) için polinomiyal eğri bulunmuştur. Çalışmanın sonucunda yüksek kazanç için lif kalite değerleri yağış miktarından olumsuz yönde daha az etkilenen ve çimlenme oranı kaybı en az olan çeşitlerin önerilebileceği sonucuna varılmıştır.

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Çimlenme Oranı
Elyaf Renk Değeri
Regresyon
Sezon Sonu Yağışlar
Yabancı Madde

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INTRODUCTION

Turkey is one of the world's important cotton producers, and cotton has a crucial role in Turkey's textile and clothing industry. Turkey ranked 6th in the production of lint cotton with a total amount of 815.000 tons in the 2019-2020 seasons in the world. One of the basic classification criteria is lint colour grade according to the Universal Cotton Standards. The color grade with fiber length, strength and fineness are critical components of the set of characteristics used to assess the overall quality of a sample of cotton, and thereby determine its value. Cotton lint color can be affected by environmental factors such as rainfall, freezes, insects, fungi, the condition of cotton storage, moisture and temperature (Xu et al., 1997; Duckett et al., 1999) and agronomical characters such as boll maturity (Heimoana and Wilson, 2017). The amount of precipitation plays an important role in cotton yield, ginning out-turn and fiber quality. Seasonal precipitation and temperature affect planting operations in early spring and also affect harvest in autumn. Adverse environmental factors on cotton fiber reduced the processing efficiency of cotton, the ability of fibers to absorb and hold dyes and finishes, and market value (Aspland and Williams, 1999). The undamaged lint of mature bolls is white and clean because of the highly reflective character of cellulose and waxy cuticle (Heimoana and Wilson, 2017). The monthly mean rainfall and the average length of wet spells in harvest season negatively affected cotton-fiber quality (Luo et al., 2016). Less than 25.4 mm of precipitation on newly opened bolls only delays the harvest without any significant damage to yield and quality. On the other hand, precipitation over 50.8 mm causes significant damage to yield and quality, decreases the color level and increases the amount of foreign matter (Hake et. al., 1992; Anonymous, 2001). Each 15 mm of precipitation causes one unit of color reduction while exposure to as little as 30 mm of rain could therefore result in significant price discounts (Grade 41 – Strict Low Middling) and exposure to about 60 mm of rain could result in severely discolored lint (Grade 61 – Strict Good Ordinary) (Anonymous, 2007). Parvin et al. (2005) found that cotton lint yield declined by 2.35 kg of lint per day and 4.09 kg of lint per centimetre of accumulated rainfall.

The short fiber content of cotton increased in fields that are harvested late due to adverse weather conditions and, length uniformity and fiber length in the gin and textile mill reduced (Hake, 1992). Fiber quality parameters, upper half mean length, uniformity index, strength and color grade components (includes Rd, +b and, to some extent, HVI trash), as measured by HVI, are reportedly most affected by delayed harvest with considerable rainfall accumulations (Bednarz et al., 2002; Columbus et al., 1990; Buxton et al., 1973; Shurley et al., 2004; Williford 1992). The reflectance and yellowness values of the fiber negatively affect if the opened bolls are exposed to long-term or heavy rain (Silvertooth 2001). Some varieties are more affected by bad weather conditions (rain, storm, etc.), while some varieties are less affected.

We arranged an experiment to evaluate the late-season precipitation on lint color grade parameters, germination, trash count and trash area in 2014 and 2015. The effects of precipitation on observed characteristics depending on the cultivars were calculated through regression analysis.

MATERIAL and METHODS

Field trials were conducted at Nazilli Cotton Research Institute (located between 37° 86' N, 28° 32' E) during the 2014 and 2015 cotton growing seasons. Cotton commercial cultivars GSN-24, Claudia, Gloria, ST-373, Flash, Carisma and ST-468 (*Gossypium hirsutum* L.) were evaluated for determining the effect of cumulative rainfall in harvest season on germination rate, yellowness, reflectance, trash count and trash area.

Soil Descriptions and Climatic Conditions

According to the soil analysis, experiment soils were sandy-loamy, light alkaline (pH: 7.54), low in salt content, total nitrogen and phosphorus and high in potassium. The climate of Nazilli-Aydin is a Mediterranean climate and monthly precipitation (mm) and average temperature of experimental years and long-term were exhibited in Figure 1. Precipitation of September, October and November when cotton was harvested varied between 5.2 mm and 119 mm and precipitation of November in 2014 and October and November in 2015 were above the long-term average. It was clearly seen that cotton bolls during the opening period were exposed to 151.8 – 183.2 mm of precipitation in the September-November period in 2014 and 2015, respectively. The total precipitation of the long term period was 138.1 mm.

Experimental Design and Management Practices

The experiments were planted on the dates 13 May 2014 and 29 April 2015 according to a split-plot design with four replications. Six different precipitation doses including control (0), 20 mm, 35 mm, 65mm, 95 mm and 125 mm were arranged as main plots. Cotton bolls were harvested when each precipitation amount targeted in the study was reached. During the harvest season, natural precipitation occurred 10 times in 2014 and 7 times in 2015. In addition, the experimental area was irrigated with sprinklers two times for ensuring the

total precipitation amount, and boll harvesting was carried out at 6 different times. The seven cotton cultivars, GSN-24, Claudia, Gloria, ST-373, Flash, Carisma and ST-468 were designed as sub-plots. Trial plots consisted of four rows of 12 m in length each with an inter-row spacing of 70 cm and inter-spacing of 20 cm. Hence, the parcel area was 33.6 m^2 for each treatment. All trials were fertilized with 400 kg ha^{-1} of composed fertilizer (20.20.0) before planting and 80 kg ha^{-1} nitrogen before first irrigation according to the long-term yield potential of the area.

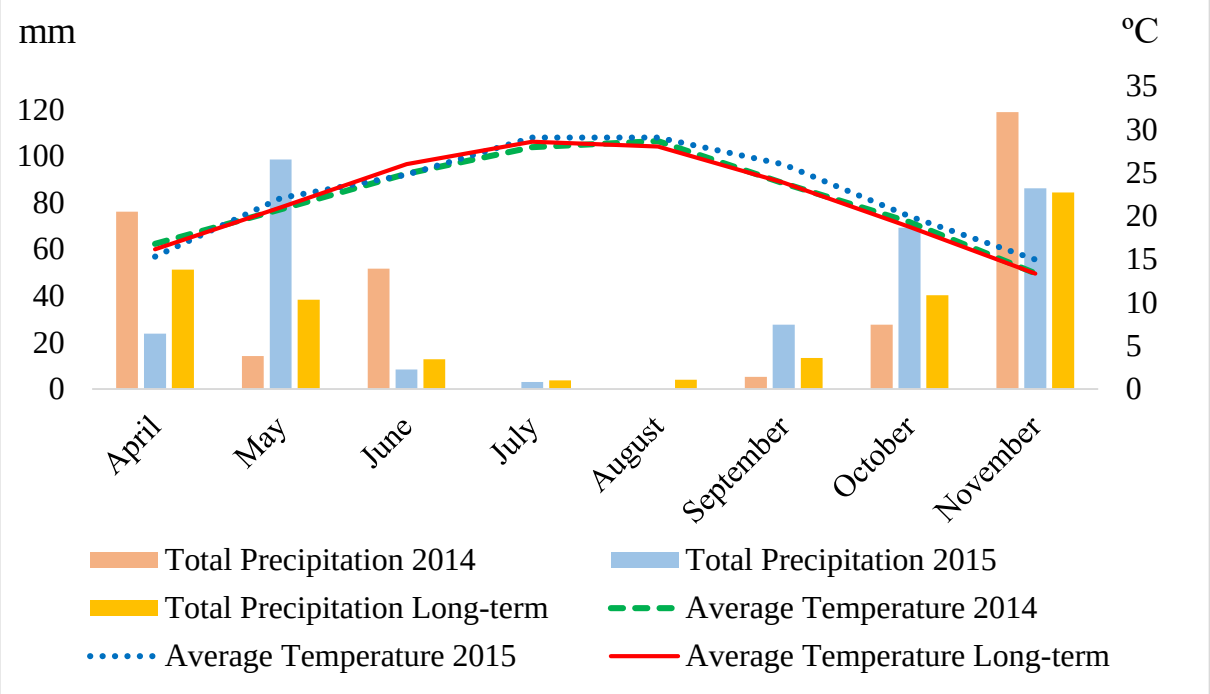


Figure 1. Temperature and precipitation values of experimental and long-term years.
Şekil 1. Deneme yılları ve uzun dönem yağış ve sıcaklık değerleri

Data Collection and Fiber Quality Analyses

At the beginning of the study, 50 bolls from the first position of the fruiting branches were picked up from 0 mm precipitation plots (not exposed to rain) and evaluated as control. The rain gauges were placed in 4 corners of the trial area, for following the precipitation during the pre-harvest period. Similarly, 50 boll samples were sampled from all target parcels when the total amount of rainfall reaches 20 mm, 35 mm, 65 mm, 95 mm and 125 mm. The samples were left to dry according to the analysis of moisture (7%) for ginning (Mayfield, 1989). The samples taken from the first boll position of each plant as 50 bolls were ginned in a roller gin machine. The reflectance (Rd), yellowness (+b), trash area (%) and trash count were determined by Uster HVI 1000 at the fiber analysis laboratory, Nazilli Cotton Research Institute. A standard germination test was used for germination rate (%). Fifty seeds from two lots for each parcel were placed in a germination cabinet at 30 °C±2°C containing moist towels. After 4 days, the wet towel was opened and healthy growing seedlings were counted and recorded. The counted seedlings were removed from the environment and the paper towels were wrapped again and left in the germination cabinet. After 8 days, the same transactions were made and the total germination was determined (Anonymous, 2005).

Statistical Analysis

The data were analyzed using JMP 14® statistical package program (SAS Institue Inc., 2018) in the experimental split-plot design. Moreover, the regression analysis due to the higher R^2 value was carried out by considering germination rate, reflectance, yellowness, trash area and trash count as dependent variables and precipitation as the independent variable in the Microsoft Excel program.

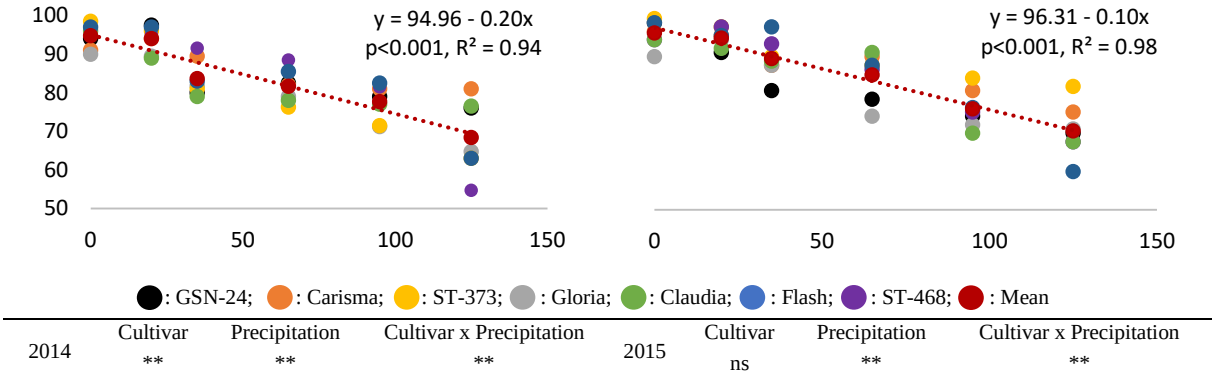
RESULTS and DISCUSSION

The significances of mean squares from variance analysis for germination rate, yellowness, reflectance, trash count and trash area were given below the figures regarding graphs of each character. The differences between the two years were significant for all observed characters according to a combined analysis of variance. Therefore, an independent analysis of variance under each level of the year was performed. Significant cultivar x precipitation interaction indicated that the differential performance of cultivars varied under different levels of precipitation.

Germination Rate

In 2014, the germination rates in the control group ranged from 98.5% (ST-373) to 90.0% (Gloria), while it varied between 97.5% (GSN-24) and 89.0% (Claudia) at 20 mm precipitation. The genotypic differences in both precipitation groups are not significant. On the other hand, the differences between the cultivars were significant at 35 mm, 65 mm and 95 mm precipitation, respectively (LSD $_{(0.05)}$ = 8.74). While the highest germination rates were obtained from the ST-468 variety in all three precipitation groups, the lowest germination rates were obtained from the Claudia variety. At 125 mm, where the heaviest precipitation is, the germination rate was 81.0% in the Carisma cultivar, 63.0% in Flash and ST-373 cultivars, and 54.75% in the ST-468 cultivar. In the Claudia cultivar, which had the lowest values in the previous rainfall amounts, the germination rate was determined as 76.5% at 125 mm rainfall (Figure 2 left).

When the germination rates of 2015 were examined, similar to 2014, the highest germination rate was found in the ST-373 variety at the beginning with 97.5%, while the lowest germination rate was found in the Gloria variety with 93.0% (LSD $_{(0.05)}$ = 7.70). It was determined that the differences between cultivars were not significant at 0 and 20 mm precipitation. The difference between Flash (96.5%) with the highest germination rate and GSN-24 (88%) with the lowest germination rate at 35 mm precipitation was significant. The least decrease in 125 mm precipitation amount was in the ST-373 variety (89.5%), whereas the germination rate was 79.5% in the Flash variety, which was significantly lower than the ST-373 variety (Figure 2 right).



2014	Cultivar	Precipitation	Cultivar x Precipitation	2015	Cultivar	Precipitation	Cultivar x Precipitation
	**	**	**		ns	**	**

Figure 2. Germination rate of precipitation x cultivar in 2014 (left) and 2015 (right)
Şekil 2. 2014(sol) ve 2015(sağ) yıllarında çeşit ve yağışlara ilişkin çimlenme oranları

In the regression analysis performed separately for both years, the R^2 values were found to be the highest for the linear equation (0.94 and 0.98, respectively). Germination rate in the first year = 94.96 - 0.20 precipitation (mm), germination rate in the second year = 96.31 - 0.10 precipitation (mm) (Figure 2). Equations indicated that the germination rate declined 0.10 - 0.20 % per milimeter of accumulated rainfall. It has been found that the amount of precipitation affects the germination rate of the seed on the seed cotton negatively depending on the years and the germination decrease rate can be estimated with the help of the equations.

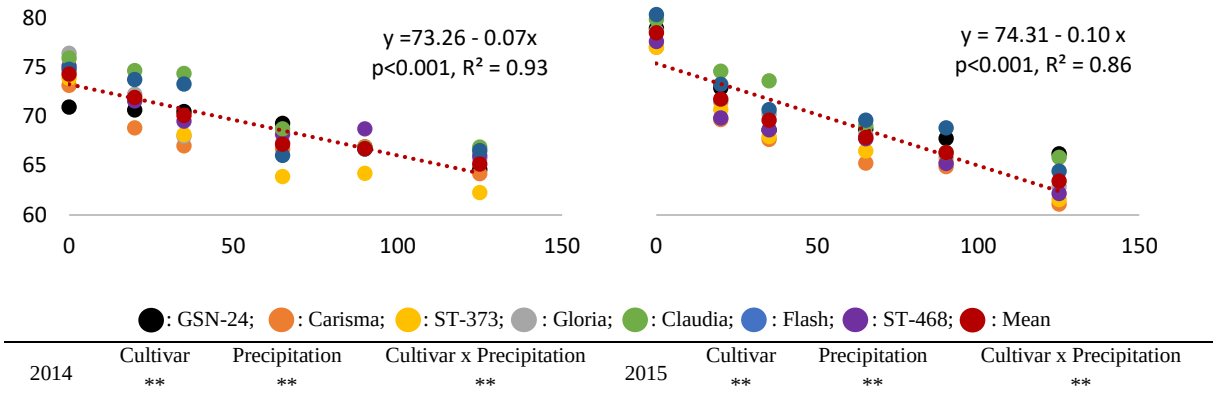
Reflectance

The reflectance values and the regression equation for both years were given in Figure 3. In 2014, Gloria (76.4), Claudia (75.9) and ST 468 (74.8) cultivars were found to have the highest reflectance values at a significant level in the control group (LSD $_{(0.05)}$ = 2.02). The lowest reflectance value was recorded in GSN-24 (71.0). It is seen that the highest reflectance values were determined in Claudia and Flash cultivars at 20 mm and 35 mm precipitation, respectively. It is noteworthy that both cultivars mentioned are the least affected by the highest rainfall. It can be seen in Figure 3 that the ST-373 variety was the most affected by the highest precipitations of 65 mm, 95 mm and 125 mm.

In 2015, the highest reflectance values were determined in Flash, Claudia, GSN-24 and Gloria in a control (non-precipitation), while Carisma, ST-468 and ST-373 varieties had the lowest reflectance values (LSD $_{(0.05)}$ = 1.67). Although all cultivars were adversely affected by an average of 8.0% in 20 mm of precipitation, the difference between cultivars did not change. The impact rate of cultivars slowed down with 35, 65 and 95 mm precipitations. GSN 24, Claudia and Flash were least affected even under the highest precipitation conditions. On the other hand, it is seen in Figure 3 that other varieties are significantly affected negatively.

R^2 values related to the linear regression curve were found to be high for both years. The regression equations were reflectance value= 73.26 – 0.07 precipitation and reflectance value= 74.31 – 0.10

precipitation in 2014 and 2015, respectively (Figure 3). Equations revealed that reflectance declined 0.07 – 0.10 per milimeter of accumulated rainfall. It was concluded that the amount of precipitation had a linear effect and, an increase in precipitation dulled the fibers.

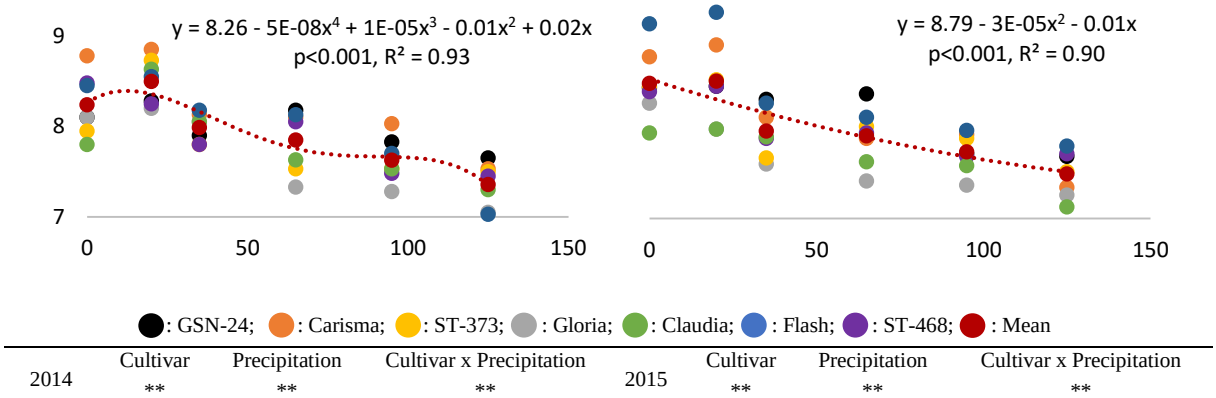


	Cultivar	Precipitation	Cultivar x Precipitation		Cultivar	Precipitation	Cultivar x Precipitation
2014	**	**	**	2015	**	**	**

Figure 3. Reflectance of precipitation x cultivar in 2014 (left) and 2015 (right)
Şekil 3. 2014(sol) ve 2015(sağ) yıllarında çeşit ve yağışlara ilişkin yansıma (Rd) değerleri

Yellowness

There were significant differences among the yellowness values of the cultivars in the control parcels (0 mm precipitation) in both years (LSD $_{(0.05)}$ = 0.21 and LSD $_{(0.05)}$ = 0.34, respectively). It is seen that Carisma (8.78) in the first year and Flash (9.5) in the second year had the highest yellowness values at the significant level. In the first year, Carisma was followed by Flash, whereas in the second year Flash was followed by Carisma. However, the Claudia variety had the lowest yellowness coefficient in both years. In the 20 mm precipitation plots, the yellowness increased, except for ST-468 in the first year and Gloria in the second year. While Carisma and ST-373 were the cultivars most affected by 20 mm precipitation in the first year, GSN 24, ST-468 and Gloria cultivars were observed to have significantly lower yellowness coefficients (Figure 4 left). In the second year, Flash and Carisma were the most affected cultivars by 20 mm rainfall, whereas Gloria and Claudia cultivars were the least affected. Significant decreases were recorded in 35 mm precipitation in both years. After this amount of precipitation, it can be said that the fluctuations depending on the yellowness of varieties have been more stable. It can be observed that the yellowness coefficient decreased significantly in 95 and 125 mm precipitation (Figure 4 right). It was determined that Flash and Gloria cultivars in the first year and Claudia, Gloria and Carisma cultivars in the second year had significantly less yellowness at 125 mm precipitation. On the other hand, it was determined that GSN-24 and Carisma varieties in the first year and, Flash and GSN-24 varieties in the second year were the varieties with the highest yellowness at the same precipitation amount. When the two-year results were evaluated together, it was noted that the Gloria cultivar had low yellowness, whereas the GSN-24 cultivar had high yellowness values.



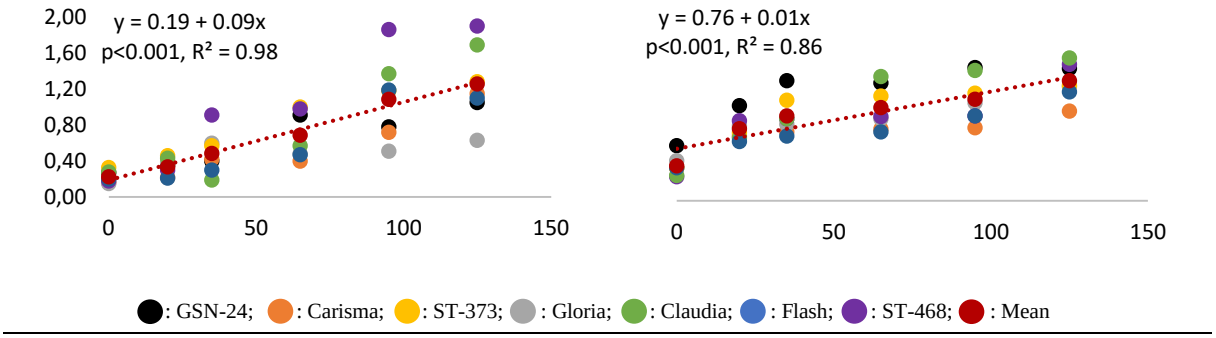
	Cultivar	Precipitation	Cultivar x Precipitation		Cultivar	Precipitation	Cultivar x Precipitation
2014	**	**	**	2015	**	**	**

Figure 4. The yellowness of precipitation x cultivar in 2014 (left) and 2015 (right).
Şekil 4. 2014(sol) ve 2015(sağ) yıllarında çeşit ve yağışlara ilişkin sarılık (+b) katsayıları

Trash Area

When the trash area values obtained in 2014 (LSD $_{(0.05)}$ = 0.17) and 2015 (LSD $_{(0.05)}$ = 0.11) were evaluated, it was seen that ST-373, Claudia and GSN-24 varieties

had significantly higher trash area values in control parcels of 2014. On the other hand, these values were significantly lower in Gloria, Carisma and ST-468 varieties. The trash area increased at 20 mm of precipitation and low values in Flash and Carisma cultivars were noted. At 35 mm, while a low trash area was observed in the Flash variety, the significant jump in the negative direction in the ST-468 variety can be observed in the same way. A significant increase in trash area was observed in ST-373 and GSN-24 varieties at 65 mm precipitation. With these two cultivars, ST-468 contains a significantly higher foreign matter area. When 95 mm and 125 mm precipitation are evaluated together, it is noteworthy that the Gloria variety was the variety that was least affected by precipitation in terms of significant foreign matter area, whereas ST 468 and Claudia varieties were the most negatively affected varieties (Figure 5 left).



2014	Cultivar	Precipitation	Cultivar x Precipitation	2015	Cultivar	Precipitation	Cultivar x Precipitation
	**	**	**		**	**	**

Figure 5. Trash area of precipitation x cultivar in 2014 (left) and 2015 (right)
Şekil 5. 2014(sol) ve 2015(sağ) yıllarında çeşit ve yağışlara ilişkin yabancı madde büyüklüğü değerleri

In 2015, at the beginning and 20 mm of precipitation, the GSN-24 cultivar had a significant trash area, whereas Claudia and Flash cultivars had the least values. In 35 and 65 mm precipitation, the GSN-24 variety had the highest foreign matter area values and it can be observed that the Claudia variety accompanied this variety. It was determined that Carisma and Flash varieties had the least values in both precipitation amounts. In 95 and 125 mm precipitation, it is seen that Claudia, GSN-24 and ST 468 varieties had significantly higher trash area values and Carisma and Flash varieties had the lowest values (Figure 5 right).

It was determined that the precipitation amount and regression equation of trash area had a linear slope in both years. Although the R^2 value was higher in the first year, trash area = 0.19 + 0.09 precipitation amount, and in the second year, trash area = 0.76 + 0.01 precipitation amount equations were obtained (Figure 5). Equations summarized that trash area increased 0.01 – 0.09 per milimeter of accumulated rainfall.

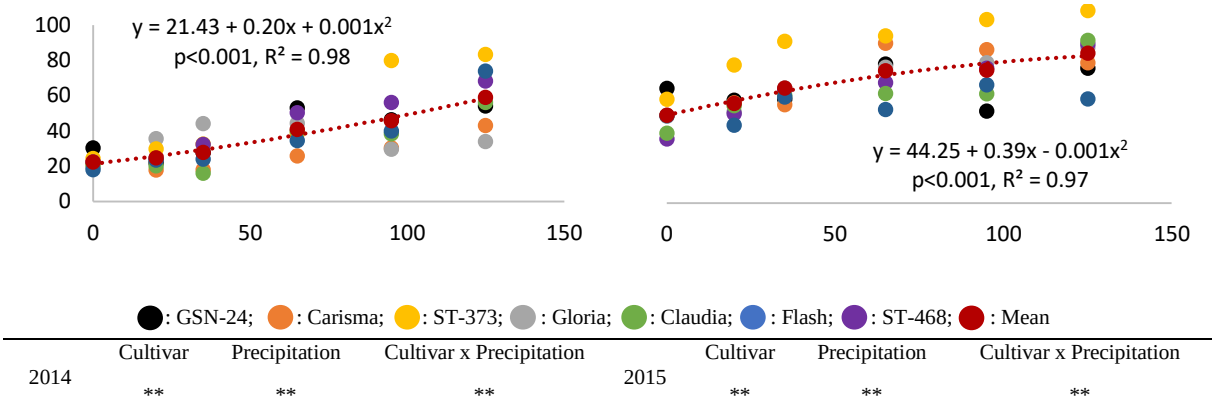
Trash Count

2014 and 2015 data in terms of trash count were given in Figure 6. When the control plots were evaluated, the GSN-24 cultivar had significantly high negative values in terms of trash count in 2014 (LSD $_{(0.05)}$ = 3.10), while other cultivars were in the same group. Similarly, in 2015 (LSD $_{(0.05)}$ = 2.38), GSN-24 and ST-373 cultivars were found to be in the highest group, while the statistical difference between other cultivars was not significant.

In both years, the polynomial regression equation between precipitation and trash count yielded the highest R^2 value (Figure 6). The equation for trash count = 21.43 + 0.20 precipitation + 0.001 precipitation^2 in the first year, trash count= 44.25 + 0.39 precipitation - 0.001 precipitation^2 in the second year was obtained. It is seen that GSN-24 and ST-373 varieties had the highest values in the control plots. Flash and Claudia in the first year and ST 468 and Claudia in the second year gave the lowest values. The response of genotypes to the increase in precipitation amounts has always been different and significant. It is noteworthy that the ST-373 variety had the worst characteristics, especially in 95 and 125 mm precipitation plots.

The results of the two-year experiment with seven cultivars and control and five precipitation amounts indicated that higher rainfall resulted in higher (unfavorable) trash count (102%) and area (311%) and resulted in a lower (unfavorable) germination rate (15.87%) and fiber reflectance (14.97%). Unlike these characters, an increase of 20 mm was observed in yellowness compared with control, and a decrease was

observed depending on the subsequent precipitation values. The linear regression curve and equation obtained for all characters except yellowness confirmed the negative increases and decreases. Previously studies revealed rainfall in the harvest season negatively affected cotton-fiber quality (Luo et al., 2016), and undamaged lint from rainfall was white and clean because of its highly reflective character (Heimoana and Wilson, 2017). Similarly, rainfall over 50.8 mm causes significant reductions in color level and increases in the foreign matter of the fiber (Hake et al., 1992; Anonymous, 2001). Also, Parvin et al. (2005) emphasized that cotton lint yield declined by 2.35 kg of lint per day and 4.09 kg of lint per centimetre of accumulated rainfall. According to Bednarz et al. (2002), HVI reflectance and yellowness decreased with weeks and light spotted color grades were recorded during the initial harvests, again corresponding to a period of significant rainfall.



	Cultivar	Precipitation	Cultivar x Precipitation		Cultivar	Precipitation	Cultivar x Precipitation
2014	**	**	**	2015	**	**	**

Figure 6. Trash count of precipitation x cultivar in 2014 (left) and 2015 (right)
Şekil 6. 2014(sol) ve 2015(sağ) yıllarında çeşit ve yağışlara ilişkin yabancı madde sayısı değerleri

Although the differences between years and the interaction of precipitation x cultivar were significant in each year, the performance of some cultivars was considerably more stable for the studied character. While the Carisma was the least affected cultivar by the increase in precipitation, significant decreases were observed in the Flash cultivar in terms of germination rate. Claudia and Flash exhibited a favorable performance for reflectance and yellowness. Gloria and Carisma for trash area; Gloria and Flash for trash count were the most suitable cultivars, whereas ST-373 performed poorly for both characters. Finally, the Flash cultivar can be recommended for minimum damage and profitable in delayed harvest conditions while seed production of the Carisma cultivar can be carried out without any problems.

CONCLUSIONS

The adverse effect of rainfall on exposed cotton boll in harvest season was clearly seen in this study. Decrease in germination rate, reflectance value and yellowness and an increase in trash count and trash area were detected with increasing precipitation amount. The different responses of cultivars to increase the precipitation amount indicated that different cultivars could be preferred in terms of seed production and lint colour grade.

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Contribution of the Authors as Summary

The authors declare the contribution of the authors is equal.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

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Crop Farm Diversification and Income Generation among Small holder: The Nigerian Experience

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ABSTRACT
The study examined crop diversification and its implications on farm productivity and income among small holder farmers in Abia State, Nigeria. A multistage sampling procedure was used to obtain a sample size of 250. Data were collected by the distribution of a structured questionnaire. Simpson Index of diversification, gross margin and land equivalent ratio were used as the analytical tools. The result revealed that crop diversification was high (Simpson Index = 0.76). Categorically, the generated net farm income of farmers was specialized diversification (₦51,472), low diversification (₦187,330), moderate diversification (₦402,300), high diversification (₦304,398) and complete diversification (₦169,130). Also, the Land Equivalent Ratio of farmers was specialized diversification (0.39), low diversification (1.16), moderate diversification (1.80), high diversification (1.40) and complete diversification (1.06). The study discovered that differences occurred in farmers' productivity and income levels as a result of crop diversification.

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INTRODUCTION
Agriculture in developing nations including Nigeria engages approximately one-half of the available labour force. A greater portion of rural communities and particularly the rural poor are directly or indirectly dependent on agriculture through farming activities, agri-business, food processing endeavour, fishing technology, forestry and wildlife, vocational skills and trade (Muhammed 2007). An enormous segment of the agricultural output is in the hands of smallholder farmers usually with possession of average holdings of roughly 1-3 hectares. Also, there is poor access to modern enhanced technical know-hows and their overall condition does not always portray excellence in noticeable investments in human capital development, raw material inputs and outputs (Ogunlela & Mukhtar 2009). This is the motive why a greater part of the smallholder farmers in the country grip a cropping pattern that is characterized by cultivating a large variety of crop mix under multiple cropping systems in space modified to various agro-ecological regions known as crop diversification (Ajibefun 2006). In Asia, it is supposed as one of the greatest ecologically achievable, cost-saving and justifiable traditions of decreasing uncertainties and risks in agriculture principally among smallholder farmers (Joshi 2004).

Crop diversification offers a wider option in varieties of crop productivity in a specified area and also lessens the threat of crop failure Tsubo Walker and Mukhala (2001). It can also offer reasonably higher net returns from crops, per unit of labour, optimization of higher land utilization efficiency (Ashaq *et al.* 2008). Agriculture is a risky professional venture due to its organic nature in practice, prone to vagaries of weather and climate including the devastating effects of pests and diseases. This risk in agriculture has led to low returns from produce and has greatly affected the income and productivity levels of farmers. This has compelled most farmers to adopt the technology for crop diversification. Crop diversification is considered as one of the tactics, methods and strategies for reducing the reported farming problems. Walelign (2004). When making choices about agricultural production, the farmer is considered to decide on a cropping plan that increases resilience and provides economic benefits, considering many forms of crop combinations at different scales (Lin 2011). The question the study sought to address was, to what extent farmers diversify their crop production enterprises and their implications on farm output? The focus is on micro farmers in Abia State Nigeria. The specific objectives were to determine the extent of diversification in crop production business and ascertain its effects on-farm productivity and farmers' income in the study area.

MATERIALS and METHODS

The study was carried out in Abia State of Nigeria. The state lies between longitudes 7°00E and 8°00E and latitude 4°45¹N and 6°17¹N of the equator. The capital is Umuahia. The state approximately occupies 6,320 square kilometers. It is characterized by low-lying tropical rainforest vegetation, agrarian with an average rainfall of about 2,400 millimeters per year and is especially intense between April through October (Federal Republic of Nigeria Official Gazette, 2007).

Sampling: The study population comprised of arable crop growers. A multistage sampling procedure was employed in the sample selection process. The *first stage* involved the selection of three LGAs each from the three agricultural zones using a simple random sampling technique. The *second stage* involved a simple random selection of four communities from each of the nine selected LGAs. *Lastly*, a technique of proportionate random sampling was used to select 250 respondents from whom primary data were collected. Ten percent of respondents were carefully chosen from each of the 36 communities. A total number of 2500 arable crop farmers formed the population size from the 36 communities.

A structured questionnaire was used to obtain primary data from the respondents. The questionnaire was designed to elicit needed information from the farmers on their socioeconomic characteristics, farm input and output.

Data generated were analysed using descriptive statistics, Simpson Index of diversification, gross margin, land equivalent ratio and analysis of variance. The Simpson Index of diversification (D) was applied to determine the extent of crop diversification [following from Joshi *et al.* (2003); Ibrahim *et al.* (2009)] and is expressed as:

$$D = 1 - \left(\frac{\sum n(n-1)}{N(N-1)} \right)$$

Where:

D is the Simpson Index of Diversification for farmer *i*

n = number of species of a crop cultivated by farmer *i*

N = the total sum of available species

The index (D) is between 0 (zero) and 1 (one) where 0 means no diversification and 1 represents infinite diversification. The greater the rate of D, the greater is the sample diversification. The categories of diversification are, 0.1–0.3 (low diversification), 0.4–0.6 (moderate diversification), 0.7–0.9 (high diversification) and one (complete diversification).

The gross margin (GM) was used to determine the effect of crop diversification on farmers' income. It is the differential value between the gross farm incomes (GFI) obtained and total variable cost (TVC). Olukosi and Erhabor (2008) recognized the GM analysis which permits the approximation of the entire expenses (costs) as well as several receipts (revenue or returns) within the production period.

It is expressed as:

GM = GFI – TVC

NFI = GM – TFC

(Where NFI = Net Farm Income and TFC = Total Farm Cost)

The land equivalent ratio (LER) was incorporated to determine the real effect of crop diversification on-farm productivity. LER as cited by Chukwuji (2008), is defined as total land area of sole crops required to produce equivalent yields as would be obtained when they are intercropped. LER as applicable here is the utmost communal index adopted and acceptable in intercropping practices to measure land productivity which is a determinant of the efficiency of intercropping models (Brintha & Seran, 2009). The LER would be calculated using the formula:

$$LER = \sum_{i-1}^{n} \left(\frac{Q_m}{Q_s} \right)$$

Where Q_m is crop yield in the intercrop farm and Q_s is specific crop yield in the sole crop farms. In a particular crop, a ratio is computed to define its partial LER, and the partial LERs are added up to equate the grand value of LER for the intercrop. A value of LER, 1.0 indicates no difference obtained in the intercrop yield and the assemblage of monocultures. Yield values greater than 1.0 indicate advantage for intercrop (Mazaheri, et al, 2006). The farmers' productivity coupled with income levels were tested across the various degrees of diversification using land equivalent ratio.

RESULTS and DISCUSSIONS

The respondents' socio-economic characteristics (Table 1) considered were gender, age, education, experience of farmers and farm size. As could be seen from the result the majority (59.2%) of farmer were males. This implies more males owned farms than females. This finding concurs with Miller (2004) and Jabil (2009) who reported that most farmlands are owned by a male. This finding is in tandem with Ayanwuyi Adeola and Oyetoro (2013) who reported that 74.1% of arable crop farmers are males. Respondents' mean age, 45 years, indicated that the crop farmers were still in their active age. This contributed to spreading of innovation practices since young people tend to accept innovations than older people and as such, they serve well as agents of innovation transfer. Ayanwuyi *et al.* (2013) also noted that more arable crop farmers were between 31-50 years old.

Farmers (60.8%) attained tertiary education. This means that a high proportion of them are literate and are more opened to absorb technologies and new farm practices, all things being equal. Nkhori (2004) pointed out that education increases the capability of households to employ their resources effectively, access, interpret and analyze information.

The mean years of farming experience was about 18 years. This suggests that the growers have the necessary experience in arable crop production. The higher the farming age, the more the farmer would have gained more knowledge and technical ideas on how to tackle farm production problems, and the higher would be his output and income (Nwaru Okoye & Ndukwu 2011).

The respondents' mean farm size was 1.42 hectares. This infers that the crop farmers were small scale farmers with high degree of land fragmentation associated with arable crop production. This finding agrees with Ajieh (2014) and Ovharhe (2020) that the small scale farm holders had between 0.5 to 3.5 hectares. Population growth forces farmers to shorten fallow periods, increase investment in land and manage soil fertility through the addition of manure (Obasi 2005).

It was detected that farmers had moderate interaction with extension activities in line with crop diversification training (54.4%). Ovharhe Emaziye and Okwuokenye (2020) reported that greater exposure of farmers to food security in line with crop diversification tends to increase farm output and income levels.

Table 1: Socio-demographic characteristics of respondents in the study area

Variables	Frequency	Percentage
Sex		
Male	148	59.2
Female	102	40.8
Age (mean: 44.85)		
Below 30 years	28	11.2
30-39 years	59	23.6
40-49 years	80	32.0
50-50 years	43	17.2
60-69 years	35	14.0
Above 70 years	5	2.0
Level of education		
No formal education	12	4.7
First Sch. Leaving Cert.	15	5.9
Snr. Sec. Cert. Exam.	71	27.8
Tertiary	152	60.8
Farming Experience (mean = 18)		
1-5 years	8	3.2
6-10 years	42	16.8
11-15 years	50	20.0
16-20 years	115	46.0
Above 20 years	35	14.0
Farm size (mean = 1.42Ha)		
Less than 1ha	11	4.4
1-1.9ha	136	54.4
2-2.9Ha	73	29.2
Above 2.9Ha	30	12.0
Contact with Extension Workers		
Monthly	11	4.4
Quarterly	136	54.4
Bi-annually	73	29.2
Annually	30	12.0

Source: Field responses

Crop Diversification (CD)

Table 2 shows respondents according to their extent of CD using the Simpson Index of Diversification. As could be seen from the Table, 4.4% of farmers engage in crop specialization, 16.0% engage in very low diversification, 34.8% carry out moderate diversification, 43.2% practice high diversification and 1.6% practice complete CD.

The farmers are engaged in the cultivation of various enterprises. The enterprises include yam, cassava, maize, pepper, melon and garden egg. The mean value of the Simpson index of diversification is 0.76. This is to say that, the arable crop farmers operated mainly diversified enterprises. Hence, the majority enjoy the benefits of CD. The finding agrees with that of Ogundari (2013) who reported that farmers were more diversified in their cropping pattern. Joshi et al. (2003) adopted the Simpson index to compare CD in several South Asian countries. Ibrahim et al. (2009) carried out research on income and CD among farming households in a rural area of north-central Nigeria using the Simpson index and they got a value of 0.82. This indicated that diversification was high in the study area as the respondents adopted multiple income-generating activities to manage risk and meet household consumption needs concerning the findings of Olukosi and Erhabor (2008).

Table 2: Distribution of respondents according to the extent of crop diversification (CD)

Simpson Index of Diversification	No. of Crops	Frequency	Percent
Zero (Specialization)	Sole crop	11	4.4
0.1–0.3 (low diversification)	Two–three crops	40	16.0
0.4-0.6 (Moderate diversification)	Four crops	87	34.8
0.7-0.9 (High diversification)	Five crops	108	43.2
One (Complete diversification)	All available enterprise	4	1.6

Source: Field responses

Crop Farm Diversification on Income

Table 3 shows the result of crop farm diversification in relation to farm income. The net farm income of the farmers was obtained as the difference between gross revenue and the total cost of production per hectare. The result reveals that 11% of the farmers who were specialized generated a net farm income of ₦51,472.73 per hectare. Farmers that had very low and moderate diversification (16.0% and 34.8% respectively) had an income of ₦187,330.35 per hectare and ₦402,300 per hectare, respectively. Also, 43.2% of the highly diversified farmers had a net farm income of ₦304,398.56 per hectare. Farmers that practiced complete diversification (1.6%) generated an average net farm income of ₦169,130.00 per hectare.

Moderate and highly diversified farms produced statistically the same amount of net farm income (NFI) per hectare that was significantly higher than others. Very low diversification and complete diversification produced the same amount of NFI that was significantly lower than others. This result implies that while enterprise diversification is good, very low and very high levels are not advisable because of income generation, a moderate level is the best. Chukwuji (2008) reported that a mixture of four enterprises considerably produced the maximum NFI, with Cassava + Yam + Maize + Vegetable combination giving the highest of about ₦21514 per hectare. He further stated that the combination of four enterprises appears to be the optimum, as all combinations less than and more than four produced lower NFI. Also, Minot Epprecht Anh and Trung (2006) and Biswajit *et al.* (2017) stated in an empirical analysis on agricultural diversification and its impact on farm income, that moderate crop diversification will increase farm income.

Table 3: Effects of crop diversification on income

Degrees of diversification	No. of crops	No. of farmers	Percent (%)	Gross income/ha(₦)	Total cost/ha (₦)	Net farm income/ha (₦)
Zero (specialization)	Sole crop	11	4.4	99473	82572	51472
0.1–0.3 (low diversification)	Two–three crops	40	16.0	242330	231233	187330
0.4–0.6 (moderate diversification)	Four crops	87	34.8	460300	442200	402300[c]
0.7–0.9 (high diversification)	Five crops	108	43.2	376399	366398	304399[c]
One (complete diversification)	All available enterprise	4	1.6	210000	201000	169130

Source: Field responses

Crop Diversification on Farm Productivity

Table 4 shows the outcome of crop enterprise diversification on-farm productivity measured as Land Equivalent Ratio (LER). The result reveals that 11% of the specialized farmers had a Land Equivalent Ratio of 0.39. Farmers that had very low and moderate diversification (16.0% and 34.8% respectively) had a LER of 1.16 and 1.80 respectively, while 43.2% of the highly diversified farmers had a LER of 1.40.

From the findings, statistically, farmers that practiced low and complete diversification had lower

and significant yield advantage. Farmers that practiced moderate and high diversification had the same and significant yield advantage, statistically. Hence, farmers with higher LER have a greater yield advantage, all things being equal. This suggests that it is most profitable to diversify on the accounts of high returns benefitted. This finding is following that of Chukwuji (2008) who stated that farms with higher economic LER were more diverse, all things being equal to those with lower economic LER. Agegnehu Ghizaw and Sinebo (2008); Dahmardeh *et al.* (2009); Brintha and Seran (2009) also reported that intercropping gives greater stability of increased yield and productivity than a sole crop cultivated in the same area of land.

Table 4: Effects of crop farm diversification on farm productivity

Degrees of diversification	No. of crops	No. of farmers	Percentage (%)	LER (Farm productivity)
Zero (specialization)	Sole crop	11	4.4	0.39
0.1–0.3 (low diversification)	Two–three crops	40	16.0	1.16
0.4–0.6 (moderate diversification)	Four crops	87	34.8	1.80
0.7–0.9 (high diversification)	Five crops	108	43.2	1.40
One (complete diversification)	All available enterprise	4	1.6	1.06

Source: Field responses

CONCLUSION

The study shows that the sampled arable crop farmers were engaged in various levels of diversification (low, moderate, high and complete). A smaller proportion also engaged in crop specialization, but majority engaged in moderate and high diversification. The land equivalent ratio result showed significant differences in the productivity and income of the farmers'. Moderate and highly diversified farms produced statistically the same amount of net farm income (NFI) per hectare that were significantly higher than others. Very low diversification and complete diversification produced the same amount of NFI that were significantly lower than others. Farmers that engaged in low and complete diversification had the same and significant yield advantage while moderate and highly diversified farmers had the same and significant yield advantage.

It is hereby recommended that farmers should be stimulated to boost their productivity by diversification to meet their income and consumption needs. This can be achieved through improving the farmers' access to credit and the extension agents through more awareness creation on the essence of CD and output increase. The monetary authority in collaboration with the government should encourage access to credits through reduced interest rates and perhaps, a little requirement for a small amount of loan. The Agronomist and Agricultural Engineers should create multiple cropping patterns that can support mechanized devices. Agrochemicals that suit multiple cropped enterprises rather than being specific should be formulated. While, as an implication to the study, extension advisers should recommend crop diversification practices mostly at moderate and high levels so as to increase income generation.

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Conflict of interest

None.

Contributions from authors

Okezie C, did the field works; Ovharhe OJ did the manuscript arrangement, presentation supervision and Chukwuji CO did the dissertation supervision and data analysis.

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Mass Modelling of Eggs Based on Shape Index Using Regression Analysis

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ABSTRACT
This study was conducted to determine the correlation between the mass of eggs based on its geometrical dimensions characteristics such as length *(L)*, width *(W)*, geometric mean diameter *(GMD)*, the first and second projection areas (PA_1, PA_2), criteria area *(CAE)*, oblate spheroid volume (V_{osp}), measured volume (V_m) and shape index *(SI)*. Based upon the *SI*, eggs were characterized as sharp (<72), normal (72 -76) and round (>76), respectively. For mass prediction, different classifications *viz.* dimension as 1st classification, projection area as 2nd classification, and volume as 3rd classification were considered. 1st classification (dimension), 2nd classification (projection area), and 3rd classification (volume) were considered. The analysis was executed using 33 linear regression models and the models were recommended by considering maximum coefficient of determination (R^2) and minimum regression standard error *(RSE)*. Based on the modelling analysis, 10 model equations based on the selected classifications were recommended for mass estimation. The findings of this investigation will be helpful for the researchers involved in the design and development of process equipments in the production and processing of eggs.

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Yumurtaların Şekil İndeksine Göre Regresyon Analiziyle Kütle Modellemesi

ÖZET
Bu çalışma, uzunluk (*L*), genişlik *(W)*, geometrik ortalama çap *(GMD)* gibi geometrik özellikleri; birinci ve ikinci projeksiyon alanları (PA_1, PA_2), kriter alanı *(CAE)* ile basık küre hacmi (Vosp) ve ölçülen hacim (Vm) ve şekil indeksi (SI) ile yumurta kütlesi arasındaki ilişkiyi belirlemek amacıyla yapılmıştır. Şekil indeksine göre yumurtalar sırasıyla sivri (<72), normal (72-76) ve yuvarlak (>76) olarak karakterize edildi. Kütle tahmini için farklı sınıflandırmalar yani boyut 1. sınıflandırma, projeksiyon alanı 2. sınıflandırma ve hacim 3. sınıflandırma olarak gözönüne alındı. Analiz 33 lineer regresyon modeli kullanılarak uygulandı ve modeller maksimum belirtme katsayısı (R^2) ve minimum regresyon standart hatası *(RSE)* dikkate alınarak önerildi. Modelleme analizine göre, kütle tahmini için seçilen sınıflandırmalara dayalı 10 model denklem önerilmiştir. Bu araştırmanın bulguları, yumurta üretimi ve işlenmesinde proseslerdeki ekipmanların tasarımı ve geliştirilmesinde araştırmacılara yardımcı olabilecektir.

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INTRODUCTION
An egg is an encapsulated source of macro and micronutrients that meet all requirements to support embryonic development until hatching (Réhault-Godbert et al., 2019). It is presumed to be a basic foodstuff due to its very high nutritive value (high protein content, nutrients, and vitamins) as reported by Rashidi et al., (2008). The major nutrients present in a whole, raw and freshly laid egg are, water (76.1%), protein, (12.6%), fat (9.5%), carbohydrates (0.7%), and ash (1.1%), respectively. Egg proteins are distributed equally between egg white and yolk, whereas vitamins, minerals and lipids are concentrated in egg yolk (Rath et al., 2015).

The factors like age and breed of the hen, weight, nutrition, maturity, and type of rearing system determine the egg quality characteristics (USDA 2018). Some environmental factors *viz.* heat, stress, overcrowding, and poor nutrition may also result in eggs having lesser weight. Even a minor variation in egg weight influences size classification which in turn affects the egg price (Altuntas & Sekeroglu, 2008).

From the consumer perspective, egg size is the most imperative characteristic as eggs having identical shapes and sizes are preferred (Rashidi et al., 2008). Sorting can help in gaining uniform size and shape, thereby reducing the costs involved in packaging and transportation and simultaneously resulted in an optimum packaging configuration (Rashidi & Gholami, 2008). It also contributes to meeting quality standards, increasing market value, and marketing operations.

Eggs can be classified into different sizes based on its weight, inclusive of peewee, small, medium, large, extra-large, and jumbo size. For the packaged egg material, mechanical strength of the eggshell is an important quality aspect to be considered. Egg shape index (SI) and shell thickness affect the proportion of damaged eggs while handling and transportation (Anderson et al., 2004; Yang et al. 2014).

Physical properties such as mass, volume, shell thickness, surface area and weight are the parameters affecting mechanical properties of chicken eggs. The correlation between the physical and mechanical properties of eggs was most significant (Altuntas & Sekeroglu, 2010). There is natural variability in egg shape and this variability can be characterized using SI. The significance of this indicator is mostly revealed in determining the direction of rotation during incubation and the movements of the embryo during the utilization of nutrients (Keranova et al., 2017).

Mass, being a relatively simple parameter, the size of any product is frequently correlated by its mass. However, sorting on the basis of selected geometrical characteristics might result in a more effective technique than mass sorting. Moreover, the mass of product can be easily estimated from geometrical attributes if the mass model of the product is known. Therefore, modelling of egg mass based on geometrical properties may be beneficial and applicable on a commercial scale (Rashidi & Gholami, 2011).

Mathematical relationships established using mass modeling will assist in grading eggs at a commercial scale making the process more accurate and less labor-intensive. This in turn will enhance the market value and commercialization potential of the eggs. Therefore, this research was inducted to determine the optimum mass models based on the shape index of the egg for mass prediction.

MATERIAL and METHODS

Raw Material

For this study, the egg materials used were obtained from a company located in Tokat, 39° 52' – 40° 55' north latitude and 35° 27' – 37° 39' east longitude, province. Eggs belong to brown layer "Atak-S" hybrid chickens developed by Ankara Poultry Research Institute in Turkey. The average air temperature and relative humidity was 20°C and 55% during the egg collection period. The chickens were 75 weeks old, and the facility housed 8 chickens per cage and brown eggs were used in this experiment.

Physical Properties

By assuming the shape of eggs as an oblate spheroid, the dimensions including length (L) and width (W) were measured using a digital vernier caliper (M/s Mitutoyo, Japan, ±0.01 mm) as shown in Figure 1.

(a) Axes of an egg (b) Normal egg (SI=72-76) (c) Round egg (SI>76)

Figure 1. Description of the axes (a), normal (b) and round (c) shape indexed egg samples.
Şekil 1. Yumurtaların eksen tanıtımı (a), normal (b) ve yuvarlak (c) şekil indeksli yumurta örnekleri.

The mass (M) of eggs was measured with a digital weighing balance (±0.001 g). The geometric mean diameter (GMD) of each egg was then calculated by Equation 1 (Altuntas and Sekeroglu, 2008; Meena et al. 2021).

$$GMD = (LW^2)^{1/3} \quad (1)$$

The shape index (SI) was calculated using the Equation 2 given below (Anderson et al., 2004):

$$SI = \frac{W}{L} \times 100 \quad (2)$$

Based upon the SI, eggs are characterized as sharp (<72), normal (72 -76), and round (>76), respectively

and 100 egg were used for each SI group (Sarica & Erensayin, 2004; Altuntas & Sekeroglu, 2008; Altuntas & Mahawar, 2021).
Two projected areas of each egg i.e. first projected area (PA_1) and second projected area (PA_2) were calculated using Equations 3 and 4, respectively. The average projected area known as the criteria area (CAE) of each egg was determined from Equation 5 as suggested by Rashidi and Gholami (2011).

$$PA_1 = \frac{\pi LW}{4} \quad (3)$$

$$PA_2 = \frac{\pi W^2}{4} \quad (4)$$

$$CAE = \frac{2PA_1+2PA_2}{3} \quad (5)$$

The volume of egg having assumed shape as oblate spheroid (V_{osp}) was calculated using Equation (6).

$$V_{osp} = \frac{\pi LW^2}{6} \quad (6)$$

For the measurement of volume (V_m), each egg was submerged into water and the volume of displaced water was measured (Rashidi and Gholami, 2011).
The relationship between M, L, W, GMD, PA_1, PA_2, CAE, V_{osp} and V_m was determined. A typical linear multiple regression model (Equatiion 7) for predicted mass for egg in this research is shown below:

$$Y = k_0 + k_1X_1 + k_2X_2 + k_3X_3 + \cdots + k_nX_n \quad (7)$$

Where:
Y= Dependent variable (for example mass of shape indexed egg)
$X_1, X_2, \ldots X_n$= Independent variables (for example physical attributes of egg)
$k_0, k_1, k_2, \ldots, k_n$ = Regression coefficients

Mass Modelling

The predicted modelling was achieved using 3 different classifications i.e. dimensions as first, projection area as second, and volume as the third classification. For dimensional model classification, mass modeling was accomplished according to the independent variables (L, W, GMD) of different eggs was taken into account. For the second projected area models classification, projected areas i.e. PA_1, PA_2 as well as CAE of eggs from each SI was considered for mass prediction. Volume parameters (V_m and V_{osp}) from each SI were used as third classification for modelling.
A total of 33 linear regression models in three classifications (12 for dimensions, 12 for the projected area, and 9 for volume) were adopted and the data was subjected to linear regression analysis using SPSS (Version 13.0). The coefficient of determination (R^2) and Regression Standard Error (RSE) and were Root mean squared error (RMSE) considered. The models having maximum R^2 and minimum RSE and $RMSE$ (Root mean squared error) values values represented the best fit (Mahawar et al., 2019).
Root mean squared error (RMSE) and Coefficient of variation [C.V(%)] was calculated as following below Equations 8,9 (Rashidi and Gholami, 2011).

$$RMSE = \sqrt{\Sigma(ni=1Mi-M*i)2n} \quad (8)$$

$$CV = (Standard\ deviation/Mean)100 \quad (9)$$

Where:
Mi = egg measured by digital balance, g
$M*i$ = egg estimated by mass model, g
n= number of samples

RESULTS and DISCUSSION

Some physical attributes of different eggs having variable shape index i.e. (72-76) (standard), (>76) (round) and mixed (72≤SI>76) which were used to determine the mass models are presented in Table 1.
For normal group, the range of parameters i.e. 59.84-79.73 g (M), 5.75-6.43 cm (L), 4.34-4.76 cm (W), 4.78-5.21 cm (GMD), 27.19-32.42 cm^2 (CAE), 67-72 cm^3 (V_m), respectively. The SI values were ranged between 71.41%-75.97%. For round SI group, the range of parameters i.e. 60.75-80.12 g (mass), 5.66-6.21 cm (length), 4.37-4.95 cm (width), 4.79-5.27 cm (GMD), 27.36-32.95 cm^2 (CAE), 73-83 cm3 (V_m), respectively. The SI values were ranged between 72.54-82.38. For mixed group, the range of parameters i.e. 59.84-80.12 g (mass), 5.66-6.43 cm (length), 4.37-4.95 cm (width), 4.78-5.27 cm (GMD), 27.19-32.95 cm^2 (CAE), 67-83 cm^3 (V_m), respectively. The SI values were ranged between 71.41%-82.38%.
Correlation coefficients (R) for these relations are given Table 2. The relationship between mass, length, width, geometric mean diameter, first projected area, second projected area, criteria area, spheroid volume and measured volume was determined as follows:

For normal egg (SI=72-76);
$M = 11.48\ L = 15.43\ W = 14.01\ GMD = 3.22\ PA1 = 4.32\ PA2 = 2.34\ CAE = 1.06\ V_{osp}$ (10)

For round egg (SI=>76);
$M = 11.69\ L = 15.13\ W = 13.91\ GMD = 3.24\ PA1 = 4.20\ PA2 = 2.33\ CAE = 1.06\ V_{osp}$ (11)

For mixed egg (72≤SI>76);
$M = 11.59\ L = 15.26\ W = 13.95\ GMD = 3.23\ PA1 = 4.25\ PA2 = 2.34\ CAE = 1.06\ V_{osp}$ (12)

The relations between M/L, M/W, M/GMD, $M/PA1$, $M/PA2$, M/CAE, M/V_{osp} have been found to be statistically significant.
Altuntas and Sekeroglu (2008) have reported the L (64.02 to 59.28 mm) and W (44.61 to 46.16 mm), GMD

(50.28 to 49.97 mm), and mass (72.34 to 70.31 g) of chicken eggs for the three *SI* categories tested. Rashidi and Gholami (2011) have reported the physical and geometrical properties of egg as, 42.05-58.33 g (mass), 5.02-5.88 cm (length), 3.85-5.23 cm (width), 4.27-5.43 cm (*GMD*), 14.50-23.19 cm² (CAE), 37.02-49.74 cm³ (V_m), respectively. Rath et al. (2015) reported different traits of White Leghorn to flock eggs as, 57.78±0.20 g (M) 54.39±0.11 mm (L), 39.92±0.07 (W), and 73.53±0.18% (*SI*), respectively. Duman et al. (2016) reported the weight of hen eggs with reference to the shape index as, 59.80 g (sharp), 60.00 g (standard), and 61.10 g (round), respectively.

Table 1. Physical attributes of different eggs having variable shape index.
Çizelge 1. *Farklı şekil indeksine sahip farklı yumurtaların fiziksel özellikleri.*

Shape Index	Parameter	Minimum	Maximum	Mean (*)	S.D.	C.V. (%)
SI 72-76 Normal (standard)	Mass (*M*), g	59.84	79.73	70.13	3.93	5.60
	Length (*L*), cm	5.75	6.43	6.11	0.126	2.06
	Width (*W*), cm	4.34	4.76	4.54	0.088	1.95
	Geometrical mean diameter (*GMD*), cm	4.78	5.21	5.01	0.092	1.85
	First projected area (PA_1), cm²	19.71	23.75	21.81	0.808	3.71
	Second projected area (PA_2), cm²	14.81	17.78	16.22	0.630	3.88
	Criteria area (*CAE*), cm²	27.19	32.42	29.92	1.11	3.69
	Oblate spheroid volume (V_{osp}), cm³	57.33	74.46	66.10	3.64	5.51
	Measured volume (V_m), cm³	67.00	72.00	68.49	1.26	1.84
	Shape Index	71.41	75.97	74.39	1.11	1.49
SI >76 Round	Mass (*M*), g	60.75	80.12	69.44	0.080	1.62
	Length (*L*), cm	5.66	6.21	5.94	0.125	2.11
	Width (W), cm	4.37	4.95	4.59	0.087	1.90
	Geometrical mean diameter (*GMD*), cm	4.79	5.27	4.99	0.081	1.62
	First projected area (PA_1), cm²	19.72	23.43	21.42	0.691	3.23
	Second projected area (PA_2), cm²	15.00	19.23	16.55	0.632	3.82
	Criteria area (*CAE*), cm²	27.36	32.95	29.70	0.959	3.23
	Oblate spheroid volume (V_{osp}), cm³	57.98	76.99	65.57	3.20	4.88
	Measured volume (V_m), cm³	73.00	83.00	76.83	2.60	3.38
	Shape Index	72.54	82.38	77.25	1.85	2.39
SI (72≤SI>76) Mixed	Mass (*M*), g	59.84	80.12	69.74	3.67	5.27
	Length (*L*), cm	5.66	6.43	6.01	0.150	2.50
	Width (W), cm	4.37	4.95	4.57	0.091	1.98
	Geometrical mean diameter (*GMD*), cm	4.78	5.27	5.00	0.086	1.73
	First projected area (PA_1), cm²	19.72	23.75	21.59	0.767	3.55
	Second projected area (PA_2), cm²	14.81	19.23	16.41	0.650	3.96
	Criteria area (*CAE*), cm²	27.19	32.95	29.70	1.029	3.45
	Oblate spheroid volume (V_{osp}), cm³	57.33	76.99	65.80	3.41	5.18
	Measured volume (V_m), cm³	67.00	83.00	73.22	4.65	6.35
	Shape Index	71.41	82.38	76.00	2.13	2.80

SD: Standard deviation; CV: Coefficient of variation; (*): 100 eggs

Mass Modelling

Models based on selected attributes (dimensions, projected area, and volume) for normal, round, and mixed-shaped eggs were screened and the one model with a higher R^2 value and lower *RSE* in each model category was selected. The linear regression equations along with R^2, *RSE and RMSE* are presented in Table 3-5.

First classification: Dimensions Based Models

Among the first classified models, for normal *SI* eggs, the model based on *GMD* i.e. *M= k0 + k1 GMD* was found best with R^2 (0.916), and lower *RSE* (1.137). The model equation was M= *- 133.047 + 40.576GMD*. For round eggs, the model based on *L* and *W* i.e. *M= $k_0 + k_1L + k_2W$* was best with R^2 (0.889), and lower *RSE* (1.187) and lowest *RMSE* (1.125). The model equation was *M= - 134.369 +11.794L +29.107W.* For mixed-shaped eggs, the model based on *GMD* as well as *L* and *W* was found best. The model equations are: *M= -132.878 + 40.529 GMD* having R^2=0.904, *RSE*=1.140 and *M= 132.076 +11.876L +28.529W* with R^2=0.904, *RSE*=1.140, respectively (Table 3). A graph of the estimated and measured values of a normal (standard) shape index egg shown in Figure 2.

Second classification: Projected Areas Based Models

Among the models based on the projected area, the linear model comprising *CAE* was the best fitted for normal *SI* eggs. The model equation was *M= -*

31.724+3.404 CAE having 0.917 (R^2) and 1.126 (*RSE*), and 1.123 *(RMSE)* respectively. For round *SI* and mixed *SI* eggs the best fitted model equations are: *M= - 32.293 + 3.245 PA1 +1.940 PA2* (R^2=0.890 and *RSE*=1.185) and *M= - 31.458 + 3.300 PA1 +1.826 PA2* (R^2=0.906 and *RSE*=1.131), respectively as also depicted in Table 4.

Table 2. Correlation coefficients of different eggs having variable shape index.
Çizelge 2. *Farklı şekil indeksine sahip farklı yumurtaların korelasyon katsayıları.*

Shape index	Particulars	Ratio	Degress of freedom	Correlation coefficient (R)
SI 72-76 (Normal)	*M/L*	11.480	128	0.850 **
	M/W	15.434	128	0.918 **
	M/GMD	14.007	128	0.957 **
	M/PA1	3.216	128	0.953 **
	M/PA2	4.323	128	0.918 **
	M/CAE	2.344	128	0.958 **
	M/Vosp	1.061	128	0.958 **
	M/Vm	1.024	128	-0.005 ns
SI >76 (Round)	*M/L*	11.685	168	0.635 **
	M/W	15.130	168	0.854 **
	M/GMD	13.905	168	0.945 **
	M/PA1	3.241	168	0.919 **
	M/PA2	4.196	168	0.854 **
	M/CAE	2.338	168	0.944 **
	M/Vosp	1.059	168	0.944 **
	M/Vm	0.904	168	0.015 ns
SI (72≤SI>76) Mixed	*M/L*	11.594	298	0.662 **
	M/W	15.261	298	0.827 **
	M/GMD	13.949	298	0.951 **
	M/PA1	3.230	298	0.926 **
	M/PA2	4.251	298	0.827 **
	M/CAE	2.341	298	0.952 **
	M/Vosp	1.060	298	0.951 **
	M/Vm	0.952	298	-0.080 ns

** Significant at 1% level. ns Non significant.

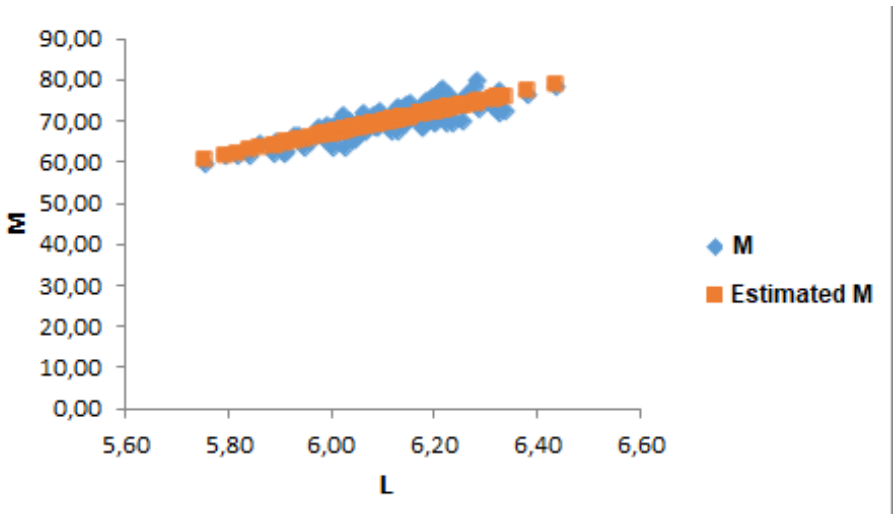

Figure 2. A graph of the estimated and measured values of a normal (standard) shape index egg.
Şekil 2. *Normal standart bir yumurtanın kütle tahmini ve ölçülen değerlerinin grafiği.*

Third classification: Volume-Based Models

Among the models based on the volume, the linear model compresing *Vm* was the best fitted for normal *SI* eggs. The model equation was, *M= 11.501+1.030Vosp - 0.139Vm* having 0.919 (R^2) and the lowest 1.126 (*RSE*) and the lowest 1.119 *(RMSE)*. For round *SI* and mixed *SI* eggs the best-fitted model equations were based on *Vosp* and *Vm* i.e. *M= 1.292 + 1.017Vosp + 0.019Vm* (R^2=0.889; *RSE*=1.142;

RMSE=1.128) and *M= 3.516+1.023 V_{osp}-0.015 V_m* (R^2=0.904 and *RSE*=1.136; *RMSE*= 1.129), respectively as also depicted in Table 5.

The recommended model equations for mass prediction of eggs based on some geometrical attributes are summarized in Table 6.

Table 3. Coefficient of determination (R^2) and regression standard error (*RSE*) and root mean squared error (RMSE) for linear regression models based on dimensions classification for normal, round and mixed-shaped eggs.

Çizelge 3. *Normal, yuvarlak ve karışık şekilli yumurtalar için boyut sınıflandırmasına göre lineer regresyon modelleri için belirtme katsayısı (R^2) ve regresyon standart hatası (RSE), .*

Shape Index	Models No	Model	Model	R^2	*RSE*	*RMSE*	Sig. M	Sig. RC
	1	$M= k_0 + k_1 L$	$M= -91.705 + 26.491 L$	0.720	2.071	2.060	*	* *
(72-76)	2	$M= k_0 + k_1 W$	$M= -113.799 + 40.472 W$	0.841	1.568	1.551	*	* *
Normal	3	**$M= k_0 + k_1 GMD$**	**$M= -133.047 + 40.576 GMD$**	**0.916**	**1.137**	1.131	*	* *
(standard)	4	$M= k_0 + k_1 L + k_2 W$	$M= -131.905 +12.273 L +27.958 W$	0.914	1.148	1.127	*	* * *
	1	$M= k_0 + k_1 L$	$M= -33.912 + 17.346 L$	0.416	2.726	2.655	*	* *
(>76)	2	$M= k_0 + k_1 W$	$M= -87.880 + 34.263 W$	0.711	1.916	1.787	*	* *
Round	3	$M= k_0 + k_1 GMD$	$M= -134.335 + 40.784 GMD$	0.889	1.190	1.125	*	* *
	4	**$M= k_0 + k_1 L + k_2 W$**	**$M= -134.369 +11.794 L +29.107 W$**	**0.889**	**1.187**	1.125	*	* * *
	1	$M= k_0 + k_1 L$	$M= -27.572 + 16.178 L$	0.435	2.760	2.749	*	* *
Mixed	2	$M= k_0 + k_1 W$	$M= -82.968 + 33.414 W$	0.682	2.070	2.059	*	* *
(72≤SI>76)	3	**$M= k_0 + k_1 GMD$**	**$M= -132.878 + 40.529 GMD$**	**0.904**	**1.140**	1.131	*	* *
	4	**$M= k_0 + k_1 L + k_2 W$**	**$M= -132.076 +11.876 L +28.529 W$**	**0.904**	**1.140**	1.126	*	* * *

M: the mass of egg; *L*: length, *W*: width; k_i is regression coefficient. *RSE*: Regression Standard Error *Sig. M*: Significant of model; *Sig. RC*: Significant of regression coefficient.

Table 4. Coefficient of determination (R^2) and regression standard error (*RSE*) and root mean squared error (RMSE) for linear regression models based on projected areas classification for normal, round and mixed-shaped eggs.

Çizelge 4. *Normal, yuvarlak ve karışık şekilli yumurtalar için projeksiyon alan sınıflandırmasına göre lineer regresyon modelleri için belirtme katsayısı (R^2) ve regresyon standart hatası (RSE).*

Shape Index	Models No	Model	Model	*R^2*	*RSE*	*RMSE*	*Sig. M*	*Sig. RC*
	1	$M= k_0 + k_1 PA_1$	$M= -30.759+4.627PA_1$	0.906	1.198	1.191	*	* *
(72-76)	2	$M= k_0 + k_1 PA_2$	$M= -22.706+5.723PA_2$	0.840	1.563	1.552	*	* *
Normal	3	**$M= k_0 + k_1 CAE$**	**$M= -31.724+3.404CAE$**	**0.917**	**1.126**	1.123	*	* *
(standard)	4	$M= k_0 + k_1 PA_1 + k_2 PA_2$	$M= -31.707+ 3.444 PA_1 +1.648PA_2$	0.916	1.132	1.123	*	***
	1	$M= k_0 + k_1 PA_1$	$M= -28.418+4.560PA_1$	0.841	1.422	1.353	*	* *
(>76) Round	2	$M= k_0 + k_1 PA_2$	$M= -9.408+4.762PA_2$	0.714	1.907	1.787	*	* *
	3	$M= k_0 + k_1 CAE$	$M= -32.388+3.425CAE$	0.889	1.188	1.131	*	* *
	4	**$M= k_0 + k_1 PA_1 + k_2 PA_2$**	**$M= -32.293+ 3.245 PA_1 +1.940 PA_2$**	**0.890**	**1.185**	1.128	*	* * *
	1	$M= k_0 + k_1 PA_1$	$M= -25.899+4.430 PA_1$	0.856	1.391	1.126	*	**
Mixed	2	$M= k_0 + k_1 PA_2$	$M= -6.872+4.670 PA_2$	0.683	2.069	2.061	*	* *
(72≤SI>76)	3	$M= k_0 + k_1 CAE$	$M= -31.427+3.396CAE$	0.905	1.131	1.128	*	* *
	4	**$M= k_0 + k_1 PA_1 + k_2 PA_2$**	**$M= -31.458+ 3.300 PA_1 +1.826 PA_2$**	**0.906**	**1.131**	1.125	*	* * *

M: the mass of egg; PA_1: first projected area, PA_2: second projected area; *CAE*: criteria area; $k_i$is regression coefficient. *Sig. M*: Significant of model; *Sig. RC*: Significant of regression coefficient.

CONCLUSION

The present study comprised of an evaluation of some physical characteristics of eggs and then correlating the measured properties with mass. The dependency of the egg mass on measured physical properties was well established by regression equations. The effect of egg shape indices on the model parameters can be observed and substantiated with the presented results. The model equations for egg mass as a function of physical parameters *viz.* dimensions, projected area and volume were predicted and based on the regression analysis, the best fit models were selected. These fundamental findings of this study will be helpful for the researchers involved in the

design and development of handling, transport and process equipments in the production and processing of eggs. Such application will make the overall process more precise, consistent and convenient, thus saving operation time, money and manpower.

Table 5. Coefficient of determination (R^2) and regression standard error (RSE) and root mean squared error (RMSE) for linear regression models based on volumes classification for normal, round and mixed-shaped eggs.

Çizelge 5. *Normal, yuvarlak ve karışık şekilli yumurtalar için hacim sınıflandırmasına göre lineer regresyon modelleri için belirtme katsayısı (R^2) ve regresyon standart hatası (RSE).*

Shape Index	Models No	Model	Model	R^2	*RSE*	*RMSE*	Sig. M	Sig. RC
(72-76)	1	$M= k_0 +k_1 V_{osp}$	$M= 2.141+1.029V_{osp}$	0.917	1.131	1.126	*	* *
Normal	2	$M= k_0 +k_1 V_m$	$M= 63.942+0.083V_m$	0.001	3.928	3.913	ns	ns
(standard)	3	$\mathbf{M= k_0 + k_1 V_{osp} + k_2 V_m}$	$\mathbf{M= 11.501+ 1.030 V_{osp}- 0.139V_m}$	**0.919**	**1.126**	1.113	*	* * *
(>76)	1	$M= k_0 +k_1 V_{osp}$	$M= 1.823+1.029V_{osp}$	0.889	1.190	1.133	*	* *
Round	2	$M= k_0 +k_1 V_m$	$M= 71.717-0.034V_m$	0.002	3.573	3.435	ns	ns
	3	$\mathbf{M= k_0 + k_1 V_{osp} + k_2 V_m}$	$\mathbf{M= 1.292+ 1.017V_{osp}+ 0.019V_m}$	**0.889**	**1.142**	1.132	*	* * *
Mixed	1	$M= k_0 +k_1 V_{osp}$	$M= 2.372+1.024V_{osp}$	0.904	1.136	1.132	*	* *
(72≤SI>76)	2	$M= k_0 +k_1 V_m$	$M= 74.335- 0.063V_m$	0.003	3.667	3.655	ns	ns
	3	$\mathbf{M= k_0 + k_1 V_{osp} + k_2 V_m}$	$\mathbf{M= 3.516+ 1.023V_{osp}- 0.015V_m}$	**0.904**	**1.136**	1.129	*	* * *

M: the mass of egg; V_{osp}: oblate spheroid volume; V_m: measured volume; k_i is regression coefficient. *Sig. M*: Significant of model; *Sig. RC*: Significant of regression coefficient; ns: not significant

Table 6. Recommended model equations for mass prediction of eggs based on SI.

Çizelge 6. *SI'ye göre yumurtaların kütle tahmini için önerilen model denklemleri*

Model/SI	Dimension	Projected area	Volume
Normal SI	$M=- 133.047 + 40.576GMD$	$M= -31.724+3.404\ CAE$	$M= 11.501+1.030V_{osp} - 0.139V_m$
Round SI	$M=-134.369+11.794L +29.107W$	$M= - 32.293+ 3.245\ PA_1 +1.940\ PA_2$	$M= 1.292+ 1.017V_{osp} + 0.019V_m$
Mixed SI	$M= - 132.878 + 40.529GMD$ and $M= 132.076 +11.876L +28.529W$	$M= - 31.458 + 3.300\ PA_1 +1.826\ PA_2$	$M= 3.516+1.023\ V_{osp}-0.015V_m$

Author Contributions

Authors declares the contribution of the authors is equal.

Conflict of Interest

The authors declare no conflict of interest.

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The Effects of Water Stress on Cotton Leaf Area and Leaf Morphology

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ABSTRACT

The most important effect of water stress on plants is that it reduces leaf area and leads to changes in leaf morphology. Decreased leaf area results in reduces crop yield through the reduction in photosynthesis. This study investigates the effects of the decrease in leaf area on seed cotton yield, evapotranspiration (ET), water use efficiency (WUE), and leaf geometry in cotton plants under water stress in different growth periods. The cotton plant was divided into three different growth periods (vegetative period (VP), flowering and boll growth period (FB), and boll opening (BO) period), and irrigation water was applied at field capacity level during the periods of full irrigation (T), while non-irrigation was applied during the water stress periods (O). In the experiment, 6 different irrigation strategies were based on: OOO, TTT, OTO, TOO, OTT, and TOT. In each treatment, five leaves were taken from three plants in every replicate during three growth periods, and the leaf area and geometric lengths of each leaf were measured. Seed cotton yield, evapotranspiration, and WUE decreased significantly depending on the severity and duration of the water stress to which the cotton was exposed. Physiologically, cotton leaves under water stress in the first stage of growth tended to increase the leaf lobe numbers while reducing the leaf area. Therefore, there were more leaf lobes numbers measured in OOO than in other treatments. Irrigation in the vegetative growth period was more effective in increasing the leaf area than the other growth periods.

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Su Stresinin Pamuk Yaprak Alanına ve Morfolojisine Etkileri

ÖZET

Su stresinin bitkiler üzerindeki en önemli etkisi yaprak alanını azal-tarak yaprak morfolojisinde değişime yol açmasıdır. Yaprak alanının azalması fotosentezdeki azalma yoluyla mahsul veriminin azal-masına neden olur. Bu çalışmada farklı gelişme dönemlerinde susuz bırakılan pamuk bitkisinde yaprak alanındaki azalmanın verim, evapotranspirasyon (ET), su kullanma oranına (WUE) ve yaprak geometrisine etkileri belirlenmeye çalışılmıştır. Pamuk bitkisi 3 farklı gelişme dönemine (vegetatif dönem, çiçeklenme ve koza oluşumu dönemi ve kozaların açılması dönemi) ayrıldı ve tam sulamanın yapıldığı dönemlerde tarla kapasitesi düzeyinde su uygu-lanırken (T), su stresli dönemlerde sulama suyu uygulan-mamıştır (O). Denemede OOO, TTT, OTO, TOO, OTT, TOT konuları olmak üzere 6 farklı sulama stratejisi esas alındı. Her konuda 3 gelişme döneminde her tekerrürdeki 3 bitkiden 5 er yaprak alındı ve her yaprağın yaprak alanı ve geometrik uzunlukları ölçüldü. Pamuğun maruz kaldığı stresin şiddetine ve süresine bağlı olarak verim, eva-potranspirasyon ve WUE önemli ölçüde azaldı. Fizyolojik olarak büyümenin ilk evresinde susuz bırakılan pamuk yaprakları alan-larını küçültürken kanat sayılarını artırma eğilimine girmiştir. Bu nedenle yaprak kanat sayısı OOO konusunda diğer konulardan daha fazla ölçüldü. Vegetatif gelişme dönemindeki sulamaların yaprak alanının artmasında gelişme dönemlerinden daha etkili olmuştur.

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INTRODUCTION

Drought stress causes morphological and physiological changes in plants. Varying depending on the duration and severity of the stress, it has effects in physiological (photosynthesis rate, stoma conductivity, leaf turgor loss, etc.), biochemical (accumulation of stress metabolite, increase in antioxidative enzymes, etc.), and molecular levels (synthesis of specific proteins, increased expression of ABA biosynthetic genes, etc.) in plant development. However, drought stress causes morphological changes in the plant by reducing plant height and leaf area (Bañona et al., 2004).

Leaf area has a fundamental role in controlling water use in plants, and it is significantly reduced under water stress. This decrease causes a decrease in the living leaf area where stomatal conductivity occurs (Babu et al., 1983; Correia et al., 2001; Meenakshi, 2005) and photosynthesis (Rucker et al., 1995). Since leaves are the most critical plant organs that use light energy to produce metabolites necessary for plant development during photosynthesis, the amount of light energy they hold is the determinant of plant production (Kanemasu et al., 1985). The change in leaf morphology also plays an essential role in the amount of water consumed by the plant. It was determined that the evapotranspiration values of the cotton plant change depending on the variety and leaf area, and less evapotranspiration occurs in the cotton of the Siokra variety, which has a small leaf area, compared to the other varieties (Can & Ödemiş, 2018). The fact that leaf area is directly related to the photosynthetic activity (Koc & Barutcular, 2000) affects the amount of dry matter, yield, and crop quality (Centritto et al., 2000). Hence, many factors that provide growth and development of the plant can be predicted by determining the leaf area.

Cotton is an extremely sensitive plant to water stress. Primarily during flowering, water shortage affects many growth parameters, especially leaf area, and flower shedding increases, while plant height, rooting depth, and canopy width decrease. One of the most obvious visual changes is the formation of redness on the stem from the point of contact with the soil to the top, depending on the level of stress (Ödemiş et al., 2018). The distinctive responses of cotton to water stress make the results of models to be established between stress and parameters affected by stress more significant. Various studies were conducted to reveal the effects of treatments on leaf area or the relationship between leaf area and plant morphological characteristics (Fournioux, 1996; Sala et al., 2015; Abd El-Mageed et al., 2016; Bozkurt & Keskin, 2018; Poşta & Sala, 2018). Cho et al. (2007) suggested that they developed nonlinear models to estimate the fresh and dry weight of cucumber and individual leaf area using leaf length, leaf width, and SPAD values, and these models had a high correlation coefficient. Sala et al. (2015) estimated the leaf area in the ratio of R^2=0.987 (for L) and R^2=0.995 (for W) using leaf length (L) and width (W) in their study on 1500 leaves in 5 different apple tree cultivars.

This study examined the amount of irrigation water, evapotranspiration and seed cotton yield in cotton plants exposed to water stress during different growth periods and the morphological changes of leaf area, width, length, leaf lobes numbers, and lobe lengths due to stress.

MATERIALS and METHODS

The experiment was carried out in the randomized blocks of the Carisma variety cotton plants belonging to the Gossypium hirsutum L. species based on the split-plot design with 3 replicates in 2015-2016. The region where the experiment area is located (Hatay/Turkiye) reflects the typical climatic character of the Mediterranean region, and the summers are hot and dry, and the winters are warm and rainy. According to long-year climate data, the annual average temperature is 20°C, the coldest month of the year is January with 8.2°C, and the hottest month is August with 29.1°C. Total precipitation during the growing season was measured as 21 mm (2015) and 149 mm (2016). The characteristics of the soils of the research area are given in Table 1.

The cotton plant was divided into three different growth periods (vegetative period (VP), flowering and boll growth period (FB), and boll opening (BO) period) (Doorenbos & Kassam, 1979), and irrigation was applied at field capacity level during the periods of full irrigation (T), while irrigation water was not applied during the water stress periods (O) (Table 2). The cotton plant was planted with a seeder with an interrow spacing of 70 cm and an intrarow spacing of 15 cm. Treatments were formed from 6 rows and 15 meters in length. There was no gap between the replicates. Harvesting was done manually from the remaining 39.2 m^2 area after leaving out one row from the right and left of each plot and 50 cm from the beginning of the plots.

The soil moisture change was determined by the gravimetric method. The first irrigation started when 50% of the available water capacity was consumed. Irrigation applications were realized using the drip

irrigation method to bring the current soil water content to the field capacity approximately once a week. Irrigation water quality was identified as C_2S_1. Irrigation water was calculated by using Equation 1.

Table 1. The physical and chemical properties of research area soils
Çizelge 1. Araştırma alanı topraklarına ilişkin bazı fiziksel ve kimyasal özellikler

Depth (cm)	Texture	pH	ECe	$CaCO_3$ (%)	Nitrate (%)	Organic mat (%)	Fc (g g^{-1})	Pwp (g g^{-1})	As (g cm^3)
0-30	SiCL	7.55	644	2.265	1.42	0.33	21.3	13.4	1.66
30-60	SiCL	7.62	560	0.680	1.65	0.34	24.1	14.2	1.68
60-90	SiCL	7.80	429	0.905	2.01	0.38	25.0	14.5	1.54
90-120	SiCL	7.65	400	0.300	2.12	0.37	25.2	14.7	1.49

Fc: Field capacity, Pwp: permanent wilting point, As: bulk density, EC_e: Electrical conductivity of soil paste (µmhos cm^{-1})

Table 2. Water stress treatments applied in different developmental stages
Çizelge 2. Farklı gelişme dönemlerinde uygulanan su stresi konuları

Treatments	Emergence*	Vegetative Growth Period (VG)	Flowering and Boll Development Period (FB)	Boll Opening Period (BO)
OOO	+	-	-	-
OTO	+	-	+	-
TOO	+	+	-	-
OTT	+	-	+	+
TOT	+	+	-	+
TTT	+	+	+	+

(+): Irrigation, (-): Non-irrigation
(T): Irrigation treatments irrigated at field capacity level, (O): Non-irrigation treatments
*: In the first year, 70 mm water was given for equal emergence, while there was no need to irrigate in the second year due to precipitation.

$$d = ((PWFC - PWAW) \times As \times D)/100 \quad (1)$$

Where; d: Soil moisture content in depth (mm); PWFC: Field capasity (%); PWAW: Moisture content of each layer (%); As: Bulk density (g cm^{-3}); D: Later depth (mm). Volume of water to be applied to each plot was calculated by Equation 2.

$$I = (d \times A \times P)/Ea \quad (2)$$

Where; I: Total irrigation water amount (L); d: Soil moisture content in depth (mm); A: Plot size (m^2); P: Wetted area (%, According to the (Yıldırım, 2008) P was taken as 35%); Ea: Irrigation efficiency (%).

The evapotranspiration of the treatments was determined according to the "Soil-Water Budget" method (James, 1988), the water use efficiency (WUE) was determined according to Howell et al., (1984).

The fertilizer treatments were performed equally to all plots with 20 kg da^{-1} of 18-46-0 (DAP) fertilizer before sowing and 4 kg da^{-1} pure nitrogen fertigation method in each of the first four irrigations after sowing (Burt et al., 1995).

Five leaves were taken from each replicates in each growth period in determining the leaf area and leaf geometry (five leaves were taken one day before irrigation from each replicates), and these leaves were drawn on sketch papers and their geometrical structures were determined. Leaf lobe lengths were identified with the help of a digital caliper (Dasqua 2310-7105 Digital Caliper (IP54 Protected)), while leaf area was determined by an electronic planimeter (Ushikata X-PLAN 380 f.c. planimeter). The distance between the two furthest points of the leaf is defined as 'height' and the widest part as 'width,' while the other lengths are called 'lobes' (Figure 1).

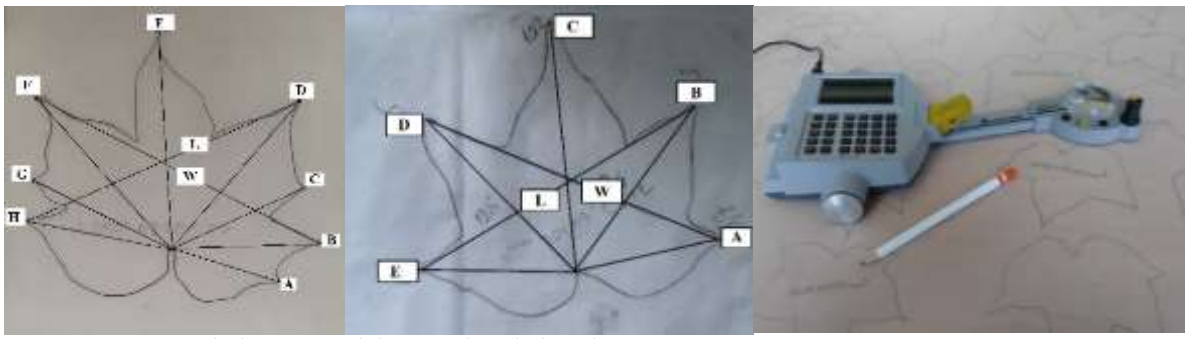

Figure 1. Width (W), length (L), lobe (A-E)-(A-H), and area measurements of cotton leaf
Şekil 1. Pamuk yaprağına ait en (W), boy (L), kanat (A-E)-(A-H) ve alan ölçümleri

RESULTS and DISCUSSION

In this research, the amount of irrigation water, evapotranspiration, seed cotton yield, and water use efficiency (WUE) changed depending on the treatments and years. The highest and lowest evapotranspiration were measured in TTT and OOO, respectively. The fact that the experimental area was windy during the irrigation season caused evaporation and evapotranspiration (ET) to be measured more than expected compared to other parts of the plain. Therefore, ET measured on TTT treatment in both years (1046 mm in the first year, 1182 mm in the second year) and was higher than ET measured in cotton cultivation areas in the region (Table 3). Evapotranspiration in cotton was determined between 449-615 mm (Ertek & Kanber, 2001), 985-1103 mm (Baştuğ & Tekinel, 1989), and 778 mm-594 mm ranges (Howell et al., 1984) in Çukurova conditions. ET was measured as 1096-995 mm between 2015 and 2016 in the experiment area (Ödemiş et al., 2018).

Table 3. Changes of the irrigation water, evapotranspiration, seed cotton yield and WUE
Çizelge 3. Deneme konularının sulama suyu, bitki su tüketimi (ET), verim ve su kullanım etkinliği (WUE) değerlerinin yıllara ve konulara bağlı değişimleri

Treat.	Irrig. Water (mm)			ET* (mm)			Seed cotton yield (kg da^{-1})			WUE** (kg m^{-3})		
	2015	2016	Mean	2015	2016	Mean	2015	2016	Mean	2015	2016	Mean
OOO	90	149	120	311	303	307	185.5±31.43	148.6±17.68	167±18.23	0.60	0.49	0.54
TTT	1135	1078	1106	1046	1182	1114	480.1±31.43	499.8±17.68	489.9±18.23	0.46	0.42	0.44
TOO	349	570	459	419	676	547	203.3±31.43	258.1±17.68	230.7±18.23	0.49	0.38	0.44
OTT	876	657	767	803	661	732	458.7±31.43	328.4±21.66	393.6±20.39	0.57	0.50	0.53
OTO	477	407	442	590	433	512	303.3±31.43	259.3±30.63	281.3±25.79	0.51	0.60	0.55
TOT	748	820	784	701	879	790	263±31.43	313.5±21.66	288.3±20.39	0.37	0.36	0.36

*ET: Evapotranspiration, **WUE: Water Use Efficiency

The irrigation strategy applied during the growth periods caused the ET to change in different treatments. Although the average ET values in TOO and OTO treatments, which were irrigated in only one of the three growth periods, were different based on the years, they were found to be at the same level on average. Similarly, in OTT and TOT treatments irrigated in two of the three growth periods, the ET value of the TOT treatment that was not irrigated during the flowering period was measured higher. On the other hand, higher crop yield was obtained in OTT. This indicates that the contribution of ET in the vegetative period to seed cotton yield is not as effective as in the flowering period. Moreover, evapotranspiration shows significant differences during growth periods. Tekinel and Kanber (1989) found out that the daily water consumption of cotton is 1-2 mm from emergence to square, 2-4 mm from square to the first flower, 3-8 mm from the beginning of flower to the first boll opening, and 8-14 mm from the first boll opening to the last effective flowering. It is known that the cotton plant is more sensitive to water during the flowering period than other periods (Karami et al., 1980). Although young leaves are more sensitive to photosynthesis in the vegetative period, stress during the peak of flowering (fruit set) weakens fruit set and increases flower shedding. Therefore, in our study, the highest seed cotton yield after TTT treatment was obtained from the OTT treatment irrigated during flowering and boll formation (393.6 kg da^{-1}) (Table 3). Although Krieg (1997) reported that water stress from the square to the time of the first flower cause a great decrease in seed cotton yield, the fact that the soil moisture did not decrease much with the effect of winter precipitation in our study caused the stress to be at a lower level than expected. The effect of stress during the flowering period was also clearly observed in WUE. The lowest WUE was measured for TOT in both years (Table 3). The WUE value was calculated higher in the treatments irrigated during the flowering period. The low calculation of WUE in the second year in the treatment of non-irrigated OOO was thought to be due to the low contribution of excessive precipitation to the seed cotton yield in the period between the last irrigation and harvest. However, many variables such as radiation load, temperature, humidity, ambient CO_2 concentration, soil type and structure, soil water availability, nutrition, and genetic makeup affect the change of WUE (Reich et al., 1985; Reddy et al., 1995; Loveys et al., 2004).

Leaf Morphological Features

Leaves have an important role in plant functions and adaptation to environmental conditions. Changes in their morphological or anatomical features may occur due to their response to environmental conditions. Although mainly composed of epidermis, stomata, and mesophyll, they exhibit marked differences in area, thickness, and shape among different species due to phylogenetic relationships and adaptation to particular environments. Some studies investigated how morphological features such as leaf area vary between different ecosystems and adapt to environmental factors (Tian et al., 2016). Our research suggested that cotton leaves showed

morphologically different responses (leaf lobe number, leaf area, leaf width, leaf length, and leaf lobe length) to water stress in different irrigation strategies.

Leaf Lobe Number

It was determined that the lobes indicated by A, B, C, D, and E were common on the leaves of all treatments, while the lobes of F, G, H were lost or not formed at all in some treatments. Therefore, F, G, and H lobes were excluded in the regression relationships regarding the number of lobes. Physiologically, cotton leaves under water stress in the first stage of growth tended to increase the number of lobes while reducing their area. Therefore, the number of lobes was measured the highest in the OOO treatment (average 6.07 units) and the lowest in the fully irrigated TTT treatment (average 5.40 units) (Table 4). In the vegetative period, the number of lobes was higher in the treatments that were non-irrigated (OTT and OTO). Fewer lobes (especially in the first year) were determined in the TTT, fully irrigated each period. Four major leaf shape alleles exist in tetraploid cotton, including normal, sub-okra, okra, and super-okra. Besides, it was found that leaf shape has consistent effects on boll rot resistance, earliness, flowering rate, chemical spray penetration, lint trash, and seed cotton yield. Nevertheless, different studies reported inconsistent effects on various insect resistances, photosynthetic rate, water use efficiency, and fiber quality (Andres et al., 2016).

Leaf Area

Leaf area decreased as water stress increased. Leaf area decreased by 40% in the first year and 22% in the second year compared to the fully irrigated treatment. Among the treatments exposed to water stress periodically, leaf area was determined the highest in TOT and lowest in OTO (Table 4). It was observed that irrigation during the vegetative period plays a significant role in increasing the leaf area. The data on TOO proves this situation. Even in the TOO treatment irrigated only in the VG period, leaf area was found to be higher than the OTT treatment irrigated in the FB and BO periods. The leaf area assessment is of higher importance for plant development. It is considered that approximately 95% of light is intercepted above an LAI of 3.

Table 4. Changes in leaf morphological characteristics by treatment
Çizelge 4. Yaprak morfolojik özelliklerinin konulara bağlı değişimleri

GP	Lobe N (units)			Area (cm²)			Width (cm)		
	2015	2016	Mean	2015	2016	Mean	2015	2016	Mean
OOO	5.89±0.25a	6.25±0.32a	6.07±0.21a	5914±357.92a	7180±551.15a	6547±366.42a	11.64±0.32a	11.61±0.40a	11.62±0.27a
TTT	5.33±0.29a	5.46±0.33a	5.40±0.23a	9847±405.84c	9201±575.66a	9524±395.77c	14.72±0.36c	13.95±0.44c	14.33±0.30c
TOO	5.67±0.25a	5.42±0.32a	5.54±0.21a	7981±357.92b	7800±551.15a	7890±366.42b	13.44±0.32b	13.14±0.40bc	13.29±0.27c
OTT	5.72±0.25a	6.04±0.35a	5.88±0.22a	6737±357.92a	8323±603.76a	7530±385.22a	12.19±0.32a	13.07±0.44bc	12.63±0.29b
OTO	5.78±0.25a	6.08±0.35a	5.93±0.22a	5976±357.92a	7610±603.76a	6793±385.22a	11.68±0.32a	12.42±0.44ab	12.05±0.29a
TOT	5.56±0.27a	5.29±0.33a	5.42±0.21a	9080±379.63c	8335±575.66a	8708±385.22c	14.18±0.34bc	13.91±0.42bc	14.04±0.29c

GP	Lenght (cm)			A (cm)			B (cm)		
	2015	2016	Mean	2015	2016	Mean	2015	2016	Mean
OOO	11.56±0.33a	11.14±0.46a	11.35±0.31a	4.98±0.20a	5.69±0.25a	5.34±0.17a	7.12±0.26a	7.47±0.27a	7.30±0.20a
TTT	14.80±0.38c	13.59±0.48c	14.19±0.34c	6.66±0.23d	7.21±0.26d	6.94±0.19c	9.58±0.30d	9.14±0.29d	9.36±0.21d
TOO	13.45±0.33b	12.62±0.46bc	13.04±0.31c	6.59±0.20cd	6.74±0.25cd	6.67±0.17bc	8.86±0.26cd	8.96±0.27bc	8.91±0.20cd
OTT	11.93±0.33a	12.54±0.50abc	12.23±0.33b	5.84±0.20bc	6.59±0.27bc	6.22±0.18b	8.34±0.26bc	9.04±0.30cd	8.69±0.21bc
OTO	11.63±0.33a	11.97±0.50ab	11.80±0.33a	5.46±0.20ab	5.95±0.27ab	5.70±0.18a	7.78±0.26ab	7.98±0.30ab	7.88±0.21ab
TOT	13.98±0.35bc	12.36±0.48abc	13.17±0.33c	6.61±0.21cd	6.80±0.26cd	6.73±0.18bc	9.37±0.28d	9.13±0.29d	9.25±0.21d

GP	C (cm)			D (cm)			E (cm)		
	2015	2016	Mean	2015	2016	Mean	2015	2016	Mean
OOO	8.69±0.29a	8.28±0.31a	8.48±0.23a	8.00±0.28a	7.84±0.33a	7.92±0.23a	5.66±0.25a	6.13±0.37a	5.89±0.24a
TTT	11.24±0.33c	10.35±0.33c	10.80±0.25c	9.71±0.31b	9.32±0.35b	9.52±0.25c	7.38±0.29b	7.28±0.41b	7.33±0.26b
TOO	9.87±0.29b	9.53±0.31bc	9.70±0.23bc	8.59±0.28ab	8.75±0.35ab	8.67±0.23bc	6.47±0.25b	6.87±0.37ab	6.67±0.24b
OTT	9.24±0.29ab	9.29±0.34bc	9.27±0.24bc	8.28±0.28a	8.98±0.39b	8.63±0.25bc	5.89±0.25a	6.62±0.41ab	6.25±0.25a
OTO	8.93±0.29a	9.02±0.34ab	8.97±0.24ab	8.01±0.28a	8.34±0.37ab	8.18±0.24ab	5.85±0.25a	6.20±0.39ab	6.02±0.24a
TOT	10.88±0.31c	9.57±0.33bc	10.22±0.24bc	9.44±0.29b	9.10±0.35ab	9.27±0.24c	6.93±0.29b	7.07±0.37b	7.00±0.25b

This indicates how efficiently intercepted light can be modified into sugar. On the other hand, photosynthesis requires water and carbon dioxide. Because gas exchanges are of primary interest, the rates of stomatal conductivity and carbon dioxide assimilation are also significant indicators of the efficiency of modification of light into sugar. The radiation use efficiency (RUE) of cotton is calculated as the division of its total dry biomass by its sum of intercepted light (Loison, 2019).

Leaf Width and Lengt

Leaf width and length were similarly affected by water stress during the growth periods. The mean values for leaf width and length are listed as TTT,

TOT, TOO, OTT, OTO, and OOO, from largest to smallest. The fact that the values in TTT and TOT treatments are at approximately the same level reflects that the water stress during the flowering and boll formation period did not cause a significant decrease in leaf width and length. On the other hand, lack of irrigation in the vegetative period (OTT and OTO) caused leaf length and width as much as the almost non-irrigated treatment (OOO) (Table 5). Studies reveal that water stress leads to an increase in specific leaf weight (Wilson et al., 1987), while it causes a decrease in leaf size (Pettigrew, 2004a). Water stress also reduces the formation of new leaves, resulting in a reduction in overall plant leaf area. Since the effect of stress is less severe on the main stem leaves, less leaf development is seen on both the main stem and the sympodial branches (Krieg & Sung, 1986).

Leaf Lobe Length

The effect of water stress on the lobe length was found to be significant in the experiment (Table 5). The lobe lengths increased over time on the dates of measurement until becoming stable. In general, five lobes were measured on all leaves (A, B, C, D, and E), while the other three lobes (F, G, and H) did not form on some leaves. Average lobe lengths were measured at the highest value in full irrigation (TTT) and lowest in non-irrigation (OOO). In lobes A, B, C, D, and E, lobe lengths from the highest to the lowest were measured for TOT, TOO, OTT, and OTO, respectively.

Table 5. Regression coefficients (r^2) between leaf area and leaf morphological characteristics
Çizelge 5. Yaprak alanı ile yaprak morfolojik özellikleri arasındaki regrasyon katsayıları (r^2)

Treat.	Leaf Width (cm)	Leaf Lenght (cm)	A (cm)	B (cm)	C (cm)	D (cm)	E (cm)
OOO	0.87** (n=9)	0.87** (n=9)	0.54 ns (n=9)	0.62ns (n=9)	0.62ns (n=9)	0.31ns (n=9)	0.25ns (n=9)
OOO	0.55* (n=12)	0.55* (n=12)	0.15ns (n=12)	0.16ns (n=12)	0.25ns (n=12)	0.27ns (n=12)	0.13ns (n=9)
OOO	0.55** (n=21)	0.55** (n=21)	0.26ns (n=21)	0.21ns (n=21)	0.19ns (n=21)	0.17ns (n=21)	0.01ns (n=18)
TTT	0.91** (n=7)	0.94** (n=7)	0.93** (n=7)	0.94** (n=7)	0.94** (n=7)	0.71* (n=7)	0.71* (n=7)
TTT	0.75* (n=11)	0.78* (n=11)	0.68* (n=11)	0.78* (n=11)	0.74** (n=11)	0.68* (n=11)	0.79** (n=9)
TTT	0.79** (n=18)	0.76** (n=18)	0.75** (n=18)	0.75** (n=18)	0.75** (n=18)	0.66** (n=18)	0.58** (n=16)
TOO	0.90** (n=9)	0.87** (n=9)	0.58ns (n=9)	0.55ns (n=9)	0.54ns (n=9)	0.57ns (n=9)	0.56ns (n=9)
TOO	0.60* (n=12)	0.62** (n=12)	0.52ns (n=12)	0.49ns (n=12)	0.51ns (n=12)	0.53ns (n=12)	0.53ns (n=12)
TOO	0.62** (n=21)	0.60** (n=21)	0.41ns (n=21)	0.40ns (n=21)	0.36ns (n=21)	0.40ns (n=20)	0.39ns (n=20)
OTT	0.86** (n=9)	0.62* (n=9)	0.64* (n=9)	0.63* (n=9)	0.65* (n=9)	0.91** (n=9)	0.63* (n=9)
OTT	0.85** (n=10)	0.63* (n=10)	0.65* (n=10)	0.63* (n=10)	0.67* (n=10)	0.68* (n=10)	0.62* (n=10)
OTT	0.84** (n=19)	0.61** (n=19)	0.52* (n=19)	0.58** (n=19)	0.58** (n=19)	0.74** (n=19)	0.55* (n=19)
OTO	0.66* (n=9)	0.67* (n=9)	0.64* (n=9)	0.63* (n=9)	0.65* (n=9)	0.63* (n=9)	0.65* (n=9)
OTO	0.75** (n=10)	0.67* (n=10)	0.76** (n=10)	0.88** (n=10)	0.80** (n=10)	0.61* (n=10)	0.69* (n=10)
OTO	0.67** (n=19)	0.47* (n=19)	0.64** (n=19)	0.69** (n=19)	0.46* (n=19)	0.46* (n=19)	0.44* (n=19)
TOT	0.67* (n=8)	0.68* (n=8)	0.59ns (n=8)	0.64ns (n=8)	0.60ns (n=8)	0.56ns (n=8)	0.53ns (n=7)
TOT	0.91** (n=11)	0.87** (n=11)	0.56ns (n=11)	0.52ns (n=11)	0.46ns (n=11)	0.56ns (n=11)	0.50ns (n=11)
TOT	0.71** (n=19)	0.85** (n=19)	0.43ns (n=19)	0.37ns (n=19)	0.36ns (n=19)	0.42ns (n=19)	0.42ns (n=18)

Relationships Between Leaf Area, Seed cotton yield and Water Use

The leaf area measurement is important in determining the plant's response to environmental conditions and predicting the development of the vegetative parts of the plant. In addition to studies investigating the relationship between the change in leaf area and seed cotton yield factors (leaf dry weight, vegetative components (stems and leaves), dry weight, and plant height) (Ghaderi & Soltani, 2007), there are also studies using the morphological features of the leaf to estimate the single leaf area (Fournioux, 1996; Sala et al., 2015; Poşta & Sala, 2018). Ghaderi and Soltani (2007) expressed that plant height is not a good determinant of leaf area, but dry leaf weight (LDW) or stem+leaf dry weight (VDW) can be used to predict leaf area. However, besides potential evaporation (Eo), leaf area index (LAI) is an important variable in determining evaporation from the soil surface on the first day after irrigation (Al-Khafaf, 1978). Marani et al. (1985) stated that the increased stress due to the decrease in the amount of irrigation water decreased the leaf expansion and leaf area, as well as decreased photosynthetic rate by increasing leaf senescence.

In our study, insignificant regression relationships in the first year and significant in the second year were found between leaf area and irrigation water amount, evapotranspiration, water use efficiency, and seed cotton yield (Figure 2-3-4-5). The average irrigation water amount and evapotranspiration values of the two years were effective in increasing the leaf area. The response of the leaves to the water stress during the growing periods was different. Leaf area was reduced by only 4.6% in the OTT treatment (767 mm), which was applied 67% more irrigation water than in the TOO treatment (459 mm). Similarly, the leaf area was found to be only 29% more in the TOT treatment (784 mm), which applied 56% more water than in the OTO treatment (442 mm). These data demonstrate that irrigation water applications during the vegetative growth and flowering periods are effective in increasing the leaf area, and the plant is more sensitive to water during the vegetative period.

In the relationship between ET-leaf area, irrigation water showed similar characteristics to the relationship between -ET (Figure 3). Based on the OOO treatment, the increase rates in the ET and leaf area are 363%-145% in TTT, 178%-121% in TOO, 238%-115% in OTT, 167%-104% in OTO, and 257%-133% in TOT. Based on this finding, the ET values of the treatments irrigated only during the vegetative growth and flowering periods caused an increase in leaf area by 121% and 104%, respectively. Compared to the OTT and TOT treatments irrigated in the two growth periods, the water consumption amounts in the TOO and OTO treatments were found to be more effective on the leaf area.

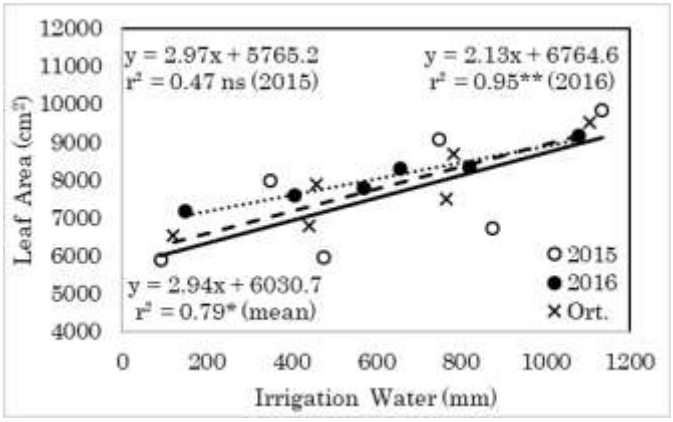

Figure 2. Relationships between leaf area and irrigation water

Şekil 2. Yaprak alanı ile sulama suyu arasındaki ilişkiler

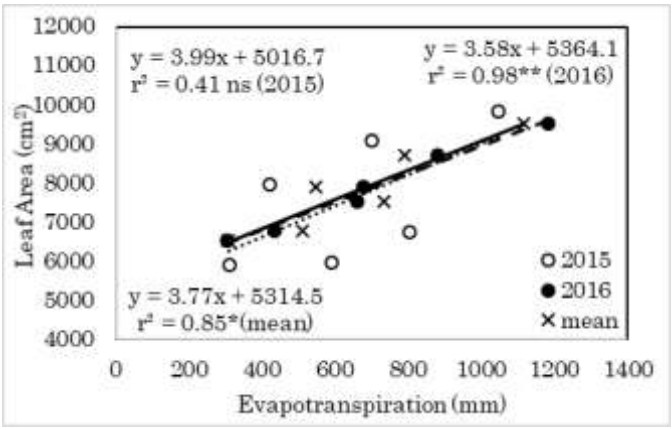

Figure 3. Relationships between leaf area and evapotranspiration

Şekil 3. Yaprak alanı ile bitki su tüketimi arasındaki ilişkiler

Water stress caused a significant regression relationship between average seed cotton yield and leaf area only in the second year (Figure 4). Compared to the non-irrigation treatment (OOO), the seed cotton yield increased by 293% in the fully irrigated treatment (TTT), while the leaf area increased by 145%. Besides, while the leaf area increased by 115% in the OTT (flowering and boll opening period), seed cotton yield increased by 236%. When OTT and TOT treatments are compared, it is seen that the flowering and boll formation period are determinative on seed cotton yield. However, it was observed that water stress in the mentioned period led to a significant decrease in WUE. No significant relationship was detected between WUE and leaf area (Figure 5). Krieg (1997) stated that water stress reduces the number and area of leaves, resulting in decreased photosynthesis and seed cotton yield. He

also pointed out that regarding the water supply that affects seed cotton yield components, the period from the square to the first flower is the most critical period of development. Drought sensitivity was highest at the peak flowering period when water stress resulted in the highest seed cotton yield reduction. The drop in seed cotton yield caused by water stress is mostly due to a fall in the number of bolls (Pettigrew, 2004b). Water stress before flowering lowers the number of fruiting sites.

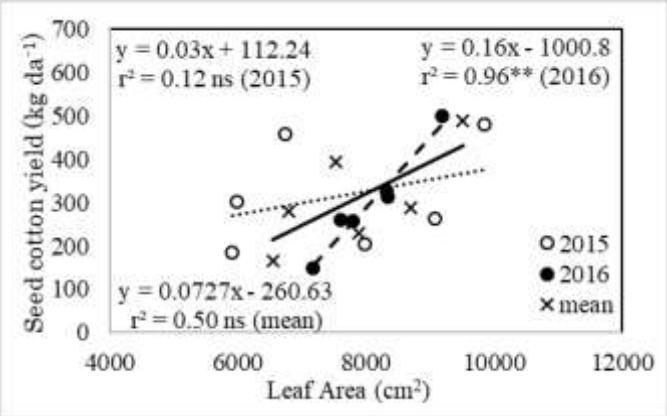

Figure 4. Relationships between leaf area and seed cotton yield

Şekil 4. Yaprak alanı ile verim arasındaki ilişkiler

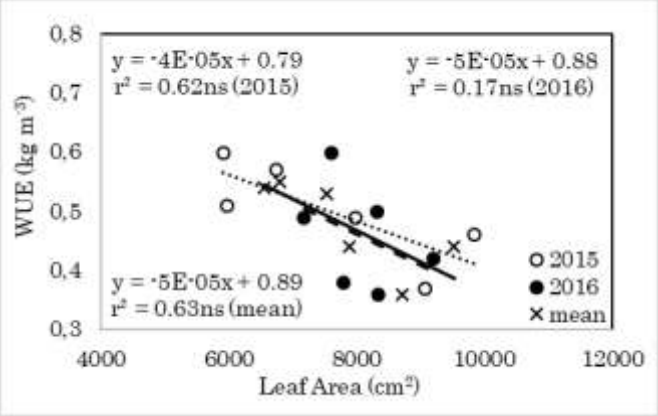

Figure 5. Relationships between leaf area and WUE

Şekil 5. Yaprak alanı ile su kullanım etkinliği arasındaki ilişkiler

Relationships Between Leaf Area and Other Morphological Parameters

The number of leaf lobes varied between five and eight depending on the treatments. Therefore, in the regression relationships between leaf area and leaf lobe lengths, only the lobes (A, B, C, D, and E) common in all treatments were taken as the basis. Lobe lengths varied according to years and irrigation. In the regression analysis, the leaf area increase did not cause a significant change in lobe lengths in the OOO, TOO, and TOT treatments. As seen in TOO and TOT treatments, non-irrigation during the flowering period did not increase the lobe lengths. Additionally, it was observed that the increase in leaf area in the treatments above was caused by irrigation, especially in the vegetative period. As the leaf area increased, a lower regression coefficient was determined between leaf lobe length and leaf area.

CONCLUSION

Many studies investigate the effects of water stress on seed cotton yield, evapotranspiration, and WUE that the cotton plant is exposed to during its growth (Howell et al., 1984; Pettigrew, 2004a; Ödemiş et al., 2018; Can & Ödemiş, 2018; Kazgöz-Candemir & Ödemiş, 2018). However, there was no study examining the correlation of these parameters with leaf area and morphological features. Hence, our study demonstrated that leaf area created significant correlation relationships with seed cotton yield, irrigation water amount, and evapotranspiration (especially in the second year of the study). However, no correlation was determined between leaf area and WUE. In the vegetative period, under stress conditions, the leaf first increased the number of lobes, while the decrease in stress and the increase in leaf width and length caused the disappearance of non-specific (small) lobes. During the flowering period, the leaf width and length became stable and reached the maximum level, and the leaf area reached the highest level. However, after the flowering period, it was observed that some lobes could maintain their length while re-stress reduced leaf area. It can be suggested that the duration and severity of the stress in that period are more effective than the development periods in the change in leaf morphology. This is more evident in the leaf area. Whether the leaf area is in the flowering period or the boll formation period, it could increase its growth under stress-free conditions until the bolls were formed. However, the most significant increase occurred with the effect of irrigation during the vegetative growth period. As the leaf area increased, a lower coefficient of regression between leaf lobe length and leaf area was determined. It was observed that irrigation during the flowering period was more effective in increasing the lobe lengths.

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Author's Contributions

The contribution of the authors is equal.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

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The Applications of Green Extraction: Production and Quality Characterization of Seed Oils Extracted From Red Pepper (*Capsicum Annuum* L.) Waste

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ABSTRACT
The study focused on the possibilities of evaluation of red pepper (*Capsicum Annuum* L.) seeds being a food industry waste. The moisture content (%), the total crude oil and ash content, color, the weight of 1000 seeds, the thickness and diameter of the seeds were characterized. The oils were extracted from the seeds using green techniques: cold-pressing, ethanol solvent, and ultrasound-assisted ethanol solvent extraction.Different techniques compared the production yield and quality characteristics of the oil samples. On average, linoleic acid (72.00%), oleic acid (11.76%), and palmitic acid (11.50%) were the predominant fatty acids in oils. The yield (16.80%) of the ultrasound-assisted technique was observed to be more effective than the others. The lowest content of acidity and the highest content of total carotenoids were found in the cold-press oil. The color, conjugated diene-triene values were higher with cold-pressing. The total phenolic contents and the antioxidant capacities were ranked in the following order: ethanol solvent (241.1 mg kg^{-1} and 79.84%), ultrasound-assisted (167.0 mg kg^{-1} and 67.18%), and cold press (131.8 mg kg^{-1} and 59.04%). The total tocopherols (1801.2 mg kg^{-1}) content was superior in the oil extracted with the ethanol solvent technique. The results were shown that the oil obtained by using the ethanol solvent extraction technique had better bioactive properties and so, antioxidant activity compared to other green extraction techniques.

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Yeşil Ekstraksiyon Uygulamaları: Kırmızı Biber (*Capsicum Annuum* L.) Atığından Ekstrakte Edilen Çekirdek Yağlarının Üretimi ve Kalite Karakterizasyonu

ÖZET
Çalışma, gıda endüstrisi yan ürünü/atığı olan kırmızı biber (*Capsicum Annuum* L.) çekirdeklerinin değerlendirilme olanaklarına odaklanmıştır. Nem içeriği (%), toplam ham yağ ve kül içeriği, renk, 1000-dane ağırlığı, çekirdeklerin kalınlığı ve çapı karakterize edilmiştir.Yağlar, yeşil teknikler uygulanarak çekirdeklerden ekstrakte edilmiştir: soğuk presleme, etanol solvent ve ultrason- destekli etanol solvent ekstraksiyonu. Yağ numunelerinin üretim verimleri ve kalite özellikleri farklı tekniklerle karşılaştırılmıştır. Ortalama olarak, linoleik asit (%72.00), oleik asit (%11.76) ve palmitik asit (%11.50) yağlarda baskın yağ asitleri olmuştur. Ultrason-destekli tekniğin, verim açısından (%16.80) diğerlerine göre daha etkili olduğu gözlenmiştir. En düşük asit içeriği ve en yüksek toplam karotenoid içeriği soğuk pres ile üretilen yağda tespit edilmiştir. Renk, konjuge dien-trien değerleri soğuk pres yöntemi ile daha yüksek elde edilmiştir. Toplam fenolik içerik ve antioksidan kapasite değerlerini şu şekilde sıralayabiliriz : etanol solvent (241.1 mg kg^{-1} ve %79.84), ultrason-destekli solvent (167.0 mg kg^{-1} ve %67.18) ve soğuk presleme (131.8 mg kg^{-1}) 1 ve %59.04). Etanol solvent tekniği ile ekstrakte edilen yağın toplam tokoferol (1801,2 mg kg^{-1}) içeriği daha üstün olmuştur. Sonuçlar, etanol solvent ekstraksiyon tekniği kullanılarak elde edilen yağın diğer yeşil ekstraksiyon tekniklerine kıyasla daha iyi biyoaktif özelliklere ve

Gıda Bilimi

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Anahtar Kelimeler
Gıda endüstrisi yan/atık ürünü
Kırmızı biber çekirdeği
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Yeşil ekstraksiyon
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dolayısıyla antioksidan aktiviteye sahip olduğunu göstermiştir.

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INTRODUCTION

Pepper (*Capsicum Annuum* L.), a popular crop plant cultivated in various countries of the world, has great importance for the consumer, producer, and processing industries. The world's chili pepper and powder production are 4.255.050 tons (FAO, 2019). The seeds are discarded during the processing of red spice pepper. Seeds constitute 30% of the dried fruit's total weight, and these data show that a significant amount of food processing by-products is produced (Yaldız, 2008). However, these byproducts were usually consumed as animal and poultry feed materials, causing resource waste and environmental pollution. Recently, value-added utilization, including industrial byproducts, is gaining interest in the food processing industry.

Red pepper seeds are the most important sources of essential nutrients such as protein, oil, nutritional fiber, and fat-soluble vitamins. They draw attention to their bioactive and functional components having antimicrobial, anti-cancerogenic, and antioxidant activities (Fıratlıgil-Durmuş, 2008). The tocopherol is responsible for reducing the levels of free radicals and preventing peroxidation reactions (Elisia et al., 2013; Baenas et al., 2019). And increasing the total tocopherol content of the oils helps to the enhancement of human health and oil quality. Chouaibi et al., (2019) also reported that red pepper seed oils are excellent sources of tocopherols such as α, ß, γ, and δ. Yang et al., (2010) reported that it has a robust antioxidant mechanism with alpha-tocopherol and capsaicin contents. Also, polyphenols, phytosterols, and aromatic compounds are included in pepper seed oils as important bioactive compounds (Chouaibi et al., 2019). Polyphenols are natural antioxidants because they remove free radicals and play an essential role in the oxidative stability of unsaturated fatty acids (Harborne et al., 1999; Kamal-Eldin 2005). Red pepper seed oil is also rich in carotenoids, especially capsanthin, lutein, and beta-carotene (Konçsek et al., 2018). Carotenoids play an important role in reducing cancer and coronary heart diseases (Reische et al., 2002). It was stated that unsaturated fatty acids are abundant in red pepper seed oil and saturated fatty acids; mainly palmitic, stearic, and myristic acid are present in it (Perez-Galvez et al., 1999; El-Adawy & Taha 2001; Nehir-Demir, 2011; Konçsek et al., 2018). It is suitable for consumption as a food owing to its high oleic and linoleic acid content. This ingredient enables it to be used in the production of margarine, salad, and cooking oil due to its nutritional quality and health benefits (Jarret et al., 2013).

The conventional solvent extraction technique is one of the most widely used methods to extract oil from food. "Soxhlet" extraction is a conventional solvent extraction method that is time-consuming and requires excessive amounts of solvents. Also, these solvents used are toxic to environment and human health. Consumers have been choosing reliable, economical, and environmentally friendly products regarding food safety. Because of that, products are tried to be produced with a green approach (Farr & Proctor, 2014; Siger et al., 2015). The development of new alternative methods has become compulsory (Wang & Weller, 2006). Several new alternatives to conventional methods have been preferred for their advantages, such as high extraction efficiency, ease, short extraction time, reliability, and high-quality products, including cold pressing extraction, ultrasound-assisted extraction, microwave-assisted extraction, and supercritical extraction as the green extraction methods (Tiwari, 2015). These green extraction techniques offer some potential to minimize or eliminate the utilization of toxic solvents and to extract bioactive lipid-soluble compounds while developing a better-quality final product (Ramadan, 2020). Cold press oils are noteworthy in terms of health since it is possible to obtain edible quality oil without the need for refining processes (Moreau & Kamal-Erdin, 2009). Recently, cold-pressed oils have received attention due to their minor bioactive compounds as natural antioxidants and characteristic natural flavor (Bozdoğan-Konuşkan, 2020). In addition, using ultrasonics for oil extraction is preferred to new modern extraction methods due to the high capital investment and high energy consumption disadvantages of these new alternatives (Tiwari, 2015). Ultrasound-assisted extraction is one of the modern, non-thermally effective techniques to obtain bioactive components and oil from different sources. The ultrasonic application enables the transfer of the material by mechanically breaking the cell walls. The extraction process is faster than other extraction methods since the cell wall disappears in this way. Today, the application of ultrasound offers a choice for a solvent that may replace toxic solvents such as hexane, chloroform, diethyl ether, and petroleum ether with alternative solvents such as ethanol and ethyl acetate, which are a GRAS solvent

with a green approach.

The study aimed to produce the oils from red pepper seeds by three green extraction techniques to examine the effect of the methods on the oil extraction yield and on the physical, chemical, and bioactive properties.

MATERIAL and METHOD

Materials

Red pepper (*Capsicum annum* L.) seeds as an industrial food waste were provided by MÜSAN Food Co. Ltd. (Kahramanmaraş, Turkey). The seeds were the post-production seeds of red pepper fruits harvested in the August-October harvest season for spicy red pepper products. The seeds were dried under the sun (moisture content ≤ 6%) within that season. The seeds were ground into a powder with an average particle size of 500 µm by an electrical grinder (model Scm 2934; Sinbo, İstanbul, Turkey). All reagents were provided by Merck (Darmstadt, Germany) and Sigma-Aldrich (St. Louis, ABD) and were of the analytical grade.

Method

First of all, the conditions for selecting the best solvent, temperature and time for the oil yield of the ultrasound assisted solvent extraction technique were determined. Then, the oil samples from the seed were obtained by using Ultrasound-assisted solvent extraction (UAE), solvent extraction (SE), and cold pressing techniques (CP).

Determination of UAE Conditions

The solvent extraction conditions were determined using an ultrasound-assisted solvent extraction technique in the ratio of 1:10 (solid: solvent; w/v). Ethanol, ethyl acetate, and hexane were used as the solvent, and an ultrasound bath (Jeiotech UC-10 brand with 40 kHz ultrasound frequency and max.300 W ultrasound power) was used for UAE. Distinct experiments were executed at 20, 40, and 60 °C of the temperatures for 20 and 40 minutes.

Ultrasound-Assisted Solvent Extraction (UAE)

The ground seeds were exposed to the ultrasound effect at high density by using the optimum conditions determined in the previous section. The supernatants were filtered, and the solvent in the extract phase was volatilized by a rotary evaporator (Hei-VAP Value model, Heidolph, Germany) under vacuum at 40 °C.

Solvent Extraction (SE)

The solvent extraction was the non-ultrasound-assisted conventional solvent extraction. It was performed using the same ultrasound bath Jeiotech UC-10 brand without ultrasonic vibrations. The extraction conditions were carried out using the same UAE procedure method.

Cold Press (CP)

The cold-pressed oil was obtained using a cold-pressing machine (model:6YL-68, Henan Double Elephants Machinery I/E Co., Ltd., China) at the range of 25-60 °C the machine operating temperatures. After the cold-pressing process, the extracted oil was passed through a cloth filter, and then the lipid fraction was centrifugated to purify from solid particles.

Analyses

Seed Analyses

The seed's moisture content (%) was determined gravimetrically according to the AOAC method (AOAC, 2000). The total crude oil and ash content were detected according to AOAC 935.47 method (AOAC, 1998) and the AOCS Ba 5a-49 method (AOCS, 1997a), respectively. Minolta Colorimeter (CR-400) was used to analyze the seeds' a*, b*, and L values. The thickness and diameter of the seeds were investigated by a digital caliper (Asimeto). The weight of 1000 seeds was determined by measuring the weight of 100 seeds (Yılmaz et al., 2015).

Seed Oil Analyses

Oil extraction yields were calculated according to Equation (1).

$$\text{oil yield } \% = [(\text{weight of oil (g)})/(\text{weight of seed (g)})] \times 100 \quad (1)$$

Free fatty acidity (as % oleic acid), peroxide value, and conjugated diene-triene values of the oil samples were measured by AOCS Ca 5a-40 method (AOCS, 1997b), AOCS Cd 8-53 method (AOCS, 1984), and AOCS Ch 5-91 method (AOCS, 1989), respectively. Instrumental color values of the oils were obtained using a Minolta CR-400 colorimeter with CIE Hunter color measurement systems. The refractive index was analyzed with a desktop Abbe refractometer (Atago Refractometer, Tokyo, Japan) at 20°C.

Fatty acid composition

Methyl esters of the fatty acids were prepared using AOCS Ce 1j-07 (AOCS, 2007) and AOAC 996.06 (AOAC, 2005) methods. The fatty acid composition of the oil samples was determined with a Gas Chromatograph (Shimadzu GC-2025) equipped with an Rt-2560 capillary column (100m 0.25mm ID with 0.2 µm film thickness).

Total phenolic content

Determination of the total phenol contents (TPC) of

seed oils was spectrophotometrically carried out by measuring the absorbance at 760 nm with the Folin-Ciocalteu reagent. TPC was expressed as mg of gallic acid (GAE) per kg seed oil (Kozlowska et al., 2016).

Antioxidant activity

The percent of inhibition was calculated by the DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging procedure with some modifications based on the method of Brand-Williams et al., (1995). The antioxidant activity was determined as the percent of inhibition by using Equation (2);

$$\% \text{ Inhibition} = \left[1 - \left(\frac{A_{sample}}{A_{control}}\right)\right] x100 \quad (2)$$

Total carotenoid content

UV-1800 model-vis spectrophotometer (Shimadzu, Kyoto, Japan) was used to measure total carotenoid content according to the method described by Guizhen et al., (2007). Total carotenoid content was calculated according to Equation (3).

$$\text{Total carotenoid content (mg kg}^{-1}) = \frac{Ay\ (ml) \times 10^6}{A^{\%}(cm) \times 1000\ g} \quad (3)$$

where A is the absorbance (at 445 nm), y is the extracting solution quantity (ml), g is the sample weight, $A^{\%}$ cm is the carotenoid molecule's average absorption coefficient 2500.

Total tocopherol content

Total tocopherol analysis was performed by determining the absorbance at 520 nm in UV–vis spectrophotometer of the reaction of iron (II), which is formed because of oxidation of ethanol with iron (III) chloride, with 2-2 dipyridyl (Wong et al., 1988). The outcomes were given as mg tocopherol kg^{-1} oil.

Statistical Analysis

The results were given as the mean ± standard error. Statistical significance was admitted at a level of $P<0.05$. The data were statistically analyzed by a three-way analysis of variance (ANOVA). Duncan tests for solvent-temperature interactions and independent student-T tests for the times were used to compare means (SPSS v.23, IBM, USA).

DISCUSSION

The results of the physical and compositional properties of red pepper seeds are shown in Table 1. The main characteristics of seeds are by the data for the capia pepper seeds reported by Arsunar (2014), except for crude oil content. The crude oil, moisture, and total ash values of the seeds were close to those observed by Chouaibi et al., (2019) and Zou et al., (2015). Especially the oil content in the seed was 20.58%, which was higher than in a *Capsicum frutescens* variety red pepper seed (19.32%) (Fıratlıgil-Durmuş, 2008) and *Capsicum annum* red pepper seed (11.04%) (Azabou et al., 2017), but lower than that in paprika variety seed (25.61%) (El-Adawy & Taha, 2001). Variations in properties may be due to the differences in origin, variety, harvesting time, and growing conditions of the seeds.

Table 1 The physicochemical properties of red pepper seeds

Tablo 1. Kırmızı biber çekirdeklerinin fizikokimyasal özellikleri

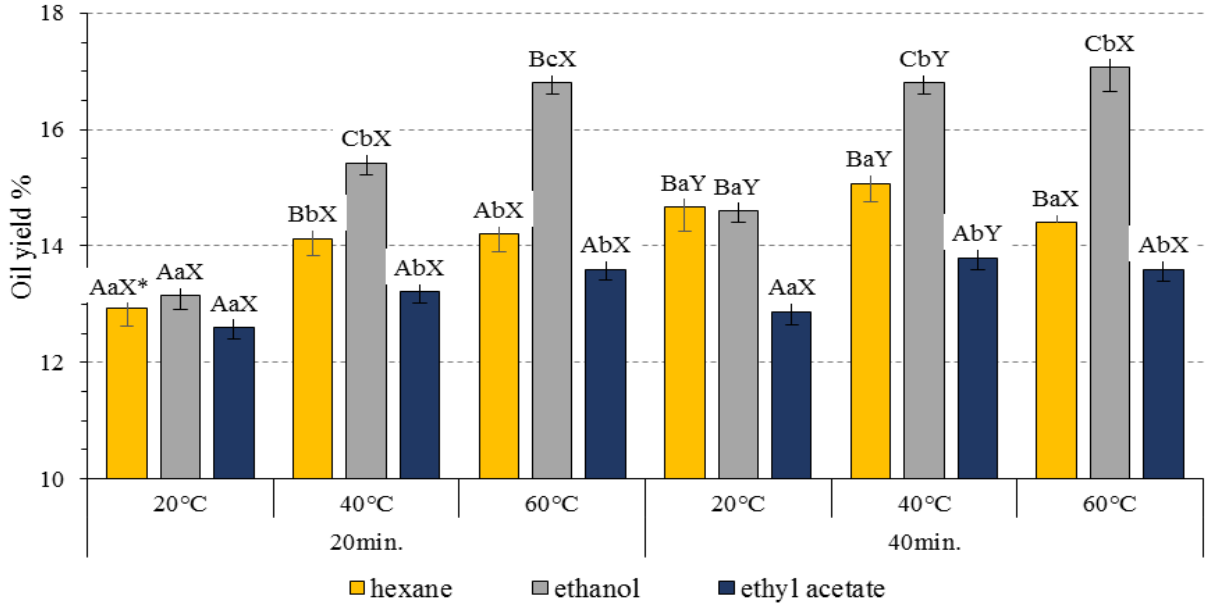
Properties	Seed
Moisture (%)	5.57±0.03
Total ash (%)	3.06±0.02
Crude oil (%)	20.58±0.13
1000-Seed weight (g)	5.69±0.08
Color	
L	53.71±1.20
a*	10.09±0.27
b*	26.52±0.26
Particle size (mm)	
Diameter	3.26±0.16
Thickness	0.74±0.03

Ethanol and ethyl acetate as green alternatives to hexane were used in ultrasound-assisted solvent extractions (UAE) to determinate solvent extraction conditions. The oil yields are presented in Fig. 1. The oil yields were 12.6-16.8% for 20 min of extraction time and 12.9-17.1% for 40 min. The triplet interaction among the time-solvent temperature was statistically not significant ($P>0.05$), but the double interactions were very significant ($P<0.05$). There is no difference between the oil yields of solvents at 20 °C for 20 minutes in the time-temperature relationship. The best yield in this period was observed in ethanol solution at 40 °C. Likewise, the ethanol solution at 40 °C for 40 minutes gave the best yield. There was no difference between the times at temperatures at 60 °C as comparing the times in the solvent-temperature relationship. Differences were found between all the oil yields at 40 °C and the others except the ethyl acetate solvent at 20 °C. It was determined no difference in the yields of 40^{th} minutes of hexane and ethyl acetate solvents as the temperatures in the solvent-time relationship were evaluated. Thus, ethanol was selected as solvent at the extraction conditions of a ratio of 1:10 (w/v), the temperature of 40 °C, and times of 40 minutes.

The previous studies related to solvent extraction support the present data. Ethanol can be used as an alternative solvent for red pepper seeds instead of hexane, commonly used to obtain oil from seeds. It was reported that using green solvents such as ethanol may compete favorably with usual organic solvents and maximize the oil yield from *Echium* seeds. The cavitation effects occur due to different

temperatures and pressures during UAE, depending on the used solvent. This situation results in different fat contents being released into the medium because of varying cell wall disruption levels and mass transfer rates. Also, it was stated that a low temperature, like 40 °C and a short time like, 40 minutes, optimum extraction conditions to obtain high oil yield by avoiding oxidation of fatty acids (Tian et al., 2013; Samaram et al., 2015; Sicaire et al., 2016; Xu et al., 2016; Castejón, 2018).

The physicochemical properties of the red pepper seed oils extracted by CP, UAE, and SE methods are presented in Table 2.



*The same letters on the bars include statistically the similarity at the level of 5% (the series 'A-C' for the differences among the solvents at the similiar time and the same temperatures, series 'a-c' for the differences among the temperatures for each solvent at the same time, and series 'X-Y' for the differences

Figure 1. Oil yield from ultrasound-assisted extraction at different times, temperatures, and solvents
Şekil 1. Farklı çözücü, sıcaklık ve sürelerde uygulanan ultrason-destekli ekstraksiyonu yağ verimleri

Table 2. Physicochemical properties of red pepper seed oils
Tablo 2. Kırmızı biber çekirdek yağlarının fizikokimyasal özellikleri

Property	CP	UAE	SE
Oil Yield (%)	11.32±0.07^{c}	16.80±0.12^{a}	14.50±0.17^{b}
Refractive Index (20°C)	1.4765±0.0001^{a}	1.4747±0.0001^{c}	1.4757±0.000^{b}
Free Fatty Acidity (% oleic acid)	3.97±0.05^{c}	6.25±0.21^{b}	8.59±0.12^{a}
Peroxide Value (meq O_2 kg^{-1} oil)	8.33±0.33^{b}	9.83±0.33^{a}	6.67±0.33^{c}
Color			
L	23.96±0.00^{b}	24.39±0.01^{a}	22.56±0.00^{c}
a*	9.00±0.03^{b}	10.66±0.01^{a}	8.76±0.03^{c}
b*	7.52±0.01^{b}	8.79±0.02^{a}	6.42±0.03^{c}
Conjugated Diene Value (K_{232})	7.69±0.03^{b}	8.63±0.02^{a}	7.07±0.03^{c}
Conjugated Triene Value (K_{270})	1.92±0.04^{c}	3.36±0.04^{a}	2.41±0.03^{b}

^{a,b,c} Values within a row with different superscripts differ significantly at P<0.05.

There are not enough similar studies to compare the data about UAE oil extraction for all the mentioned analyses. The ultrasonic procedure provides easy transfer of the oil out of the cell by mechanically breaking the cell walls, which is the leading oil extraction hurdle by pressing from oilseeds and increasing efficiency. Thus, oil extraction was faster than other methods with ultrasound applications. Higher oil yield was obtained with ultrasound application from chia seeds compared to the extraction procedure without ultrasound in a study by De Mello et al., (2015). Additionally, the results obtained in the present study based on the effect of

extraction methods on oil yield agree with the results by Li et al., (2015), Sicaire et al., (2016), and Moradi et al., (2018).

The free fatty acidity values of the oil samples varied between 3.97% and 8.59%, and the free fatty acid content of the cold press extracted seed oil was lower than the values reported by other methods ($P<0.05$). This is thought to be due to the polarity of the solvent used in solvent extractions. According to the determined results, the free fatty acidity value of cold extracted seed oil was like the one found by Yılmaz et al., (2015) but higher than those reported by Chouaibi et al., (2019) and Domokos et al., (1993). The results for solvent extractions were higher than those obtained by El-Adawy and Taha (2001) and show like results obtained by Jarret et al., (2013).

The refractive index (20 °C) value for CP (1.4765) was significantly higher than those obtained by UAE (1.4747) and SE (1.4757) ($P<0.05$). A study by Shahidi (2005) suggested that the refractive index values of oils are varied depending on the degree of unsaturation, molecular weight, chain length of the fatty acids, and degree of conjugation. The refractive index value of CP oil in the present study was higher than that obtained by Chouaibi et al., (2019) and Arsunar (2014). It was observed that the refractive index values were slightly lower than the values obtained in the present study comparing the study with pepper seed oil by Ma et al., (2019).

Peroxide value (PV) is one of the main quality criteria of oils (Codex Alimentarius Commission, 1982). It is due to the concentration of peroxides and hydroperoxides that occurred in the initial phase of lipid oxidation (Zhang et al., 2010). PV of the oil samples ranged from 6.67 to 9.83 meq O_2 kg^{-1} oil. The peroxide values of CP and SE oils were significantly lower than that of UAE-extracted oil ($P<0.05$). The formations of primary oxidation products (conjugated diene, peroxide) increase in oils as the intensity of ultrasound increases (Hosseini et al., 2015). The results were within the average values defined by the Food Standards Commission for vegetable oils; that is, peroxide values of the oil samples did not exceed 15 meq O_2 kg^{-1} oil limitation (Turkish Food Codex 2012).

The UAE oil had higher L*, a*, and b* values. A significant difference in luminosity was determined in the samples ($P<0.05$). L*, a*, and b* values of the SE oil were lower than that of the oils extracted by other methods. The results showed that the UAE was darker-colored and had more redness-yellowness than the other oils. That darker color may be related to Maillard's reactions. This result was in agreement with the report of Hosseini et al., (2015).

The oxidative degradation level in oils can be determined more effectively by measuring the conjugated diene (K_{232}) and triene (K_{270}) formation together with the peroxide value (Kıralan & Kıralan 2017). The conjugated diene (CD) values of the oil samples ranged from 7.07 to 8.63, and also the conjugated triene (CT) values ranged from 1.92 to 3.36. Other oils extracted were significantly lower than UAE oil's CD and CT values ($P<0.05$). There are no studies about CD and CT values of CP and UAE oil. The results obtained from SE were higher than that of solvent extraction with hexane by Azabou et al., (2017). These high values indicate that *C. annum* seed oil contains high amounts of primary and secondary oxidation products, such as hydroperoxides and aldehydic carbonyl components (Azabou et al., 2017). There was a good correspondence between these results and the findings of Hosseini et al., (2015), who studied the effect of ultrasound on the physicochemical properties of some edible oils. Similarly, Hosseini et al., (2015) found that the UAE increased CD and CT values compared to the control methods. These results demonstrated that as the intensity of ultrasound, the formation of primary oxidation products (peroxides and conjugated dienes) increases, and the ultrasound (cavitation phenomenon) causes the rapid increase of primary oxidation of edible oils.

The fatty acid compositions of the oils extracted by CP, UAE, and SE techniques are presented in Table 3.

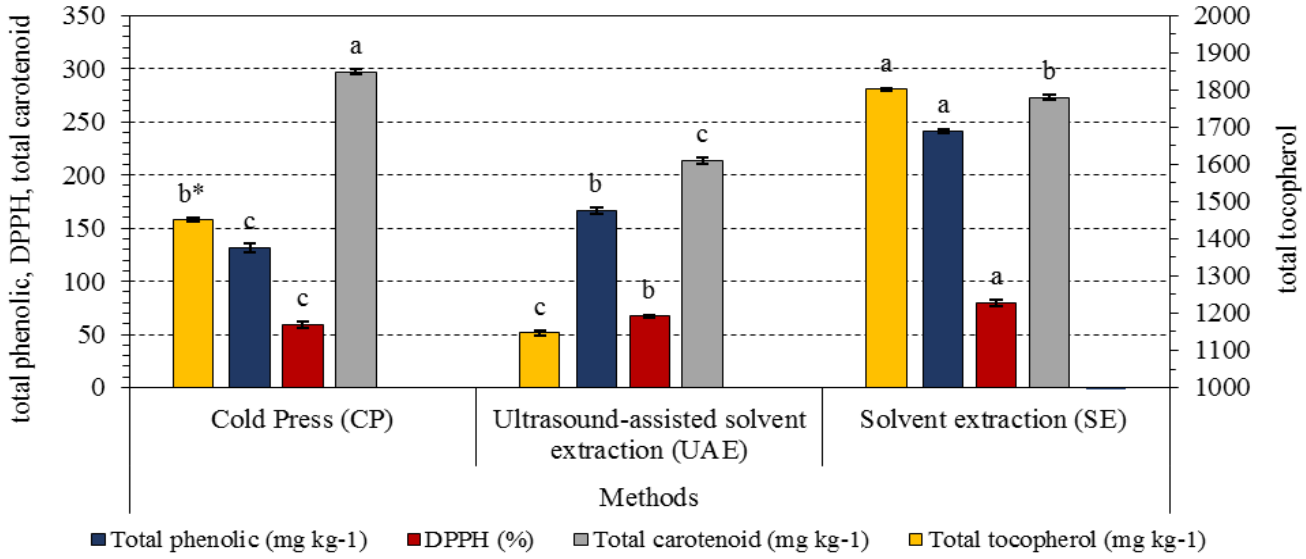
While the predominant unsaturated fatty acids of all samples were oleic and linoleic acid, the predominant saturated fatty acids were stearic and palmitic acid. The linoleic acid contents were determined to be 72.194, 72.052, and 71.826% for SE, CP, and UAE oils, respectively. The oleic acid contents were found to be 11.840, 11.808, and 11.670% for SE, UAE, and CP oils, respectively. Alfa-linolenic acid ($C_{18:3\ n-3}$), palmitoleic acid ($C_{16:1}$), arachidic acid ($C_{20:0}$), myristic acid ($C_{14:0}$), cis-11-eicosenoic ($C_{20:2}$), lignoceric ($C_{24:0}$), and behenic ($C_{22:0}$) acids were present in minor amounts. Nevertheless, there are no significant differences between extraction techniques applied to the oils in terms of prominent fatty acids (linoleic and palmitic acid) and also cis-11-eicosenoic acid content ($P>0.05$). The differences in the composition and ratio of fatty acids may be related to the affinity of the fatty acids to the solvent and the solubility. Solubility may depend on different concentration ranges, extraction temperature, and pressure. When compared with these studies reported for the samples, it was observed that the palmitic, linoleic acid, linolenic, stearic, and oleic acid content was in the range of 11.08-13.84%, 67.77-80%, 0.278-0.380%, 2.71-3.40% and 7.90-14.56%, respectively. The results obtained from the present study are in agreement with those reported by Domokos et al., (1993), Pérez-Gálvez et al., (1999), El-Adawy and Taha (2001), Fıratlıgil-

Durmuş (2008), Matthaus and Özcan (2009), Li et al., (2011), Embaby and Mokhtar (2011), Arsunar (2014), Konçsek et al., (2018), Azabou et al., (2017), Chouaibi et al., (2019), Ma et al., (2019).

Table 3. The fatty acid compositions of red pepper seed oils (%)
Tablo 3. Kırmızı biber çekirdek yağlarının yağ asit kompozisyonları (%)

Fatty acid	CP	UAE	SE
Myristic acid ($C_{14:0}$)	0.238±0.002^{a}	0.223±0.005^{b}	0.196±0.000^{c}
Palmitic acid ($C_{16:0}$)	11.290±0.040	11.702±0.211	11.290±0.004
Palmitoleic acid ($C_{16:1}$)	0.203±0.002^{b}	0.273±0.031^{a}	0.213±0.001^{ab}
Stearic acid ($C_{18:0}$)	3.211±0.029^{a}	3.026±0.009^{c}	3.115±0.020^{b}
Oleic acid ($C_{18:1n9c}$)	11.670±0.045^{b}	11.808±0.021^{a}	11.840±0.044^{a}
Linoleic acid ($C_{18:2n6c}$)	72.052±0.037	71.826±0.200	72.194±0.075
Arachidic acid ($C_{20:0}$)	0.324±0.003^{a}	0.281±0.002^{c}	0.291±0.001^{b}
cis-11-eicosenoic ($C_{20:2}$)	0.132±0.001	0.137±0.007	0.133±0.002
α-Linolenic acid ($C_{18:3n3}$)	0.344±0.003^{a}	0.297±0.000^{b}	0.285±0.000^{c}
Behenic acid ($C_{22:0}$)	0.232±0.002^{a}	0.189±0.003^{c}	0.199±0.001^{b}
Lignoceric acid ($C_{24:0}$)	0.304±0.003^{a}	0.238±0.006^{b}	0.244±0.010^{b}

^{a,b,c} Values within a row with different superscripts differ significantly at P<0.05.



*The same letters on the bars include statistically the similarity among the extraction methods at the level of

Figure 2. The total phenolic content, DPPH%, total carotenoid content, and total tocopherol content of the oils
Şekil 2. Yağların toplam fenollik içeriği, %DPPH, toplam karotenoid ve toplam tokoferol içerikleri

It was shown that the total phenolic content (TPC), antioxidant activities (DPPH%), total carotenoid content (TCC), and total tocopherol content (TTC) of the oil samples are in Figure 2. TPC ranged from 131.8 to 241.1 mg kg^{-1} of oil (P<0.05). SE oil included excellent amounts of total phenolics, followed in decreased order by UAE and CP oils. The results show that the CP technique is unsuitable for producing an oil rich in total phenol. The reason is the inability of polyphenols to leak from the cell wall, as the pressure can sometimes be applied irregularly in cold pressing. However, it was observed that the application of ultrasound caused a statistically significant decrease in the phenolic content of the seed oil (P<0.05). Polyphenol content positively affects oxidative stability, nutrition, and health. The amount and types of phenolic substances contained in edible oils may alter depending on many factors, such as seed type, growing conditions, climate, processing, or extraction techniques. The TPC of the samples was higher than those reported by Chouaibi et al., (2019) but agree with those obtained by Jimenez et al., (2007). Already, Jimenez et al., (2007) reported that ultrasound assessment had a significant effect on polyphenols, which can lead to a decrease in TPC.

The samples's equivalent radical scavenging activities

(DPPH%) ranged from 59.04 to 79.84%. The antioxidant capacity was observed at the highest level in SE oil and the lowest level in CP oil (P<0.05). Red pepper seed oil has a high antioxidant capacity due to its high content of vitamin E, polyphenols, and unsaturated fatty acids; mainly phenolic and tocopherol components are the most effective antioxidants (Fazel et al., 2008). There is a positive correlation between the samples' DPPH % and TPC values. Ma et al., (2019), Zhong et al., (2018), and Delfan-Hosseini et al., (2017) also reported similar results.

TCC of the CP, SE, and UAE oils were 296.80, 272.67, and 213.73 mg kg^{-1} oil, respectively. The techniques have statistically significant effects on the carotenoid content of the extracted oils (P<0.05). The variety of seeds, cultivation conditions, seasonal factors, extraction techniques, and these factors' interactions significantly affect the carotenoid content of oil. Further, it is thought that thermal-sonication applications decrease carotenoid content due to causing for destroying some carotenoid compounds (Konçsek et al., 2018). While there is not any study related to the carotenoid content of SE and UAE oils, the findings for CP oil are lower than that obtained by Domokos et al., (1993) and Konçsek et al., (2018) and higher than the results of Chouaibi et al., (2019).

The extraction techniques significantly affected the tocopherol contents (P<0.05). TTC of the oils were 1450.2, 1146.8, and 1801.2 mg kg^{-1} oil for CP, UAE, and SE, respectively. These results showed that tocopherols are more extractable by chemical techniques using organic solvents. In general, it has been observed that ultrasound application affects and causes statistically a decrease in the total tocopherol content (P<0.05). This situation may come from the increased temperature during the ultrasound application. TTC of CP oil sample was significantly higher than that reported by Domokos et al., (1993), Konçsek et al., (2018), Chouaibi et al., (2019), but was lower than that found by Arsunar (2014).

CONCLUSION

This study examined oil extraction efficiency from red pepper seed assisted by ultrasound, ethanol solvent, and cold-pressing as green techniques. The results revealed that red pepper seeds might be an excellent potential food source thanks to their valuable content and chemical, biological and ecological importance. So, there more studies are needed for their functional properties. It has been observed that the applied techniques provide different advantages in terms of oil yield and quality parameters. By ultrasound-assisted solvent extraction, a modern non-thermal procedure, it was possible to obtain maximum yield oil. It was concluded that the solvent extraction method is more effective producing oils by preserving their bioactive components and that it is preferable to obtain red pepper seed oil. The use of toxic solvents was eliminated by these green extraction techniques applied in this study, and better-quality seed oils were produced with a green approach regarding food safety. Although one of the most widely used methods for obtaining edible quality vegetable oils is the cold pressing method, this method yields oil with low efficiency. Research on the discovery of new resources needed by the food industry and efficient use of available resources should be increased. At this point, industrial waste products have been evaluated with this study, and necessary steps have been taken.

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Contribution of the Authors as Summary

The consultancy of this study was carried out by ALI, and the planning was carried out by ALI and ABA. Laboratory analyzes of the study were done by ABA. The authors contributed to the article writing and ALI reviewed and approved the final version.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

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Arıtma Çamurunun Kadife Çiçeği (*Tagetes erecta* L.) ve Yer Minesi (*Verbena hybrida*) Bitkileri ile Toprağın Besin Elementleri ve Ağır Metal Üzerine Etkisi

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ÖZET

Bu çalışma, kadife çiçeği (*Tagetes erecta* L.), Yer minesi (*Verbena hybrida*) süs bitkilerinin Biyokonsantrasyon faktörü (BCF), bitki besin elementi ve ağır metal konsantrasyonları ile hasattan sonra her saksıdan alınan topraktaki ağır metal konsantrasyonunu üzerine arıtma çamurunun etkilerini belirlemek amacıyla yapılmıştır. Saksı denemesi tesadüf blokları deneme desenine göre 3 tekrarlı olarak yürütülmüştür. Arıtma çamuru/toprak karışımları (w/w) aşağıdaki şekilde karıştırılmıştır: %0 arıtma çamuru+%100 toprak (kontrol), %3 arıtma çamuru+%97 toprak (%3 SS), %6 arıtma çamuru+94 toprak (%6 SS) ve %9 arıtma çamuru +%91 toprak (%9 SS). BAC değerlerine göre Marigold Zn, Cd elementleri ve Garden mineçiçeği ise Zn elementi için hiperakümülatör bitki olabilir. Kadife çiçeği ve Yer minesinin N, P, K, Mg ve Ca makro bitki besin elementleri en yüksek değerleri sırasıyla %9 SS ve %6 SS uygulamalarından almıştır. Kadife çiçeği, en büyük Na (784 mg kg^{-1}) %9 SS, Fe (2236 mg kg^{-1}) %9 SS, Cu (7. 4 mg kg^{-1}) %9 SS, Zn (136 mg kg^{-1}) %6 SS, Mn (142 mg kg^{-1}) %6 SS ve B (42 mg kg^{-1}) kontrol uygulamalarından elde edilmiştir. Yer minesi bitkisinde, en büyük Na (696 mg kg^{-1}) %6 SS uygulamasından, Fe (1700 mg kg^{-1}) %6 SS, Cu (12 mg kg^{-1}) %6 SS, Zn (115 mg kg^{-1}) %6 SS, Mn (100 mg kg^{-1}) %3 SS ve B (47 mg kg^{-1}) kontrol uygulamalarından saptanmıştır. Ağır metaller (Ni, Cd, Cr, Pb, As ve Hg) bakımından kadife çiçeği ve bahçe mineçiçeği bitkilerinde toksisite etkilerine rastlanmamıştır. Toprak ağır metal (Ni, Cd, Pb, As ve Hg) seviyeleri limit değerlerin altında belirlenmiştir.

Toprak Bilimi

Araştırma Makalesi

Makale Tarihçesi
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Anahtar Kelimeler
Ağır metal
Arıtma çamuru
Biyokonsantrasyon faktörü
Bitki besin elementleri
Süs bitkileri

Effect of Sewage Sludge on Marigold (*Tagetes erecta* L.) and Garden Verbena (*Verbena hybrida*) Plant and Soil Nutrient Elements and Heavy Metal

ABSTRACT

This study was carried out to determine the effects of sewage sludge (SS) treatments on bioconcentration factor (BCF), plant nutrients, heavy metal marigold (*Tagetes erecta* L.), garden verbena (*Verbena hybrida*) ornamental plants and soil taken from the pot after harvest is to determine heavy metal concentration. Pot experiments conducted randomized blocks design with 3 replications. Sewage sludge/soil mixtures (w/w) was arranged: 0% SS+%100 soil (control), 3% SS+ 97% soil (3 % SS), 6% SS+94 % soil (6% SS) and 9% SS +91% soil (9% SS). According to BAC values Marigold can be for Zn, Cd and Garden verbena can be hyperaccumulator plant for Zn element. Marigold and garden verbena, the greatest N, P, K, Mg, and Ca plant nutrients were respectively obtained from 9% SS and 6% SS treatments. Marigold, the greatest Na concentration (784 mg kg^{-1}) was obtained from 9% SS, Fe (2236 mg kg^{-1}) 9% SS, Cu (7.4 mg kg^{-1}) 9% SS, Zn (136 mg kg^{-1}) 6% SS, Mn (142 mg kg^{-1}) 6% SS and B (42 mg kg^{-1}) control treatments. Garden verbena, the greatest Na concentration (696 mg kg^{-1}), was

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obtained at 6% SS, Fe (1700 mg kg^{-1}) 6% SS, Cu (12 mg kg^{-1}) 6% SS, Zn (115 mg kg^{-1}) 6% SS, Mn (100 mg kg^{-1}) 3% SS and B (47 mg kg^{-1}) control treatments. Heavy metals (Ni, Cd, Cr, Pb, As and Hg) toxicity impacts were not encountered on marigold and garden verbena plants. Soil heavy metal (Ni, Cd, Pb, As and Hg) levels were below the threshold values.

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INTRODUCTION

Together with the global increase in population, food demands are also increasing. To meet such increasing needs, agricultural production should be increased. The world population is expected to rise by 9.3 billion by the year 2050. Thus food demand is expected to increase by about 60% within the next 30 years (Lee, 2011). Urbanization and industrialization increase sewage sludge generation of these processes. Just because of human and environmental health concerns, proper methods should be used in the disposal of sewage sludge. Sewage sludge (SS) is an inevitable by-product of wastewater treatment and a severe pollution source if not disposed of properly (Song et al., 2014). There are three primary methods used in the disposal of sewage sludge: soil application, storage and incineration. In some cases, agricultural use as a fertilizer is seen as the best option for disposal of sewage sludge (Sanchez et al., 2004). Increasing inorganic fertilizer costs and a large quantity of SS generations worldwide have led researchers to investigate the potential use of the land application as an alternative disposal method for sewage sludge (Petersen et al., 2003). In recent years, it has been observed that treatment sludge began to be used as a source of organic matter in the creation of landscapes and green areas in the world. Agricultural use of sewage sludge not only offers an affordable means of disposal but also improves soil fertility and physical properties, thereby enhancesing crop productivity. Moreover, it facilitates nutrient recycling and reduces the cost of cultivation (Swain et al., 2021). Sewage sludge use in agriculture has been recognized as an environment-friendly management technique (Beidokhti et al., 2019). It acts as a vital source of organic matter (OM) for agricultural soils; moreover, it provides essential macro and micronutrients for plant growth and development (Latare et al., 2018; Karkush & Aljorany, 2019). The positive response of various crops, such as rice and wheat (Latare et al., 2017), spinach (Golui et al., 2014), Grass-legume (Bozkurt et al., 2020) apple trees (Bozkurt & Yarılgaç, 2003) and Maize (*Zea mays* L.) (Çakır & Çimrin, 2020) sewage sludge application has been reported. Sewage sludge was applied as 20 kg m^2 year-1 dose on plant growth of Liquidambar Orientalis species (Demirkan & Söğüt, 2018). The sewage sludge (sludge and soil mixed at a volume ratio of 1:10, 3:10 and 5:10) was applied to the Sedum lineare ornamental plant at different ratios (Peng et al., 2017). Ornamental plants are the best choices because they provide environment-enhancing green spaces and offer commercial products (Mir et al., 2019). On the other hand, being an ornamental crop due to the interest in its flowers reduces the risk of contamination of the food chain (Chitraprabha & and Sathyavathi, 2018).

The aim of this study was to evaluate the effects of different doses of municipal sewage sludge applications on Bioconcentration factors (BCF), nutrient concentration (N, P, K, Mg, Ca, Na, Fe, Cu, Zn, Mn and B) and heavy metal levels of concentration (Ni, Cd, Cr, Pb, As and Hg) ornamental plants of marigold (Tagetes erecta L.) and garden verbena (Verbena hybrida) and soil taken from each pot after harvest is to determine the heavy metal concentration (Zn, Cu, Ni, Pb, Cd, As and Hg) in the samples according to the applications.

MATERIAL and METHOD

Material

This study started in the April 2015 pot experiments under greenhouse conditions. Bornova town of Izmir province (38° 27' 12.5" N, 27° 13' 40.2" E). The soil used in this study was taken from a depth of 0-20 cm in a farm in the city of the Research, Application and Production Farm of Ege University Agriculture Faculty and transferred to the laboratory and dried at laboratory temperature. The physical and chemical characteristics of soil are presented in Table 2. After air drying, the soil samples were passed through a 4 mm sieve. The soil is classified as Typic xerofluvent (Soil Survey Staff, 2010). The physicochemical

properties of soil amended by municipal sewage sludge (SS) and growth of Marigold (*Tagetes erecta* L.) and garden verbena (*Verbena hybrida*) plants were grown in two separate plots in amended soil with pot experiments under greenhouse (1.50 m x 21 m) conditions. The granulated dry municipal sewage sludge was supplied from the Wastewater Treatment Plant of İzmir Greater City Municipality. Sewage sludge was applied at different doses to Marigold (*Tagetes erecta* L.) and garden verbena (*Verbena hybrida*) plants. Analysis results of treated sewage sludge and experiments soil are given in table 1 and table 2.

Table 1. Analysis results of sewage sludge
Çizelge 1. Arıtma çamurunun analiz sonuçları

Properties	Values	Properties	Values
pH	7.18±0.14	Na (mg kg^{-1})	1391±22.11
EC (dS m^{-1})	1.95±0.11	Fe (mg kg^{-1})	12755±124.53
C:N ratio	9.90±0.51	Cu (mg kg^{-1})	177±7.21
C_{org} (%)	29.66±1.18	Zn (mg kg^{-1})	1377±32.14
		Mn (mg kg^{-1})	350±20.12
N(%)	2.99±0.50	Ni (mg kg^{-1})	69.73±3.01
P (%)	0.23±0.12	Pb (mg kg^{-1})	17.44±1.11
K (%)	0.34±0.18	Cr (mg kg^{-1})	112.5±2.32
Ca (%)	6.36±1.15	Cd (mg kg^{-1})	2.83±0.21
Mg (%)	2.04±0.21	B (mg kg^{-1})	16.10±0.34

According to the Turkish directives, the heavy metal limits (mg kg^{-1}) for sewage sludge use in agriculture are as follows: Cd 10, Cr 1000, Ni 300, Pb 750, Cu 1000 and Zn 2500. The sludge used for this study contains the heavy metal concentrations (mg kg^{-1}) as follows: Cd (2.83±0.21), Cr (112.5±2.32), Ni (69.73±3.01), Pb (17.44±1.11), Cu (177±7.21) and Zn (1377±32.14). Thus, the concentrations of sewage sludge heavy metals in this study were lower than the permitted limits (Anonymous, 2010). Since sewage sludge was brought to suitable physical conditions to be used in plant production, it was directly used in growing medium mixtures without any further processing. Experiments were conducted in randomized blocks design with 3 replications. Soil and sewage sludge mixtures prepared at specific proportions were placed on top of this drainage layer. To measure the performance of sewage sludge as a growing medium, essential fertilizers were not used in the present experiments. About 5 cm gravel drainage layer was placed beneath the sowing boxes. Soil and sewage sludge mixtures were placed on top of this drainage layer. Fertilizers were not applied to measure the sole performance of sewage sludge as a growing medium. Marigold (*Tagetes erecta* L.) and garden verbena (*Verbena hybrida*) plants were grown in two separate plots. Five seedlings were planted in each plot, and a total of 20 seedlings (5 seedlings x 4 replicates) were planted on 28.04.2015.

Table 2. Analysis results of experimental soil
Çizelge 2. Deneme toprağının analiz sonuçları

Properties	Values	Properties	Values
pH	7.64±0.12	Ca (mg kg^{-1})	2162±25.83
EC (dS m^{-1})	0.44±0.10	Na (mg kg^{-1})	31.17±3.16
Texture	Sandy-loam	Fe (mg kg^{-1})	2.65±0.51
Sand (%)	55.84	Cu (mg kg^{-1})	0.89±0.11
Silt (%)	31.44	Zn (mg kg^{-1})	2.08±0.15
Clay (%)	12.72	Mn (mg kg^{-1})	1.83±0.32
$CaCO_{3\ (\%)}$	4.74±0.32	Ni (mg kg^{-1})	53.09±2.73
C_{org} (%)	0.876±0.21	Pb (mg kg^{-1})	13.34±1.21
N (%)	0.081±0.04	Cr (mg kg^{-1})	25.57±1.54
P (mg kg^{-1})	14.66±2.13	Cd (mg kg^{-1})	0.75±0.18
K (mg kg^{-1})	237±10.20	B (mg kg^{-1})	1.07±0.08

In this experiment, pots with a top diameter of 40 cm, the height of 40 cm, and a capacity of 36 kg were used. Soil moisture content was maintained at 70% water holding capacity (WHC). Experiments had different treatments sewage sludge/soil mixtures (v/v) was mixed with soil in the following proportions: 0%

sewage sludge+%100 soil (36 kg) (applications control), 3% sewage sludge (0.850 kg)+ 97% soil (35.15 kg) (applications 3 % SS), 6% sewage sludge (1.94 kg)+94 % soil (34.06 kg) (applications 6% SS) and 9% sewage sludge (3.03 kg)+91% soil (32.97 kg) (applications 9% SS). Throughout the experiments, irrigations were performed based on soil field capacity. Pots were watered every 4 days with deionized water to maintain soils close to 70% of WHC throughout the experimental period and the leaked water returned into the pots in order to prevent losses. Chemicals were applied against louse afid and pests. The study was carried out between 28 April and 15 July 2015. The total duration of the experiment was 79 days.

Method

The soil material used in the present experiments was sieved and made available to be used in mixtures. Three wooden sowing boxes with 12 cells were used in the present experiments. Each cell has dimensions of 40x40x20 cm and was filled with 4 different growing media all, including gravel, garden soil and sewage sludge. Soil samples were air-dried and passed through a 2 mm sieve. pH was measured in (1:2.5 soil: water) extract, and soil salinity (EC, dS m^{-1}) was measured in 1:2.5 soil: water extracts. Organic matter (C_{org}) was determined using the Modified Walkler-Black method (Jackson, 1973). Lime ($CaCO_3$) was measured with a Scheibler calcimeter (Nelson, 1982). The texture was determined with the hydrometer method (Bouyoucos, 1962). Total nitrogen (N) with modified Kjeldahl method (Bremner, 1965). Available phosphorus was determined by Olsen method (Olsen & Dean, 1965). Soil available Na, K and Ca concentration were determined using 1 N ammonium acetate extraction (NH_4OAc, pH=7) (Pratt, 1965). Sample K, Ca, and Na were determined in a flame photometer. Soil Fe, Cu, Zn and Mn were determined through the extractions with DTPA solution (Lindsay and Norvell, 1978). Soil Fe, Cu, Zn and Mn concentrations were determined in atomic absorption spectrophotometer (AAS) (Hanlon, 1992). Soil total Zn, Cu, Ni, Pb, Cd, Cr, As and Hg concentrations were determined in HCl and HNO_3 (aqua regia 3:1, v/v) extracts, and Hg concentration was determined in cold-vapor atomic absorption spectrophotometer (Kacar & İnal, 2008). Boron concentration was determined by hot-water extract with azomethine-H method (Wolf, 1971). The principal chemical properties of sewage sludge sample In particular, the pH was measured on mixtures of sewage sludge: water 1:5; the EC was measured on a 1:5 sewage sludge sample: water ratio extract and the organic matter (C_{org}) Modified Walkler-Black method (Jackson, 1973). Total nitrogen (N) with modified Kjeldahl method (Bremner, 1965). Sewage sludge samples after nitric and perchloric acid digestion(HNO_3; $HClO_4$; 4:1, v/v). Total P spectrophotometric analysis with the use of vanadomolibdo phosphoric yellow color method (Lott et al., 1956). Total K, Ca, and Na concentrations were determined in a flame photometer, Mg, Fe, Zn, Cu, Mn, Ni, Cd, Cr, Pb, As and Hg measurements were performed by using cold vapour atomic absorption spectrophotometer (AAS) (Kacar & İnal, 2008). Following dry-ashing, sample B concentration was determined spectrophotometrically with the use of an azomethine-H method (Wolf, 1971). The plants were harvested carefully for analysis after 13 weeks of planting. Leaves were collected from the mid-third sections of each of the plants (Coelho et. al., 2017). All samples were washed to remove any adhering soil particles and rinsed with distilled water. The plant samples were dried at 65-70 °C for 48 h and then grinded and made ready for analysis. Total Nitrogen (N) analysis was conducted by modified Kjeldahl method (Bremner, 1965). Plant samples acid-digestion (HNO_3; $HClO_4$; 4:1, v/v) was performed before nutrient analysis. Total P spectrophotometric analysis with the use of vanadomolibdo phosphoric yellow color method (Lott et al., 1956). Total K, Ca and Na concentration were determined in a flame photometer, and Mg, Fe, Zn, Cu, Mn, Ni, Cd, Cr, Pb, As were determined by atomic absorption spectrophotometer. Mercury (Hg) measurement was performed by using a cold vapour atomic absorption spectrophotometer (Kacar & İnal, 2008). Boron (B) concentration dry-ashing was determined spectrophotometrically with the azomethine-H method (Wolf, 1971).

Bioconcentration Factors (BCF); Metal loads were calculated using the bioconcentration factor (BCF) as: BCF=metal concentration in the plant (mg kg^{-1}dw)/metal concentration in soil (Chang et al., 2014).

Statistical Analysis; Statistical analyses were conducted with the use of SPSS Statistics 20.0 software in accordance with randomized blocks design. Significant means were compared with the use of Duncan's multiple range test at α=0.05 significance level. Differences between the treatments were significant at $P < 0.01$ or $P < 0.05$.

RESULTS and DISCUSSION

Bioconcentration Factors (BCF)

In Marigold (*Tagetes erecta* L.) plants, bioconcentration factors (BCF) were identified as Zn (1.11±0.11-1.34±0.16), Cu (0.27±0.05-0.34±0.02), Ni (0.17±0.02-0.28±0.05), Pb (0.10±0.04-0.14±0.04), Cd (0.16±0.08-1.17±0.21), As (0.02±0.005-0.03±0.006) and Hg (0.22±0.10-0.43±0.12). Bioconcentration factor (BCF) was as follows for Marigold (*Tagetes erecta* L.): Zn>Cd>Hg>Cu>Ni>Pb>As. In garden verbena (*Verbena hybrida*) plants, bioconcentration factors (BCF) were identified as Zn (1.04±0.10-1.17±0.15), Cu (0.34±0.03-0.52±0.07), Ni (0.16±0.04-0.19±0.03), Pb

(0.10±0.03-0.14±0.04), Cd (0.22±0.11-0.27±0.14), As (0.03±0.001-0.04±0.005) and Hg (0.19±0.10-0.31±0.12) (Table 3). Bioconcentration factor (BCF) was as follows for garden verbena (*Verbena hybrida*): Zn>Cu>Hg>Cd>Ni>Pb>As. In both plants, the highest BAC value was determined for Zn element and the lowest BAC value for As. Analyzing the results of the bioconcentration factor (BCF) for the tested plants, it was noted that the Marigold plant accumulated Zn and Cd more easily, followed by Hg, Cu, Ni and Pb, and then As to a lesser extent. The values of factors for Cd and Zn were correlated with the high mobility of these elements compared to other metals and their relatively easy plant uptake. The lowest values of the BCF found for Pb and As are due to the lowest mobility of Pb and As from soil to plant tissues (Pusz et al., 2021). The content of metals in plants can vary depending on their ability to move from soil to aboveground parts (Awa & Hadibarata, 2020). Khan et al. (2008) also suggested that the BCFs of Cd and Zn were high in brassica plants. Cd is the metal most susceptible to accumulation from the soil by plants (Kabata-Pendias & Mukherjee, 2007). Translocation factor and BCF are a vital indices to determine the phytoremediation capability of plants, and it was well reported that plants revealing BCF values >1 could be favorable for phytoextraction (Chanu & Gupta, 2016).

BCF of less than 1 means more heavy metals concentration in soil than those taken up by plants (Hellen and Othman, 2016). It further enables the categorization of plants as accumulators (BAC>1) or excluders (BAC<1) of trace elements (Olowoyo et al., 2010).

In the present study for Zn and Cd, BCF was>1. Therefore, marigold plant can be a hyperaccumulator plant for Zn, and Cd elements and the Garden verbena plant can be a hyperaccumulator plant for Zn element (Biswal et al., 2022).

Table 3. Average BCF values of plants
Çizelge 3. Bitkilerin ortalama BCF değeri

Plants	Treatments	Zn	Cu	Ni	Pb	Cd	As	Hg
Marigold	Control	1.17±0.10	0.34±0.02	0.25±0.04	0.11±0.04	0.16±0.08	0.02±0.005	0.39±0.11
	% 3 SS	1.11±0.11	0.31±0.02	0.17±0.02	0.10±0.03	0.62±0.12	0.02±0.005	0.34±0.10
	% 6 SS	1.34±0.16	0.28±0.04	0.27±0.04	0.14±0.04	0.99±0.14	0.03±0.006	0.22±0.10
	% 9 SS	1.12±0.14	0.27±0.05	0.28±0.05	0.12±0.04	1.17±0.21	0.03±0.006	0.43±0.12
G.verbena	Control	1.04±0.10	0.34±0.03	0.16±0.04	0.14±0.04	0.22±0.11	0.03±0.001	0.26±0.11
	% 3 SS	1.05±0.10	0.45±0.05	0.16±0.05	0.10±0.03	0.23±0.12	0.04±0.005	0.25±0.11
	% 6 SS	1.17±0.15	0.52±0.07	0.18±0.04	0.13±0.05	0.27±0.13	0.03±0.002	0.31±0.12
	% 9 SS	1.13±0.12	0.43±0.08	0.19±0.03	0.13±0.04	0.27±0.14	0.03±0.003	0.19±0.10

Plant nutrients

Experimental treatments had significant (P<0.01) effects on plant leaf total nitrogen (N) concentration. In marigold (*Tagetes erecta* L.) plants, the most significant N (%) concentration (3.0±0.13) was obtained from 9% SS and the lowest (2.4±0.10) from the control treatments. In garden verbena (*Verbena hybrida*) plants, the greatest value (3.0±0.14) was obtained from 9% SS treatments and the lowest (2.3±0.11) from the control treatments (Table 4).

Table 4. Macro plant nutrient concentrations of marigold and garden verbena plants
Çizelge 4. Kadife çiçeği ve yer minesi bitkilerinin makro bitki elementi konsantrasyonları

Plants	Treatments	N	P	(%) K	Mg	Ca
Marigold	Control	2.4±0.10b	0.39± 0.13	2.00±0.14ab	0.42±0.10b	3.2±0.11c
	3% SS	2.7±0.11ab	0.34±0.10	1.97±0.16 b	0.43±0.11b	3.8±0.14bc
	6% SS	2.9 ±0.11a	0.37±0.11	2.30± 0.10a	0.60±0.12a	5.2 ±0.20a
	9% SS	3.0 ±0.13a	0.42±0.15	2.33 ±0.22a	0.61±0.24a	5.0±0.15ab
	S. level	**	ns	*	*	*
G.verbena	Control	2.3 ±0.11b	0.26±0.12c	2.27±0.17ab	0.60±0.11b	3.5±0.10
	3% SS	2.5 ±0.10b	0.33±0.13bc	2.30±0.20ab	0.64±0.12ab	3.9±0.12
	6% SS	2.6±0.12ab	0.43±0.16ab	2.60±0.23a	0.67±0.10ab	4.3±0.16
	9% SS	3.0 ±0.14a	0.51±0.21a	2.20± 0.11b	0.70± 0.14a	4.8±0.20
	S. level	**	**	**	*	ns

Significant level (S. level),* p<0.05 ** p<0.01 ns: not significant

Applications of the marigold plant on phosphorus (P) concentration had no significant effect. Plant P (%) concentration varied between 0.34±0.10-0.42±0.15. On the other hand, treatments had significant effects on

the P concentration of garden verbena plants (P<0.01), with the greatest value (0.51±0.21) in 9% SS treatments and the lowest value (0.26±0.12%) in the control treatments. Marigold potassium (K), magnesium (Mg) and calcium (Ca) concentrations were significantly affected by treatments (P<0.05). The greatest plant K (2.33±0.22) and Mg (0.61±0.24) concentration were obtained from 9% SS treatments, followed by 6% SS treatments. Both treatments (6% SS and 9% SS) were placed into the same statistical group. The greatest Ca (%) concentration (5.2±0.20) was obtained from 6% SS treatments, followed by 9% SS treatments (5.0±0.15). On the other hand, in garden verbena plants, treatments had a significant effects on plant K concentration at significant (P<0.01) level and on Mg at (P<0.05) significant level but did not have significant effects on plant Ca. Plant K (%) concentration varied between 2.20±0.11-2.60±0.23 with the greatest value in 6% SS treatments and Mg (%) varied between 0.60±0.11-0.70±0.14 with the greatest value in 9% SS treatments. The most remarkable plant Ca (%) (4.80±0.20) was obtained from 9% SS and the lowest concentration (3.5±0.10) value was obtained from control treatments. Increasing SS doses had significant effects on Na concentrations of marigold (*Tagetes erecta* L.) and garden verbena (*Verbena hybrida*) plants significant (P<0.01) level. In marigold and garden verbena plants, the lowest Na (mg kg^{-1}) concentration was obtained from the control (349±11.93) treatments and the greatest values (377±15.36) were obtained from 9% SS treatments of marigold plants (784±26.41) and 6% SS treatments of garden verbena plants (696±25.16). Microelement concentration of marigold and garden verbena plants also increased with increasing SS doses (Table 5).

Table 5. Micro element concentrations of marigold and garden verbena plants
Çizelge 5. Kadife çiçeği ve yer minesi bitkilerinin mikro element konsantrasyonları

Plants	Treatments	Na	(mg kg^{-1}) Fe	Cu	Zn	Mn	B
Marigold	Control	349±11.93c	1698±35.41	7.3±0.21	95±10.17c	104±11.82b	42±10.21
	3% SS	493±13.57c	1503±28.52	7.3±0.25	102±12.25bc	109±14.26ab	39±8.14
	6% SS	625±15.12ab	2300±50.95	7.3±0.25	136±16.32a	142±20.74a	39±9.63
	9% SS	784±26.41a	2236±40.24	7.7±0.30	130±14.50ab	129±15.21ab	37±8.95
	S. level	**	ns	ns	*	**	ns
G.verbena	Control	377±15.36c	1521±25.38	8 ±0.11b	84±10.66b	74±12.64	47±11.15
	3% SS	456±18.72bc	1587±30.64	10±0.15ab	101±11.23ab	100±15.26	46±9.28
	6% SS	696±25.16a	1700±50.42	12± 0.21a	115±14.18a	96±20.51	46±10.65
	9% SS	611±20.51ab	1423±26.45	10±0.18ab	108±13.25a	92±18.40	46±12.84
	S. level	**	ns	*	**	ns	ns

Significant level (S. level),* p<0.05 ** p<0.01 ns: not significant

Plant Fe (mg kg^{-1}) concentrations varied between 1503±28.52-2300±50.95 in marigold plants and between 1423±26.45-1700±50.42 in garden verbena plants. The highest amount of Fe was determined in 6% SS applications for the marigold plant (2300±50.95) and the G.verbena plant (1700±50.42), respectively. Applications of the marigold plant had no significant effect on Cu (mg kg^{-1}) concentrations and varied between 7.3±0.21-7.7±0.30. Treatments had significant effects on G.verbena plant Cu concentration at P<0.05 level. The lowest Cu (mg kg^{-1}) concentration was found in the control (8 ±0.11) treatment, and the highest value was 6% SS (12± 0.21) application. The effect of applications on Zn in the marigold plant was found to be significant (P<0.05) level. The most minor Zn (mg kg^{-1}) concentration was determined in control (95±10.17) and the highest in 6% SS (136±16.32) application. Treatments had significant (P<0.01) effects on Zn concentration in garden verbena plants. The smallest Zn concentration in the control (84±10.66) and the highest value (115±14.18) in the 6% SS treatment. Marigold plant Mn (mg kg^{-1}) concentrations significantly (p<0.01) increased with increasing SS treatments. The lowest Mn concentration was obtained from the control treatment (104±11.82), and the most excellent Mn value (142±20.74) was obtained from the 6 % SS treatment. G.verbena Mn concentrations did not significantly change with SS treatments. The highest Mn was obtained from the 3 % SS treatment (100±15.26) and the lowest value (74±12.64) from the control treatment. In both plant species, the effects of sewage sludge treatments on plant B (mg kg^{-1}) concentration were not significant. The highest B concentration in the marigold plant was seen in the control treatment (42±10.21) and the lowest value in the 9% SS treatment (37±8.95 mg kg-1). In the G.verbena, plant the greatest B concentration (47±11.15) was obtained from control treatments, followed by 3% SS, 6% SS and 9% SS treatments. Afonso et al. (2018) reported plant nutrients for lemon verbena. The sufficiency ranges set for the macronutrients (%) N, P, K, Ca, and Mg were respectively 2.80–4.30, 0.09–0.38, 1.00–2.80, 0.75–3.00, and 0.20–0.80. The sufficiency ranges found for

the micronutrients B, Cu, Fe, Zn, and Mn were, respectively, 35–200, 7–22, 60–300, 25–125, and 40–250 mg kg^{-1}. Present study, N and Fe elements differed according to these values, especially in the Fe element. Soares et al. (2018) concentration of Na (321-513 mg kg^{-1}), but Amed et al. (2012) differed from the Fe (836.74 mg kg^{-1}) concentration value. The herb is a good source of Fe as well, where roots contain notably higher quantity (1763 mg kg^{-1}) than leaves (836.74 mg kg^{-1}), stems (324.39 mg kg^{-1}), seeds (893.25 mg kg^{-1}) and seeds husk (476.21 mg kg^{-1}) (Amed et al., 2012). Eaton et al. (2013) reported plant nutrients for the marigold plants. The sufficiency ranges set for the macronutrients (%) N, P, K, Ca, and Mg were respectively 2.40–5.70, 0.30–1.40, 0.90–5.30, 1.70–3.60, and 0.60–1.90. The sufficiency ranges found for the micronutrients (mg kg^{-1}) B, Cu, Fe, Zn, and Mn were, respectively, 30-53, 8–23, 61–233, 67–229 and 44–541. Present study, Fe elements differed according to these values, especially in terms of Fe element. Similar to Na (595 mg kg^{-1}) element, as stated by Sonmez et al (2017), but differed from Fe (971.6 mg kg^{-1}). These differences are thought to be due to soil characteristics, sewage sludge treatments (12755±124.53 mg kg^{-1} Fe) and plant variety. It can be concluded that Marigold (*Tagetes erecta* L.) and Garden verbena (*Verbena hybrida*) plants have high Fe concentration, and these plants can be hyper accumulator for Fe element.

Plant heavy metal concentration

Treatments had significant (P<0.05) effects of marigold Ni (mg kg^{-1}) concentration. The most significant value (13±1.23) was obtained from 9% SS treatments and the lowest (8±1.35) in 3% SS treatments. In garden verbena plants, the greatest Ni (8.9±0.93) was obtained from 9% SS treatments and the lowest (7.0±0.52) in the control treatments (Table 6).

Table 6. Heavy metal concentrations of marigold and garden verbena plants
Çizelge 6. Marigold ve garden verbena bitkilerinin ağır metal konsantrasyonları

Plants	Treatments	Ni	Cd (mg kg^{-1})	Cr	Pb	As	Hg (µg kg^{-1})
Marigold	Control	11±0.71ab	0.16±0.08b	19±0.28b	2.5 ±0.10b	0.35±0.02	48.79±2.34
	3% SS	8±1.35b	0.69±0.10ab	24±0.32ab	2.7±0.16ab	0.32±0.05	43.11±8.32
	6% SS	12±1.12a	1.08±0.06ab	25± 0.43a	3.8±0.21a	0.49±0.06	45.61±9.11
	9% SS	13±1.23a	1.21± 0.09a	28± 0.51a	3.2±0.20ab	0.52±0.07	61.82±10.13
	S. level	*	**	*	*	ns	ns
G.verbena	Control	7.0±0.52	0.3±0.05	7.2 ±0.46b	3.0±0.21	0.40±0.03	27.04±3.46
	3% SS	7.3±0.61	0.3±0.06	8.2±0.62ab	2.6±0.15	0.52±0.06	28.59±4.73
	6% SS	8.3±0.75	0.4±0.07	9.4±0.81ab	2.9±0.20	0.42±0.04	37.11±5.30
	9% SS	8.9±0.93	0.4±0.09	10.0±1.02a	3.2±0.28	0.49±0.05	24.15±9.28
	S. level	ns	ns	*	ns	ns	ns

Significant level (S. level), * p<0.05 ** p<0.01 ns: not significant

Experimental treatments had significant effects on marigold Cd (mg kg^{-1}) concentration (P<0.01), with the greatest value (1.21±0.09) in the 9% SS treatments and the lowest value (0.16±0.08) in the control treatments. Effects of SS treatments on garden verbena Cd concentration were not found to be significant. Plant Cd value 0.3±0.05-0.4±0.09. Marigold plant Cr (mg kg^{-1}) concentration significantly (P<0.05) varied between 19±0.28–28±0.51, the lowest value in the control treatment and the greatest value in 9% SS application dose. Effects of SS treatments on garden verbena Cr concentrations were not found to be significant. Plant Cr concentration 7.2±0.46-10.0±1.02. There were significant (P<0.05) differences in Marigold Pb (mg kg^{-1}) concentration. The most significant plant Pb concentration (3.8±0.21) was obtained from 6% SS treatments and the lowest (2.5±0.10) in the control treatments. On the other hand, in garden verbena plants, the greatest Pb concentration (3.2±0.28) was obtained from 9% SS treatments and the lowest (2.6±0.15) from 3% SS treatments. Sewage sludge treatments did not have significant effects on marigold As and Hg concentration. Plant As (mg kg^{-1}) concentration varied between 0.32±0.02-0.52±0.07 and Hg (µg kg^{-1}) concentration range from 43.11±8.32 to 61.82±10.13. Similarly, the effects of SS treatments on garden verbena As and Hg value were not found to be significant. Plant As concentration 0.40±0.03-0.52±0.06 and Hg concentration 24.15±8.28-37.11±5.30. Present As the concentration of marigold and garden verbena plants were lower than the EU threshold values for As in fodder (2-4 mg kg^{-1}) (Adamse et al., 2017; Dradrach et al., 2020). Accoding to Kabata-Pendias & Pendias (2011) Normal values of total heavy metals in plants (mg kg^{-1}) 0.02-5.0 Ni, 0.1-2.4 Cd, 0.03-14.0 Cr and 0.2-20.0 Pb concentration in plant. Marigold plant concentrations of Ni, Pb and Garden verbena plant Ni concentration were determined to be higher than the specified average values. Mercury concentrations determined in Marigold and G. verbena

plants were below the maximum (0.5 mg kg^{-1}) limit value specified for contaminated food by the World Health Organization (WHO, 2004).

Soil heavy metal concentration

The effect of sewage sludge applications on the total Zn of marigold plant soils after harvest was statistically significant ($P<0.05$) and differed according to the applications. The smallest Zn (mg kg^{-1}) value was determined at 81.38±1.22 in the control application, and the highest Zn value was determined at 116.58±2.47 in the 9% SS application. The applications did not have a significant effect on the total Zn of garden verbena plant soils after harvest. The lowest Zn concentration was 80.47±2.73 in the control application and the highest value was obtained at 97.97±3.2 in the 6 % SS application respectively. The effect of the treatments on marigold soil the total Cu was statistically significant ($P<0.05$), with the highest Cu (mg kg^{-1}) value being 28.49±1.14 in 9% SS application and the lowest Cu value in control application with 21.23±2.15. The applications did not have a significant effect on the total Cu garden verbena soil. The concentration of Cu (mg kg^{-1}) was determined in the range of 22.28±2.20-23.35±2.12. The effect of sewage sludge doses on the total Ni concentration of the soil was not significant. In marigold soil, the lowest soil Ni (mg kg^{-1}) concentration (44.32±1.95) was determined in control, and the highest Ni concentration (46.70±2.02) was determined in 3% SS application. In garden verbena soil, the lowest Ni (mg kg^{-1}) value (43.95±1.28) was obtained from the control and the greatest Ni concentration (45.88±1.10) from 9% SS treatments. These values were below the toxic level of 100 mg kg^{-1} specified by Özbek et al. (1995). Soil total Pb significantly increased with increasing SS doses ($P<0.05$) compared to the control. In marigold soil, the lowest Pb (mg kg^{-1}) concentration (22.42±1.66) was obtained from the control and the greatest (27.58±0.39) from 9% SS treatments. In garden verbena soil, the lowest value (21.63±0.87) was obtained from the control and the greatest (25.13±2.88) from 3% SS treatments (Table 7).

Table 7. Heavy metal concentration of post-harvest soils according to applications
Çizelge 7. Hasat sonrası toprakların uygulamalara göre ağır metal konsantrasyonları

Plants	Treatments	Zn	Cu	Ni	Pb (mg kg^{-1})	Cd	As	Hg (µg kg^{-1})
Marigold	Control	81.38±1.22b	21.23±2.15b	44.32±1.95	22.42±1.66b	1.02±0.15	17.76±0.83	125.71±21.77
	3% SS	91.57±1.14ab	24.22±2.41ab	46.70±2.02	25.75±0.45a	1.12±0.17	18.81±0.17	128.04±13.47
	6% SS	101.86±3.14ab	25.62±2.12ab	44.98±1.12	27.04±1.05a	1.09±0.26	19.41±0.10	207.79±59.37
	9% SS	116.58±2.47a	28.49±1.14a	46.58±1.45	27.58±0.39a	1.03±0.38	19.81±0.05	143.33±3.90
	S. level	*	*	ns	*	ns	ns	ns
G.verbena	Control	80.47±2.73	23.35±2.12	43.95±1.28	21.63±0.87	1.38±0.02	14.39±0.10	104.92±12.27
	3% SS	95.74±2.81	22.28±2.20	45.17±1.27	25.13±2.88	1.28±0.13	14.62±1.32	114.25±6.54
	6% SS	97.97± 3.24	23.12±3.41	45.58±0.46	22.79±0.96	1.47±0.02	14.93±0.72	121.13±12.87
	9% SS	95.46±3.12	23.32±3.56	45.88±1.10	23.71±3.37	1.50±0.01	15.15±2.44	126.04±25.31
	S. level	ns	ns	ns	ns	ns	ns	ns

Significant level (S. level) * $p<0.05$ ** $p<0.01$ns: not significant

Present Pb values were all below the toxic level of 100 mg kg^{-1} specified by Kabata-Pendias & Pendias (1992). Total Cd (mg kg^{-1}) concentration in marigold soil and garden verbena soil did not significantly change with increasing SS doses. Soil total Cd varied between 1.02±0.15-1.12±0.17 in marigold soil and 1.28±0.13-1.50±0.01 in garden verbena soil. Present Cd values were within the normal limits of 3 mg kg^{-1} specified by Kabata-Pendias (2011). Soil total Cd, Ni and Pb values were different from the values of Delibacak & Ongun (2018), probably because of varying treatment doses and soil characteristics. Soil pH also influences the presence and uptake of heavy metals (Khan et al., 2015; Eid & Shaltout, 2016). In marigold soil, the lowest soil total As (mg kg^{-1}) concentration (17.76 ±0.83) was obtained from the control and the greatest concentration (19.81±0.05) from 9% SS treatments. In garden verbena soil the lowest value (14.39±0.10) was obtained from the control and the greatest concentration (15.15±2.44) from 9% SS treatments. Present As values were all below standard value of 20 mg kg^{-1} of WHO and FAO (Chiroma et al., 2014). Soil total Hg (µg kg^{-1}) concentration did not change significantly with increasing SS doses. In marigold soil, the lowest soil Hg concentration (125.71±21.77) was obtained from the control and the greatest concentration (207.79±59.37) from 6% SS treatments. In garden verbena soil, the lowest value (104.92±12.27) was obtained from the control and the greatest concentration (126.04±25.31) from 9% SS treatments. Present Hg values were below the standard limit of 1.5 mg kg^{-1} of Turkey (Anonymous, 2010; Chiroma et al., 2014). Threshold values of heavy metals (mg kg^{-1}) in soil were given in Turkey as 200 Zn, 100 Cu, 70 Ni, 100 Pb, and 1.5 Cd, respectively (Anonymous, 2010). Soil heavy metal (Zn, Cu, Ni, Cd, Pb, As and Hg) concentrations were below the allowable limits. Depending on the treatment sludge application dose, it has been reported that EC and exchangeable Na increase in soils in long-term soil application (Cucina et al., 2019). The positive relationship between sludge addition and EC increase has been reported by several

studies (Dhanker et al., 2021). The soil EC significantly increased with sludge dosage. Since the organic acids produced during the decomposition of solid waste caused the accumulation of dissolved salts (Hamdi et al., 2019). Sewage Sludge application did not affect total Cd, Pb and Ni concentrations in treated soils; total Hg, Zn and Cu accumulated proportionally with the amount of sewage sludge applied (Cucina et al., 2019). The total concentration of trace elements showed the following variation in the soil over the 5 yr (mg kg^{-1}): Cd (16.8–20.0), Co (19.5–21.5), Cr (98.2–125.7), Cu (8.1–17.1), Mn (62.9– 85.7), Ni (20.3–35.0), Pb (27.0–52.4), and Zn (20.3–35.8) (Chagas et al. 2020). It was stated that Sarğın (2011), seven years after the treatment sludge application, the heavy metal Zn, Cu, Pb, Ni and Cd concentrations in the soil were determined below the limit values for all soil depths (0-30 cm, 30-60 cm and 60-100 cm). Changes in heavy metal accumulation in soils depend primarly on the characteristics of the sewage sludge, the rate applied and soil texture (Wu et al., 2012). Heavy metals bioavailability in soils after amendment varies according to soil characteristics, such as soil pH, and organic matter content (Achiba et al., 2009).

CONCLUSION

The BCF values of marigold for Zn, Cd element and garden verbena for Zn were >1. Thus, marigold (*Tagetes erecta* L.) and garden verbena (*Verbena hybrida*) were considered as a potential accumulator plants and can be used for the remediation of contaminated soils. In terms of heavy metals (Ni, Cd, Cr, Pb, As and Hg), toxicity symptoms were not encountered in marigold (*Tagetes erecta* L.) and garden verbena (*Verbena hybrida*) plants. Soil heavy metal (Ni, Cd, Pb, As and Hg) were below the limit values. Based on the present findings, it is recommended that sewage sludge treatments could be applied at 3% SS for marigold (*Tagetes erecta* L.) and garden verbena (*Verbena hybrid*) plants.

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Statement Contribution of The Authors

Authors declares the contribution of the authors is equal.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

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Limon ve Portakal Kabuğu Ekstraktları Içeren Yenilebilir Film Kaplamaların Kalamar (*Loligo vulgaris*) Halkaları ve Gökkuşağı Alabalığı (*Oncorhynchus mykiss*) filetolarında Mikrobiyolojik Kalitesi ve Raf Ömrü Üzerine Etkisi

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ÖZET
Bu çalışmanın amacı, meyve kabuklarının (portakal ve limon) ekstrakt olarak değerlendirerek antimikrobiyal özelliklere sahip yenilebilir filmler üretmek ve gökkuşağı alabalığı filetoları ile kalamar halkalarında raf ömrüne etkisinin belirlenmesidir. Bu amaçla ksantan, keçiboynuzu ve karagenan kullanılarak portakal ve limon kabuğu ekstraktlarından antimikrobiyal film üretimi gerçekleştirilmiştir. Gökkuşağı alabalığı filetoları (*Oncorhynchus mykiss* 1792) ve kalamar halkaları (*Loligo vulgaris* 1798), bu ürünlerin raf ömrünü uzatmak amacıyla meyve kabuklarından üretilen antimikrobiyal yenilebilir filmlerle kaplanmıştır. Sonuç olarak, kalamar halkalarının (CS) toplam mezofilik bakteri sayısı (TMC), 6. günde mikrobiyolojik tüketim sınırını aşarken, limon kabuğu+keçiboynuzu (LLS) ve limon kabuğu+karagenan (LKS) ile kaplı kalamar halkalarının TMC sayısının 8. günde bile bu sınırı aşmadığı belirlenmiştir. Bu çalışma, sadece balıkçılık ürünlerinden fonksiyonel ürünlerin üretilebilmesine değil, aynı zamanda meyve kabuklarının da bu amaçla değerlendirilebilmesine neden olmuştur. Bu çalışmanın sonuçları, su ürünleri ve meyve suyu işleme tesislerinin yanı sıra gıda üreticileri tarafından da değerlendirilebilir.

Su Ürünleri

Araştırma Makalesi

Makale Tarihçesi
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Kabul Tarihi : 18.03.2022

Anahtar Kelimeler
Meyve kabukları,
Mikrobiyal flora,
Kalamar halkaları,
Gökkuşağı alabalığı filetoları,
Yenilebilir filmler

The Impact of Edible Film Coatings With Lemon and Orange Peel Extracts on Microbiological Quality and Shelf-Life of Squid (*Loligo vulgaris*) Rings and Rainbow Trout (*Oncorhynchus mykiss*) Fillets

ABSTRACT
The aim of this study is to produce edible films with antimicrobial properties by using the discarded fruit peels (orange and lemon) as extracts and to determine the effect on shelf life of rainbow trout fillets and squid rings. For this purpose, antimicrobial edible films were performed the orange and lemon peels extracts by using xanthan, locust bean and carrageenan gums. Rainbow trout fillets (*Oncorhynchus mykiss* 1792) and squid rings (*Loligo vulgaris* 1798) were covered with this antimicrobial edible films from discarded fruit peels to extend the shelf-life of these products. As a result, total mesophilic bacteria count (TMC) of squid rings (CS) exceeded the microbiological limit of the consumption on day 6, whereas TMC of squid rings covered with limon peel+locust bean (LLS) and limon peel+carrageenan (LKS) did not exceed this limit on day 8. This study gave rise to not only can be produced of functional products from the fishery products but also the fruit peels residues can also be evaluated for this purpose. The results of this study can be evaluated by seafood and fruit juice processing plants as well as food producers.

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INTRODUCTION

Fish and fishery products are included in food products that have been deteriorated very quickly, as they are vulnarable to chemical enzymatic and microbiological degradation. (Ashie et al., 1996). For this reason, nowadays it is very important to use natural products instead of chemicals preservatives in order to extend the shelf life of seafood products, to ensure their quality and safety (Mohan et al., 2016; Viji et al., 2017; Kılınç et al., 2017; Mei et al., 2019). There have been found some natural inhibitors in herbal foods such as some spices and herbs have active ingredients with antimicrobial effect. Some examples can be given for these natural inhibitors: eugenol in cloves, allicin in garlic and onion, cinnamic aldehyde in cinnamon, isothiocionate in mustard, timol and isotimol in sage, anetol in anise, vanillin in vanilla etc (Ünlütürk & Turantaş, 2003). A large number of studies have recently investigated the effect of plant extracts on the pathogenic microorganisms (Fernandez-Lopez et al., 2005; Ertürk et al., 2010; Bhalodia & Shukla, 2011; Khan et al., 2013; Gonelimali et al., 2018). Some plants have been contained essential oils with antimicrobial effect (Akarca & Şevik, 2021). Essential oils in citrus fruits (citral etc.) are located in the fruit peels. Essential oils in lemon and orange have been stated to prevent the development of certain concentrations of bacteria (Ünlütürk & Turantaş, 2003). Many fruit juice production factories have been found in Türkiye. Fruit and vegetable peels have various positive effects on health in terms of bioactive components such as polyphenols, carotenoid etc. Peels have more biological activity than other fractions of fruit and vegetables therefore, it is very important to evaluate them (Kılınç et al., 2018). Furthermore, fruit peels are quite valuable because of the antimicrobial properties. For this purpose, the aim of this study was to evaluate fruit peels (oranges and lemons) which have been discarded and to create edible films with antimicrobial properties. This study gave rise to not only produce antimicrobial films (food coating materials) for producing of functional fishery products but also fruit peels were evaluated in this purpose. Another aim was to determine the impact of edible films with lemon and orange peels by using xanthan, locust bean and carrageenan on the microbial flora and the shelf-life of squid rings and rainbow trout fillets.

MATERIALS and METHODS

Preparation of lemon and orange peels extracts

In this study lemon (*Citrus limon*) and orange (*Citrus sinensis*) peels were supplied and in shade dried naturally. THE Dried lemon and orange peels were powdered at using an electric mill. The lemon and orange peels were pulverized separately and exracted with ethanol using in a mechanical mixer for 24 hours. The obtained resulting mixture was filtered and evaporated with a rotary evaporator according to method of Baytop, 1999. Prepared extracts were kept in the refrigerator until used.

Production of Edible Film Solutions From Fruit Peels

The edible film solutions were produced from orange and lemon peels because of which have the highest antimicrobial effect (Kılınç et al., 2018). Preparation of edible films was proceeded according to Sothornvit et al. (2011). Film solution was prepared by using at the ratio of 1% (w v-1) xanthan, locust bean, carrageenan gums in distilled water at 90°C for 30 minutes. The mixture was homogenized with magnetic mixer. Glycerol was added into the mixture at the ratio 3 % as a plasticizer. 3.2% (g 100ml-1) natural antimicrobial in concentrations obtained as the result of Minimum Inhibition Concentration (MIC) of fruit peel extracts were added according to Kılınç et al. (2018). The solution was left to cool at room temperature.

Coating Fishery Products with Edible Film Solutions

In order to improve the sensory quality and extend the shelf-life of trout fillets and squid rings, edible film coatings containing lemon and orange peels by using xanthan, locust bean and carrageenan gums were used. Edible film solutions were applied on fishery products. Frozen-thawed rainbow trout fillets (*Oncorhynchus mykiss* Walbaum, 1792) and squid rings (*Loligo vulgaris* Lamarck, 1798) were obtained from fish markets. They were brought to the Laboratory of Fish Processing Technology of Ege University Fisheries Faculty in cold chain by using cooler box containing ice in approximately 30 minutes. The samples were put into the refrigerator at 4±1°C for thawing process for 15 hours. After thawing process, the samples were aseptically soaked into the edible film solution at room temperature (18°C) for two minutes. The samples were coated with orange and lemon peels by using xanthan, locust bean and carrageenan gum containing edible films, separately (Fig. 1). The coated fishery products without extract were identified as control.

After coating the samples with edible films, approximately 150-200 g of two trout fillets and 10 piece of squid rings were placed in strafor plates separately. The samples were incubated at 4±1°C for

10 days. Analysis was carried out on the 1st, 3rd, 6th and 8th day of storage. Total mesophilic, psychrotrophic, Enterobactericeae and lactic acid bacteria counts of samples were made in triplicate. Three strofor plates were used on each analysis day for microbiological analyses. The results were given as the mean value of three analyses.

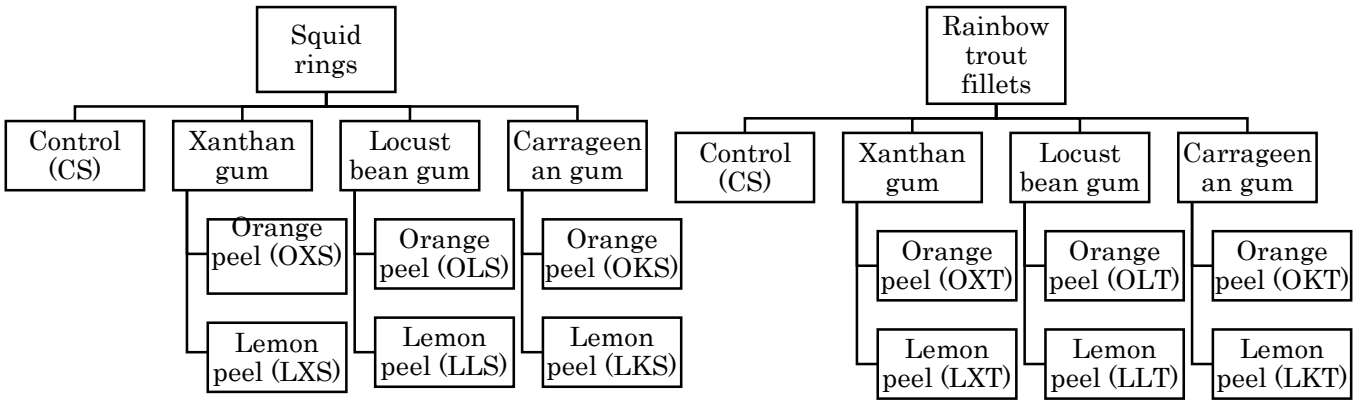

Figure 1. It is a group of coated (control) and uncoated aquaculture products with different extracts and edible films.

Şekil 1. Farklı ekstrakt ve yenilebilir filmler ile kaplanmış ve kaplanmamış (kontrol) su ürünleri grupları.

Analytical Methods

Microbiological analysis and bacteria identifications

Samples (10 g) were added aseptically to 90 ml of sterile Buffered Peptone Water solution (1 g ml-1 bacteriological peptone, Merck 1.07228.0500, Germany) and mixed in a Stomacher (IUL, Barcelona, Spain) at high speed for 1 min. Plate Count Agar (PCA, Merck 1.05463.0500, Germany) was used to evaluate total mesophilic bacterial counts (TMC) and total psychrotrophic bacterial counts (TPC). Violet red bile dekstrose Agar (VRBD-A, Merck 1.10275.0500, Germany) and De Man Rogosa Sharpe Agar (MRS, Merck 1.10660.0500, Germany) were used to asses Enterobacteriaceae (ENT), total coliform bacteria and lactic acid bacteria (LAB) respectively. On the other hand, Yeast Extract Glucose Chloramphenicol Agar (YGC, Merck 1.16000.0500, Germany) were used to grow molds and yeasts (MY). Plates were incubated 30°C at 24 h for TMC, 7 °C at 10 d for TPC. LAB were proceeded after incubation at 30 °C at 72 h under anaerobic conditions. Yeasts and fungi were incubated at 25 °C for 72 h (Harrigan & Mc Cance, 1976). After incubation periods, the means of counts with standard deviations, were reported as logarithms of the number of colony forming units (log CFU g-1). Furthermore, the isolated microorganisms were identified with API test kits with 20 NE (30 °C for 24 h), 20 E (36 °C for 24 h), 50 CH and 50 CHL (30 °C for 24 h) (Biomérieux, France) from bacterial flora. These kits were used according to the instructions of the manufacturer and the database provided by bioMerieux.

Sensory analysis

The sensory evaluation of the edible films coated rainbow trout fillets and squid rings were evaluated by 5 experienced panelists at the Ege University Fisheries Faculty Department of Fish Processing Technology. Prepared edible film coated and uncoated fishery products were presented to panelists in random order. On each analysis day, each sample was coded differently and then served to the panelits for evaluating the sensory characteristics of samples. The sensory evaluation of the samples was proceeded according to Paulus et al. (1979). In sensory evaluation, the panelists evaluated the following characteritics (color, odour, texture) and general acceptability defining criteria 9 to 1 of the samples. A hedonic scale ranging from 9 (very good completely fresh fish) to 1 (very bad, completely degraded fish) was employed in the evaluation.

pH analysis

The pH value was measured by using a Hanna 211 model pH meter (Cluj-Napoca, Romania), with the glass electrode applied directly to the homogenate (5 g

of fish/5 mL of distilled water). The experiment was proceeded in triplicate.

Statistical analysis

The data were analysed by one-way ANOVA. Tukey's multiple range test was applied for determining group differences at 95% significance level. Analysis was performed using SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

The total mesophilic bacterial counts (TMC) of the coated and uncoated rainbow trout fillets and squid rings are given in Table 1. Edible films with orange and lemon peels by using xanthan, locust bean and carrageenan on the rainbow trout fillets and squid rings delayed the growth of bacteria, when compared with uncoated samples. TMC of groups (OXS, LXS, CS) exceeded the microbiological limit of consumption on day 6, whereas TMC of groups (OXT, LXT, CT, OLS, OLT, LLT, OKS, OKT, LKT) exceeded this limit (7 log CFU g-1) according to the ICMSF (1986) on day 8. However, TMC of squid rings covered with limon peel+locust bean (LLS) and limon peel+carrageenan (LKS) did not exceed the limit of consumption on day 8. In another words, the lowest TMC was determined on squid rings covered with lemon peel+ locust bean (LLS) and lemon peel+ carrageenan (LKS) films, when compared with other groups.

Table 1 Total mesophilic bacterial counts (log CFU g-1) of coated and uncoated rainbow trout fillets and squid rings during storage

Çizelge 1. Kaplamalı ve kaplamasız gökkuşağı alabalığı filetolarının ve kalamar halkalarının depolama sırasındaki toplam mezofilik bakteri sayıları (log CFU g-1)

Storage Time	Total Mesophilic Bacteria Counts (log CFU g^{-1})					
	OXS	LXS	CS	OXT	LXT	CT
Day 1	4.22 ± 0.03^{b1}	3.82 ± 0.24^{c1}	4.55 ± 0.21^{a1}	3.43 ± 0.55^{ab1}	3.05 ± 0.19^{b1}	3.55 ± 0.16^{a1}
Day 3	5.81 ± 0.19^{b2}	5.15 ± 0.02^{c2}	6.02 ± 0.14^{a2}	4.89 ± 0.05^{b2}	4.26 ± 0.07^{a2}	4.69 ± 0.06^{b2}
Day 6	7.15 ± 0.01^{b3}	7.02 ± 0.02^{c3}	7.90 ± 0.40^{a3}	6.50 ± 0.07^{ab3}	6.31 ± 0.44^{b3}	6.78 ± 0.23^{a3}
Day 8	8.38 ± 0.08^{b4}	8.11 ± 0.14^{c4}	8.93 ± 0.06^{a4}	7.40 ± 0.18^{ab4}	7.06 ± 0.06^{b4}	7.93 ± 0.04^{a4}
	OLS	LLS	CS	OLT	LLT	CT
Day 1	3.58 ± 0.47^{c1}	3.86 ± 0.05^{b1}	4.06 ± 0.61^{a1}	3.88 ± 0.10^{a1}	3.45 ± 0.51^{b1}	3.79 ± 0.51^{ab1}
Day 3	3.86 ± 0.40^{c1}	4.43 ± 0.07^{b2}	5.42 ± 0.31^{a2}	5.04 ± 0.22^{a2}	4.10 ± 0.05^{b2}	5.86 ± 0.05^{ab2}
Day 6	5.74 ± 0.23^{b2}	5.49 ± 0.14^{c3}	6.89 ± 0.12^{a3}	6.47 ± 0.26^{a3}	5.65 ± 0.28^{b3}	6.93 ± 0.28^{ab3}
Day 8	7.12 ± 0.11^{b3}	6.97 ± 0.83^{c4}	7.47 ± 0.08^{a4}	7.42 ± 0.64^{b4}	7.00 ± 0.15^{a4}	7.67 ± 0.15^{b4}
	OKS	LKS	CS	OKT	LKT	CT
Day 1	4.20 ± 0.42^{a1}	3.65 ± 0.21^{b1}	3.80 ± 0.14^{c1}	3.35 ± 0.21^{a1}	3.70 ± 0.14^{b1}	3.75 ± 0.35^{b1}
Day 3	5.22 ± 0.89^{b2}	4.55 ± 0.26^{c2}	6.84 ± 0.05^{a2}	4.95 ± 0.07^{b2}	5.11 ± 0.33^{b2}	6.37 ± 0.39^{a2}
Day 6	6.65 ± 0.64^{b3}	5.80 ± 0.42^{c3}	7.25 ± 0.21^{a3}	6.00 ± 0.14^{b4}	6.25 ± 0.07^{b3}	7.65 ± 0.21^{a3}
Day 8	7.40 ± 0.55^{b4}	6.73 ± 0.24^{c4}	8.42 ± 0.29^{a4}	7.46 ± 0.15^{b4}	7.09 ± 0.06^{b4}	8.27 ± 0.02^{a4}

n=3; Mean value ± standart deviation $^{a-c}$: a-c: different letters in the same row show statistically significant difference between the groups ($p<0.05$). $^{1-4}$: 1-4: different numbers in the same column show statistically significant difference for each group according to storage ($p<0.05$).

There was a reduction of approximately up to 1.5 log cycles between control and coated samples (LLS and LKS) after 8 days of storage. The edible films that were produced using locust bean with lemon peel extract and carrageenan with lemon peel extract caused a statistically significant ($p<0.05$) decrease in the TMC of coated samples compared to other groups. The inhibition of the microbial growth in coated samples was observed in the study and this result was thought to be related with the antimicrobial activiy of the lemon and orange peels. It was also determined that the differences between the groups of the TMC were significant ($p<0.05$) according to the coating types and the storage period ($p<0.05$). Total psychrotrophic bacterial counts (TPC) of the coated and uncoated rainbow trout fillets and squid rings are shown in Table 2. The highest TPC of groups were determined in the uncoated samples during the storage period, when compared with coated groups. The initial TPC (log CFU g^{-1}) of coated and uncoated samples increased signicantly during the storage period ($p<0.05$). *Enterobactericeae* bacteria counts (EBC) of coated and uncoated rainbow trout fillets and squid rings are given in Table 3.

Gradual increase in the number of EBC have been observed throughout the storage in all groups except for the groups (OXT, LXT, CT), in which EBC counts of samples were determined below the detectable value (<1 log CFU g^{-1}) during storage period.

Lactic acid bacteria (LAB) counts (log CFU g^{-1}) obtained from coated and uncoated rainbow trout fillets and squid rings during storage are shown in Table 4. While there was no statistically significant difference ($p>0.05$) in the lemon and orange coated groups during storage, the difference between the

control group and the other groups was found to be significant ($p<0.05$). The lactic acid bacteria counts were determined statistically ($p<0.05$) higher in edible film coated groups, in which they thought to be stopped the other microbial growth due to the antimicrobial effect of lactic acid bacteria.

Table 2. Total psychrotrophic bacteria counts (log CFU g^{-1}) of coated and uncoated rainbow trout fillets and squid rings during storage

Çizelge 2. Kaplamalı ve kaplamasız gökkuşağı alabalığı filetolarının ve kalamar halkalarının depolama sırasındaki toplam psikrotrofik bakteri sayısı (log CFU g^{-1})

Storage Time	Total Psychrotrophic Bactria Counts (log CFU g^{-1})					
	OXS	LXS	CS	OXT	LXT	CT
Day 1	4.21 ± 0.22^{b1}	3.62 ± 0.29^{a1}	4.41 ± 0.25^{b1}	3.43 ± 0.36^{a1}	3.29 ± 0.38^{a1}	3.51 ± 0.03^{a1}
Day 3	5.01 ± 0.22^{b2}	5.61 ± 0.46^{b2}	6.21 ± 0.17^{a2}	5.59 ± 0.30^{a2}	5.51 ± 0.49^{a2}	5.80 ± 0.08^{a2}
Day 6	7.55 ± 0.13^{b3}	7.46 ± 0.06^{b3}	7.75 ± 0.47^{a3}	7.30 ± 0.11^{a3}	7.65 ± 0.25^{a3}	7.34 ± 0.07^{a3}
Day 8	8.20 ± 0.09^{b4}	8.06 ± 0.08^{b4}	$8.72\pm0,29^{a4}$	8.20 ± 0.14^{a4}	7.10 ± 0.14^{a4}	8.53 ± 0.11^{a4}
	OLS	LLS	CS	OLT	LLT	CT
Day 1	4.53 ± 0.12^{a1}	4.16 ± 0.51^{b1}	4.53 ± 0.21^{a1}	3.64 ± 0.25^{b1}	3.49 ± 0.09^{b1}	4.11 ± 0.43^{a1}
Day 3	5.40 ± 0.03^{a2}	4.76 ± 0.97^{b2}	5.96 ± 0.77^{a2}	4.71 ± 0.22^{b2}	5.00 ± 0.48^{b2}	6.48 ± 0.54^{a2}
Day 6	6.75 ± 0.30^{a3}	6.72 ± 0.14^{a3}	6.99 ± 0.60^{b3}	6.46 ± 0.07^{b3}	6.86 ± 0.05^{b3}	7.10 ± 0.15^{a3}
Day 8	7.48 ± 0.20^{a4}	7.12 ± 0.16^{b4}	7.75 ± 0.25^{a4}	7.27 ± 0.23^{b4}	7.07 ± 0.08^{b4}	7.79 ± 0.23^{a4}
	OKS	LKS	CS	OKT	LKT	CT
Day 1	4.77 ± 0.04^{a1}	3.55 ± 0.08^{a1}	4.45 ± 0.54^{a1}	3.60 ± 0.26^{b1}	3.08 ± 0.06^{b1}	4.16 ± 0.20^{a1}
Day 3	$5.44\pm0,02^{a2}$	4.70 ± 0.44^{a2}	5.15 ± 0.59^{a2}	4.05 ± 0.08^{b2}	3.99 ± 0.06^{b2}	5.26 ± 0.14^{a2}
Day 6	6.76 ± 0.29^{a3}	6.84 ± 0.72^{a3}	6.05 ± 0.23^{a3}	5.87 ± 0.07^{b3}	5.47 ± 0.07^{b3}	7.32 ± 0.20^{a3}
Day 8	8.30 ± 0.08^{a4}	7.06 ± 0.52^{a4}	8.70 ± 0.14^{a4}	8.03 ± 0.13^{b4}	7.78 ± 0.06^{b4}	8.47 ± 0.72^{a4}

n=3; Mean value ± standart deviation $^{a-c}$: a-c: different letters in the same row show statistically significant difference between the groups ($p<0.05$). $^{1-4}$: 1-4: different numbers in the same column show statistically significant difference for each group according to storage ($p<0.05$).

Table 3. Enterobacteriaceae bacteria counts (log CFU g^{-1}) of coated and uncoated rainbow trout fillets and squid rings during storage

Çizelge 3. Kaplamalı ve kaplamasız gökkuşağı alabalığı filetolarının ve kalamar halkalarının depolama sırasındaki Enterobactericeae bakteri sayıları (log CFU g^{-1})

Storage Time	*Enterobactericea* Bacteria Counts (log CFU g^{-1})					
	OXS	LXS	CS	OXT	LXT	CT
Day 1	2.35 ± 0.38^{a1}	2.44 ± 0.20^{a1}	2.21 ± 0.13^{a1}	$<1^{a1}$	$<1^{a1}$	$<1^{a1}$
Day 3	3.00 ± 0.09^{b2}	2.65 ± 0.36^{a1}	3.20 ± 0.03^{b2}	$<1^{a1}$	$<1^{a1}$	$<1^{a1}$
Day 6	3.60 ± 0.17^{a3}	3.56 ± 0.01^{a2}	3.48 ± 0.19^{a2}	$<1^{a1}$	$<1^{a1}$	$<1^{a1}$
Day 8	4.38 ± 0.04^{b4}	4.17 ± 0.09^{a3}	4.64 ± 0.05^{c3}	$<1^{a1}$	$<1^{a1}$	$<1^{a1}$
	OLS	LLS	CS	OLT	LLT	CT
Day 1	$2.16\pm0,02^{a1}$	2.15 ± 0.15^{a1}	2.38 ± 0.33^{a1}	$2.62\pm0,04^{a1}$	2.27 ± 0.52^{a1}	2.57 ± 0.70^{b1}
Day 3	2.65 ± 0.11^{a2}	2.66 ± 0.28^{a2}	2.60 ± 0.05^{a2}	2.80 ± 0.57^{b1}	2.55 ± 0.35^{b2}	3.05 ± 0.21^{a2}
Day 6	3.42 ± 0.24^{a3}	3.29 ± 0.05^{a3}	3.37 ± 0.04^{a3}	3.20 ± 0.85^{b2}	2.95 ± 0.35^{b43}	3.80 ± 0.09^{a3}
Day 8	3.91 ± 0.16^{a4}	3.83 ± 0.18^{a4}	$4.19\pm0,01^{a4}$	3.90 ± 0.42^{b3}	3.37 ± 0.52^{b4}	4.26 ± 0.23^{a4}
	OKS	LKS	CS	OKT	LKT	CT
Day1	1.81 ± 0.47^{c1}	2.23 ± 0.12^{b1}	2.53 ± 0.59^{a1}	2.23 ± 0.12^{b1}	2.03 ± 0.11^{c1}	2.41 ± 0.10^{a1}
Day 3	2.41 ± 0.10^{c2}	2.91 ± 0.06^{b2}	3.14 ± 0.14^{a2}	3.21 ± 0.18^{a2}	2.57 ± 0.15^{c2}	3.13 ± 0.21^{b2}
Day 6	2.74 ± 0.66^{c3}	3.45 ± 0.18^{b3}	4.45 ± 0.21^{a3}	3.66 ± 0.30^{b3}	3.57 ± 0.12^{c3}	3.82 ± 0.11^{a3}
Day 8	3.76 ± 0.08^{c4}	3.98 ± 0.04^{b4}	4.80 ± 0.14^{a4}	3.97 ± 0.07^{b4}	3.98 ± 0.07^{b4}	4.74 ± 0.06^{a4}

n=3; Mean value ± standart deviation $^{a-c}$: a-c: different letters in the same row show statistically significant difference between the groups ($p<0.05$). $^{1-4}$: 1-4: different numbers in the same column show statistically significant difference for each group according to storage ($p<0.05$).

pH values of the coated and uncoated rainbow trout fillets and squid rings showed initial decrease followed by an increase as reported in Table 5. The sensory evaluation of the coated and uncoated rainbow trout fillets and squid rings are presented in (Table 6-8). The sensoy evaluation of groups was determined by the sensorial characteristics such as color, odour and texture, as well as general acceptability. Color, odour, texture and general accetability characteristics of all the groups (OXT, OXS, LXT, LXS, CS, CT, OLS, OLT, LLT, LLS, OKS, LKS, OKT, LKT) were statistically significant ($p<0.05$) decrease during the storage period.

Table 4. Lactic acid bacteria counts (log CFU g^{-1}) of coated and uncoated rainbow trout trout fillets and squid rings during storage

Tablo 4. Kaplamalı ve kaplamasız gökkuşağı alabalığı filetolarının ve kalamar halkalarının depolama sırasındaki laktik asit bakteri sayıları (log CFU g^{-1})

Storage Time	Lactic acid bacteria Counts (log CFU g^{-1})					
	OXS	LXS	CS	OXT	LXT	CT
Day 1	<1 ^{a1}	<1 ^{a1}	<1 ^{a1}	2.28±0.02^{a1}	2.75±0.06^{b1}	2.31±0.33^{a1}
Day 3	2.89±0.02^{a2}	2.60±0.28^{a2}	2.09±0.12^{b2}	3.18±0.20^{a2}	2.40±0.32^{b2}	2.41±0.29^{b1}
Day 6	3.19±0.27^{a3}	3.55±0.10^{a3}	2.59±0.16^{b3}	3.77±0.04^{a3}	3.76±0.05^{a3}	3.50±0.16^{b2}
Day 8	3.75±0.03^{a4}	3.97±0.10^{a4}	3.50±0.19^{b4}	4.29±0.16^{a4}	4.44±0.06^{a4}	3.87±0.12^{b3}
	OLS	LLS	CS	OLT	LLT	CT
Day 1	2.30±0.43^{c1}	2.61±0.01^{b1}	2.33±1.02^{a1}	3.09±0.13^{a1}	3.07±0.21^{a1}	3.45±0.13^{b1}
Day 3	3.41±0.04^{b2}	4.78±0.20^{a2}	2.72±0.90^{c2}	4.08±0.20^{c2}	4.84±0.04^{a2}	3.43±0.25^{b1}
Day 6	4.40±0.25^{b3}	5.30±0.09^{a3}	3.80±0.10^{c3}	5.80±0.40^{a3}	5.30±0.05^{b3}	4.30±0.30^{c2}
Day 8	6.70±0.32^{b4}	7.74±0.25^{a4}	5.20±0.27^{c4}	7.37±0.36^{a4}	7.59±0.20^{a4}	5.84±0.52^{b3}
	OKS	LKS	CS	OKT	LKT	CT
Day 1	2.86±0.37^{b1}	3.30±0.28^{a1}	2.19±0.83^{c1}	2.79±0.23^{a1}	2.76±0.81^{a1}	1.90±0.84^{b1}
Day 3	3.82±0.05^{b2}	4.09±0.12^{a2}	2.72±0.90^{c2}	3.60±0.26^{a2}	3. ±0.02^{a2}	3.16±0.01^{b2}
Day 6	4.95±0.32^{b3}	5.69±0.03^{a3}	3.89±0.78^{c3}	4.55±0.60^{a3}	4.80±0.14^{a3}81	4.24±0.19^{b3}
Day 8	6.55±0.01^{b4}	6.56±0.12^{a4}	5.04±0.08^{c4}	5.92±0.18^{a4}	6.47±0.57^{a4}	4.84±0.08^{b4}

n=3; Mean value ± standart deviation $^{a-c}$: a-c: different letters in the same row show statistically significant difference between the groups ($p<0.05$). $^{1-4}$: 1-4: different numbers in the same column show statistically significant difference for each group according to storage ($p<0.05$).

Table 5. pH values of coated and uncoated rainbow trout fillets and squid rings during storage

Çizelge 5. Kaplamalı ve kaplamasız gökkuşağı alabalığı filetoları ve kalamar halkalarının depolama sırasındaki pH değerleri

Storage Time	pH Values					
	OXS	LXS	CS	OXT	LXT	CT
Day 1	7.47±0.12^{b1}	7.17±0.15^{a1}	7.53±0.21^{b1}	6.30±0.10^{b1}	6.43±0.15^{b1}	6.64±0.15^{a1}
Day 3	7.44±0.30^{b1}	7.25±0.23^{b2}	8.07±0.15^{a2}	6.55±0.01^{b2}	6.57±0.01^{b2}	6.61±0.05^{a1}
Day 6	7.15±0.03^{b2}	7.16±0.02^{b1}	7.51±0.03^{a1}	6.96±0.12^{b3}	6.63±0.04^{a3}	6.83±0.01^{b2}
Day 8	7.26±0.05^{b3}	7.26±0.04^{b2}	7.59±0.02^{a3}	6.68±0.11^{b4}	6.82±0.05^{b4}	6.99±0.02^{a3}
	OLS	LLS	CS	OLT	LLT	CT
Day 1	6.67±0.01^{b1}	5.90±0.14^{c1}	7.05±0.12^{a1}	6.20±0.05^{b1}	6.29±0.08^{c1}	6.33±0.03^{a1}
Day 3	6.94±0.09^{b2}	6.26±0.06^{c2}	7.31±0.05^{a2}	6.32±0.06^{b2}	6.28±0.04^{c1}	6.58±0.07^{a2}
Day 6	7.11±0.11^{b3}	6.68±0.17^{c3}	7.42±0.12^{a3}	6.86±0.11^{b3}	6.41±0.11^{c2}	7.21±0.23^{a3}
Day 8	7.39±0.13^{b4}	6.85±0.06^{c4}	7.79±0.18^{a4}	7.23±0.22^{b4}	6.96±0.17^{c3}	7.35±0.17^{a4}
	OKS	LKS	CS	OKT	LKT	CT
Day1	7.03±0.35^{c1}	7.17±0.15^{b1}	7.77±0.15^{a1}	6.20±0.36^{b1}	6.45±0.24^{ab1}	6.64±0.15^{a1}
Day 3	7.03±0.21^{b1}	6.96±0.05^{c1}	7.67±0.29^{a2}	6.40±0.35^{b2}	6.49±0.35^{ab1}	6.50±0.14^{a2}
Day 6	6.72±0.16^{b2}	6.11±0.06^{c2}	7.17±0.20^{a3}	6.30±0.07^{b3}	6.22±0.14^{ab2}	6.42±0.03^{a3}
Day 8	7.57±0.31^{b3}	7.26±0.04^{c3}	7.63±0.55^{a4}	6.69±0.16^{b4}	6.89±0.04^{ab3}	7.13±0.14^{a4}

n=3; Mean value ± standart deviation $^{a-c}$: a-c: different letters in the same row show statistically significant difference between the groups ($p<0.05$). $^{1-4}$: 1-4: different numbers in the same column show statistically significant difference for each group according to storage ($p<0.05$).

According to the sensory evaluation, the groups LKS, LLS and LXS were the most preferred group in terms of the odour, whereas the groups OXT, OLT and OKT were showed the best favorable sensorial characteristics in terms of color from the panelists at the beginning of the storage. At the end of the storage period (on day 8), general acceptability scores of the groups decreased to 1.80, 1.00, 1.60, 1.20, 1.40, 1.60 for the groups OXS, LXS, CS, OXT, LXT and CT, respectively, while general acceptability scores of the groups decreased to 1.60, 1.60, 1.80, 2.00, 2.40, 1.00 for the groups OLS, LLS, CS, OLT, LLT and CT. In addition to this, Sensory evaluation of the groups OKS, LKS, CS, OKT, LKT and CT decreased to 1.60, 2.60, 1.20, 1.80, 2.60, 1.20, respectively on the 8th day of storage. When compared with coated and uncoated samples, the lowest general acceptability scores were determined in uncoated samples.

Table 6. Sensory evaluation of coated by using orange and lemon peels with xanthan and uncoated rainbow trout fillets and squid rings during storage

Çizelge 6. Depolama sırasında ksantanlı portakal ve limon kabukları ile kaplanmış ve kaplanmamış gökkuşağı alabalığı filetoları ve kalamar halkalarının duyusal değerlendirmesi

Storage Time		Sensorial Quality					
		OXS	LXS	CS	OXT	LXT	CT
Day 1	color	7.20±1.30^{a1}	7.20±1.30^{a1}	8.60±0.89^{b1}	8.80±0.45^{c1}	7.20±0.84^{a1}	8.00±1.00^{b1}
	odor	7.80±1.30^{a1}	8.00±1.00^{a1}	8.40±0.55^{b1}	8.00±0.71^{b1}	7.60±0.89^{a1}	7.60±0.55^{a1}
	texture	8.40±0.89^{a1}	8.20±0.45^{a1}	8.80±0.45^{b1}	8.20±0.45^{a1}	8.40±0.55^{ab1}	8.60±0.55^{bc1}
	general	8.00±0.71^{b1}	7.60±1.14^{a1}	8.60±0.55^{c1}	8.00±0.71^{b1}	7.20±0.84^{a1}	8.60±0.55^{c1}
Day 3	color	5.20±0.84^{a2}	5.60±0.89^{b2}	6.80±1.10^{c2}	6.80±1.10^{b2}	5.80±0.45^{a2}	7.80±0.45^{c2}
	odor	6.40±0.55^{b2}	6.20±1.10^{b2}	5.80±0.84^{a2}	5.80±1.10^{a2}	6.20±0.45^{b2}	5.80±0.45^{a2}
	texture	7.20±0.84^{b2}	7.00±0.71^{b2}	6.60±0.89^{a2}	5.80±1.30^{b2}	7.00±1.00^{c2}	5.40±0.89^{a2}
	general	5.40±0.89^{a2}	5.60±1.14^{a2}	7.40±0.55^{b2}	7.20±0.84^{b2}	6.40±0.55^{a2}	7.40±0.55^{b2}
Day 6	color	3.60±0.89^{b3}	2.80±0.84^{a3}	4.80±1.30^{c3}	5.00±0.71^{b3}	4.20±1.30^{a3}	4.00±1.58^{a3}
	odor	5.20±0.84^{b3}	7.00±1.00^{c3}	4.20±0.84^{a3}	4.00±0.71^{b3}	5.00±0.71^{c3}	3.40±1.52^{a3}
	texture	5.40±0.89^{b3}	5.20±0.84^{c3}	4.40±1.14^{a3}	4.40±1.14^{a3}	4.80±0.45^{b3}	5.40±1.14^{c3}
	general	5.60±0.89^{c3}	4.80±0.45^{b3}	3.60±1.34^{a3}	5.40±0.89^{c3}	4.80±0.84^{b3}	4.20±1.30^{a3}
Day 8	color	1.40±0.55^{a4}	1.40±0.55^{a4}	2.40±0,89^{b4}	2.00±1.00^{ab4}	1.80±0.84^{a4}	2.20±0.84^{b4}
	odor	3.20±1.10^{c4}	2.80±0.84^{b4}	1.20±0.45^{a4}	2.00±0.71^{a4}	1.80±0.84^{a4}	1.80±0.84^{a4}
	texture	2.40±0.55^{c4}	2.00±0.71^{b4}	1.40±0.55^{a4}	2.40±0.55^{b4}	2.20±0,84^{ab4}	1.80±0.84^{a4}
	general	1.80±0.84^{ab4}	1.00±0.71^{b4}	1.60±0.89^{a4}	1.20±0.84^{b4}	1.40±0.55^{b4}	1.60±0.89^{a4}

n=3; Mean value ± standart deviation $^{a-c}$: a-c: different letters in the same row show statistically significant difference between the groups ($p<0.05$). $^{1-4}$: 1-4: different numbers in the same column show statistically significant difference according to storage ($p<0.05$).

Table 7. Sensory evaluation of coated by using orange and lemon peels with locust bean gum and uncoated rainbow trout fillets and squid rings during storage

Çizelge 7. keçiboynuzu gam ile portakal ve limon kabuklarıyla kaplanmış ve kaplanmamış gökkuşağı alabalığı filetoları ve kalamar halkalarının depolama sırasında duyusal değerlendirilmesi

Storage Time		Sensorial Quality					
		OLS	LLS	CS	OLT	LLT	CT
Day1	color	8.00±1.22^{b1}	7.20±0.84^{a1}	8.20±0.84^{b1}	8.60±0.55^{a1}	7.60±0.55^{b1}	8.60±0.55^{a1}
	odor	8.00±0.71^{b1}	8.60±0.55^{a1}	8.00±0.71^{b1}	7.60±0.55^{a1}	8.20±0.84^{b1}	8.50±0.58^{c1}
	texture	8.20±0.84^{a1}	8.20±0.84^{a1}	8.40±0.55^{b1}	8.00±0.71^{a1}	8.00±0.71^{a1}	8.60±0.55^{b1}
	general	7.80±0.84^{a1}	8.20±0.84^{b1}	8.60±0.55^{c1}	8.20±0.84^{a1}	8.20±0.84^{a1}	8.60±0.55^{b1}
Day 3	color	5.60±1.14^{a2}	6.20±0.84^{b2}	6.20±1.10^{b2}	5.80±1.30^{a2}	6.40±0.55^{b2}	6.20±1.30^{b2}
	odor	6.40±0.55^{b2}	6.20±1.10^{b2}	5.80±0,84^{a2}	5.80±1.10^{a2}	6.20±0.45^{b2}	5.80±0.45^{a2}
	texture	6.20±1.48^{a2}	6.20±1.30^{a2}	6.80±1.10^{b2}	5.80±1.79^{a2}	6.20±1.48^{b2}	5.60±1.14^{a2}
	general	6.20±0.84^{a2}	6.00±1.00^{a2}	6.80±0.45^{b2}	6.00±1.22^{a2}	6.20±0,84^{a2}	6.80±0.45^{b2}
Day 6	color	3.80±1.48^{b3}	3.00±1.00^{a3}	4.60±1.67^{c3}	4.00±0.71^{a3}	4.40±2.07^{b3}	3.80±1.79^{a3}
	odor	3.20±1.30^{a3}	3.60±1.82^{b3}	3.60±1.14^{b3}	3.20±1.30^{b3}	3.60±0.89^{c3}	2.80±0.84^{a3}
	texture	4.80±1.48^{a3}	5.00±1.22^{a3}	4.80±1.30^{a3}	4.80±1.30^{a3}	5.40±0.55^{b3}	4.80±1.64^{a3}
	general	5.00±0.71^{b3}	4.80±1.10^{b3}	3.80±1.10^{a3}	4.80±1.30^{b3}	4.60±1.34^{b3}	4.20±1.30^{a3}
Day 8	color	1.80±0.84^{a4}	1.60±0.55^{a4}	2.20±0.84^{b4}	2.40±0.55^{b4}	2.20±0.84^{a4}	2.40±1.14^{b4}
	odor	2.20±0.84^{b4}	2.80±0.45^{c4}	1.40±0.55^{a4}	2.40±0.55^{b4}	2.20±0.84^{b4}	1.40±0.55^{a4}
	texture	2.40±0.55^{c4}	2.00±0.71^{b4}	1.40±0.55^{a4}	2.40±0.55^{b4}	2.20±0.84^{b4}	1.80±0.84^{a4}
	general	1.60±0.55^{a4}	1.60±0.55^{a4}	1.80±0.84^{b4}	2.00±0.71^{a4}	2.40±0.55^{b4}	1.00±1.00^{a4}

n=3; Mean value ± standart deviation $^{a-c}$: a-c: different letters in the same row show statistically significant difference between the groups ($p<0.05$). $^{1-4}$: 1-4: different numbers in the same column show statistically significant difference according to storage ($p<0.05$).

Samples of colonies with different morphological characteristics were analyzed throughout the storage period. After the stage of purified and the colonies were identified with API 20 E bacterial identification test kits. According to the results taken from the computer Identification Program, isolated bacteria with identification rates were given as follows: In the groups of CS and CT, the isolation rates of *Moraxella* spp., *Ochrobacretrum anthropi* and *Enterobacter aerogenes* were equivalent to 85.7%, 85.9 and 96.2%, respectively. Bacteria identified in squid rings and rainbow trout fillets covered with extracts of orange and lemon peels and films made by using xanthan, locust bean and carrageenan were *Serratia*

liquefaciens 98.0%, *Serratia marcescens* 98.0%, *Pasteurella aerogenes* 97.6%, *Photobacterium damselae* 84.7%, *Pantoe spp*. 95.8%, *Erwinia spp*. 95.8%. On the other hand, the bacterial strains identified in the samples, which were covered with orange extracts, lemon peels and edible films were equivalent to 98.0%, 98.0%, 97.6%, 84.7%, 95.8% and 95.8% for *Serratia liquefaciens, Serratia marcescens, Pasteurella aerogenes, Photobacterium damselae, Pantoe* spp., *Erwinia* spp, respectively. Hence *Serratia liquefaciens* and *Serratia marcescens* were the most important species of the *Enterobacteriaceae* family.

Table 8. Sensory evaluation of coated by using orange and lemon peels with carrageenan gum and uncoated rainbow trout fillets and squid rings during storage

Çizelge 8. Karagenan gam ile Portakal ve limon kabuklarıyla kaplanmış ve kaplanmamış gökkuşağı alabalığı filetoları ve kalamar halkalarının depolama sırasında duyusal değerlendirilmesi

Storage Time		Sensorial Quality					
		OKS	LKS	CS	OKT	LKT	CT
Day 1	color	7.60±0.89^{b1}	7.80±0.84^{b1}	7.00±0.71^{a1}	8.50±0.71^{b1}	8.20±0.84^{b1}	7.40±0.55^{a1}
	odor	8.00±0.71^{a1}	8.40±0.55^{b1}	8.40±0.89^{b1}	7.80±0.84^{a1}	8.00±0.71^{a1}	8.60±0.55^{b1}
	texture	8.40±0.55^{b1}	8.20±0.45^{a1}	8.60±0.55^{b1}	8.20±0.45^{a1}	8.40±0.55^{ab1}	8.60±0.55^{b1}
	general	8.40±0.55^{b1}	7.80±0.84^{a1}	8.60±0.55^{b1}	8.40±0.55^{a1}	8.40±0.89^{a1}	8.80±0.45^{b1}
Day 3	color	5.60±0.55^{b2}	6.00±1.00^{c2}	5.00±1.22^{a2}	5.00±0.71^{a2}	7.20±0.84^{b2}	5.20±1.10^{a2}
	odor	6.80±0.84^{a2}	7.40±0.55^{b2}	6.60±0.55^{a2}	5.60±0.89^{a2}	7.40±0.55^{c2}	6.40±0.55^{b2}
	texture	6.80±0.45^{a2}	8.00±0.71^{b2}	7.60±0.55^{c2}	6.60±1.14^{a2}	7.40±0.55^{b2}	6.40±0.55^{a2}
	general	7.80±0.45^{a2}	7.80±0.45^{a2}	7.40±0.55^{b2}	7.60±0.55^{ab2}	7.80±0.84^{b2}	7.40±0.55^{a2}
Day 6	color	3.60±1.14^{a3}	4.40±1.52^{b3}	5.40±1.52^{c3}	3.60±0.89^{a3}	4.00±0.71^{b3}	4.80±1.30^{c3}
	odor	6.00±1.00^{a3}	5.60±1.52^{b3}	4.00±0.71^{c3}	5.00±1.22^{c3}	3.80±1.10^{b3}	3.20±1.30^{a3}
	texture	4.80±0.45^{b3}	4.60±0.55^{b3}	3.60±0.55^{a3}	4.00±0.71^{a3}	5.40±1.14^{b3}	4.00±1.22^{a3}
	general	5.20±1.30^{b3}	5.60±1.52^{c3}	4.00±1.22^{a3}	3.20±1.30^{a3}	5.40±1.82^{c3}	3.60±0.55^{b3}
Day 8	color	2.20±0.84^{b4}	1.80±0.84^{a4}	2.20±0.84^{b4}	2.00±0.71^{a4}	2.00±0.71^{a4}	2.00±1.00^{a4}
	odor	3.00±0.71^{b4}	3.00±0.71^{b4}	1.60±0.55^{a4}	2.20±0.84^{b4}	2.20±0.84^{b4}	1.40±0.55^{a4}
	texture	3.00±0.71^{b4}	2.40±1.14^{a4}	2.20±0.45^{a4}	2.20±0.84^{b4}	1.80±0.45^{a4}	1.80±0.84^{a4}
	general	1.60±0.55^{b4}	2.60±0.55^{b4}	1.20±0.45^{a4}	1.80±0.45^{b4}	2.60±0.55^{b4}	1.20±0.45^{a4}

n=3; Mean value ± standart deviation $^{a-c}$: a-c: different letters in the same row show statistically significant difference between the groups ($p<0.05$). $^{1-4}$: 1-4: different numbers in the same column show statistically significant difference according to storage ($p<0.05$).

According to the API 50 CH bacteria identification test kit, the identified lactic acid bacteria species as follows: *Lactobacillus acidophilus* 89.1%, *Lactobacillus salivarius* 99.9%, *Lactococcus lactis* %81.8 and *Lactobacillus brevis* 99.6% in rainbow trout fillets and squid rings after coating with orange and lemon peels extract and xanthan. Bacteria isolated in rainbow trout fillets and squid rings after coating with orange and lemon peels extract and locust bean were *Lactobacillus paracasei* 97.9%, *Lactococcus lactis* 96.5%, *Lactobacillus brevis* 98.0% and *Carnobacterium maltaromaticum* 99.9%. After coating with orange and lemon peel extracts and carragenaan, the identified bacteria in rainbow trout fillets and squid rings were *Lactobacillus pentosus* 86.0%, *Lactobacillus brevis* 95.6%, *Leuconostoc mesenteroides* 78.4%, *Lactobacillus paracasei* 95.0%, *Lactococcus lactis* 72.4%.

DISCUSSION

Hassanzadeh et al. (2018) studied the effect of 2.00 % chitosan and 0.10% grape seed extract on the shelf-life of rainbow trout fillets. The authors reported in their study that the coatings had significant effect on reducing the total bacteria counts of samples. This result was very similar to our findings that the edible films with orange and lemon peels were also inhibited the growth of bacteria on rainbow trout fillets and squid rings. The results obtained in our study were well correlated with the previous studies (Chamanara et al., 2013; Korkmaz et al., 2019; Socaciu et al., 2018; Song et al., 2011) about observing slower increase in TMC of the coated fishery products, when compared with uncoated samples. The group of *Entebactericeae* was reported to be an indicator of the hygienic conditions of the fresh rainbow trout by Mexis et al., (2009). In addition to this, this group of microorganisms was also reported to be plant sourced bacteria (Ünlütürk & Turantaş, 2003). Uçak (2019) reported that the coating of rainbow trout fillets with gelatin-based film either alone or in combination with of garlic peel extract (GPE) inhibited the growth of *Enterobactericeae* during the storage. However, in this study the lowest bacterial count was found in gelatin coated samples incorporated with GPE. Volpe et al. (2015) and Chytiri et al. (2004) also reported in their study that slow growth of Enterobacteriaceae was observed during storage period. Our results were very similar to those of the above studies (Chytiri et

al. 2004; Uçak 2019; Volpe et al. 2015), in which edible films inhibited the growth of *Enterobactericeae* on fishery products during the storage. In addition to this, the group of LAB generally was recognized as safe for human consumption, and they also could be found naturally dominate microflora of many foods (Ghanbari et al., 2013). Raeisi et al. (2015) studied the application of carboxymethyl cellulose (CMC) coatings incorporated with *Zataria multiflora Boiss*. essential oil (ZMEO) and grape seed extract (GSE) to extend the shelf life of rainbow trout fillets. Researchers reported in their study that high concentrations of ZMEO and GSE rapidly increased the LAB counts of the samples, thereby LAB counts showed a strong synergistic effects against spoilage microorganisms. Likewise, Joukar et al. (2017) reported in their study that LAB counts developed at refrigerator temperatures. The initial LAB counts of trout fillets was reported to be 1 log CFU g^{-1}, whereas this value reached to 6.28 log CFU g^{-1} at the end of storage period. Our results were very similar with the above study, which was reported by Joukar et al. (2017). Based on the period of storage there were various rates of decrease or increase in the pH values of rainbow trout were reported by Aksoy and Sezer 2019; Chamanara et al. 2013; Hosseini et al. 2016. The authors also reported that a slight increase in the pH value of all samples were observed at the end of the storage due to the growth of spoilage related bacteria, which caused the alkaline compounds to be increased. Yu et al., (2018) reported that edible coatings were represented an effective and environmentally friendly alternative that can be used to extend the shelf-life of all types of fishery products. Frangos et al., (2010) stated that salt and oregano oil (0.2%) that were used in cooked trout samples had sensorially acceptable pleasant odor and were also well accepted by the panellists. In one report; the shelf-life of eel was specified as 16 days for laurel and 20 days for myrtle whereas 12 days for the control group (Özoğul et al., 2014). In another report; Alparslan et al. (2019) reported that the results of sensorial and microbiological analysis revealed that the shelf-life of gelatin coated shrimp had 12 days, while gelatin film with orange peel essential oil coated shrimp was 15 days. Previous studies reported that it was advised the use of edible films enriched with plant extracts and essential oils to extend the shelf-life of fishery products (Korkmaz et al., 2019; Mei et al., 2019; Mohan et al., 2012; Uçak, 2019). Sallam (2007) reported that the major group of microorganisms, which was responsible for spoilage of stored fresh fish at chilled temperatures, was the gram-negative psychrotrophic bacteria. The results obtained in this study were in accordance with the other studies, in which the authors reported an increase in psychrotrophic bacterial growth on fishery products during cold storage (Bulat et al., 2020; Chamanara et al., 2013; Granda, 2015; Kılınç & Altaş, 2016; Kılınç et al., 2017). *Erwinia* and *Serratia* species reported to be the plant-based microorganisms and they also reported not to be indicated the fecal contamination (Surengil, 2014). In our study, the groups of identified bacteria species such as *Serratia liquefaciens* (98%), *Serratia marcescens* (98%) and *Erwinia* spp. (95.8%) were thought to be originated from the orange and lemon peels. Lacticacid bacteria can be found in vegetable-derived products and spices. Lactic acid bacteria was isolated from plants and spices, which were reported by (Fuselli et al., 2003). In this study, *Lactobacillus paracasei* was isolated from black pepper, *L. cellobiosus* was isolated from bay leaf and *L.acidophilus* was isolated from red pepper (Fuselli et al., 2003).

CONCLUSION

In our study the impact of edible films with orange and lemon peels on microbial flora and shelf-life of squid rings (*Loligo vulgaris*) and rainbow trout fillets (*Oncorhynchus mykiss*) were evaluated. When compared with the uncoated samples, edible film coated samples had determined longer shelf-life. In terms of microbiological and sensory evaluation; the results of microbiological count were lower and sensory attributes were determined much more favorable in edible film coated samples, when compared to control samples. Especially, the groups LKS, LLS and LXS were the most preferred group in terms of the odour, whereas the groups OXT, OLT and OKT were showed the best favorable sensorial characteristics in terms of color. TMC of squid rings (CS) exceeded the microbiological limit of the consumption on day 6, whereas TMC of squid rings LLS and LKS did not exceed this limit on day 8.

The differences of the TMC of the groups were determined statistically significant ($p<0.05$) according to the coating types and the storage period. The most important bacteria species of rainbow trout fillets and squid rings covered with edible films prepared by using lemon and orange peels were identified as: *Lactobacillus paracasei* 97.9%, *Lactococcus lactis* 96.5%, *Carnobacterium maltaromaticum* 99.9%, *Lactobacillus pentosus* 86.0%, *Leuconostoc mesenteroides* 78.4%, *Lactobacillus acidophilus* 89.1%, *Lactobacillus salivarius* 99.9%, *Lactobacillus brevis* 99.6%, *Serratia liquefaciens* 98.0%, *Serratia marcescens* 98.0%, *Erwinia spp.* 95.8%. Discarded fruit peels in fruit juice processing plants can be evaluated for producing functional fishery products, which would a good alternative for being produced edible films. The results of this study can be evaluated by seafood and fruit juice processing plants as well as food producers.

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Statement of Conflict Of Interest

Authors have declared no conflict of interest.

Author's Contributions

The contribution of the authors is equal.

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Diklofenak Sodyumun Zebra Balığı (*Danio rerio*) Larvaları Üzerindeki Teratojenik ve Gelişimsel Toksisitesinin Değerlendirilmesi

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ÖZET

Bu çalışmada tıpta ve veteriner hekimlikte ağrı ve iltihabı kontrol etmek için kullanılan diklofenak sodyumun (DKFS) zebra balığı embriyoları ve larvaları üzerindeki etkileri değerlendirilmiştir. Embriyolar 96 saat süreyle 0.21-5.33 mg L^{-1} DKFS'ye maruz bırakılmış ve bu bireylerin hayatta kalma oranları, kalp atım sayıları, kuluçkadan çıkma oranları ve vücut malformasyonları belirlenmiştir. LC_{50}, EC_{50} ve teratojenik indeks (TI) değerleri sırasıyla 1.55 ve 0.81, 1.91 olarak hesaplanmıştır. DKFS, hesaplanan TI değerine göre zebra balığı embriyoları için teratojendir. 0.47 mg L^{-1} ve daha yüksek konsantrasyonlarda DKFS zebra balıklarında, perikardiyal ödem, yolk kesesi ödemi, kuyruk malformasyonu ve omurga eğriliğine neden olmuştur. En sık rastlanan malformasyonlar perikardiyal ve yolk kesesi ödemi olarak belirlenmiştir. 0.7 mg L^{-1} ve daha yüksek konsantrasyonlarda zebra balıkları larvalarının boy uzunluklarında ve dakikadaki kalp atım sayılarında önemli oranda inhibisyona neden olmuştur. 2.37 mg L^{-1} ve daha yüksek konsantrasyonlarda DKFS'nin ise zebra balıklarının kuluçkadan çıkma oranlarını %50'nin altına düşürdüğünü göstermiştir. Bu sonuçlar, DKFS'nin zebra balığı gelişimi üzerinde olumsuz etkilere neden olduğunu ve sucul ortama girmesi durumunda su ekosistemini olumsuz etkileyebileceğini göstermektedir.

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Anahtar Kelimeler
Diklofenak sodyum
Zebra balığı embriyo testi
Toksisite

Evaluation of Teratogenic and Developmental Toxicity of Diclofenac Sodium on Zebrafish (*Danio rerio*) Larvae

ABSTRACT

In this study, the effects of diclofenac sodium (DKFS), used in medicine and veterinary medicine to control pain and inflammation, on zebrafish embryos and larvae were evaluated. Embryos were exposed to 0.21-5.33 mg L^{-1} DCFS for 96 hours and the survival rates, heart rate, hatching rates and body malformations of these individuals were determined. LC_{50}, EC_{50} and teratogenic index (TI) values were calculated as 1.55 and 0.81, 1.91, respectively. DKFS is teratogenic for zebrafish embryos based on the calculated TI value. DKFS at concentrations of 0.47 mg L^{-1} and higher caused pericardial edema, yolk sac edema, tail malformation and spinal curvature in zebrafish. The most common malformations were determined as pericardial and yolk sac edema. At concentrations of 0.7 mg L^{-1} and higher, it caused significant inhibition in the length and heart rate of zebrafish larvae. It has been shown that DKFS at concentrations of 2.37 mg L^{-1} and higher reduced the hatching rate of zebrafish below 50%. These results show that DKFS causes adverse effects on zebrafish development and may adversely affect the aquatic ecosystem if it enters the aquatic environment.

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GİRİŞ

Farmasötikler, sucul ekosistemde yaygın olarak bulunan ksenobiyotik bileşiklerdir. Bu bileşiklerden kaynaklanan çevre kirliliği, sucul organizmalar ve insan sağlığı için potansiyel riskler oluşturabilmektedir (Daou ve ark., 2020). Bu bileşikler ve metabolitleri, evsel, endüstriyel ve hastane atıkları ile su kütlelerine ve besin zinciri yolu ile insana kadar ulaşabilmektedir (Santos ve ark., 2021).

Diklofenak sodyum (DKFS), ağrı ve iltihabı kontrol etmek için kullanılan, steroid olmayan anti-inflamatuar bir ilaçtır. Fenilasetik asidin bir türevi olan DKFS (sodyum 2-(2-(2,6-diklorofenilamino) fenil) asetik asit), 1970 yılından beri tıpta genellikle sodyum veya potasyum tuzu olarak kullanılmaktadır. Dünya genelinde çok yaygın olarak kullanıldığından dolayı çoğu sucul ekosistemde sıklıkla tespit edilmektedir (Mirzaee ve ark., 2021).

DKFS, yüzey sularında 0.0002 -100 µg L^{-1} konsantrasyon aralığında bulunabilmektedir. Sucul ekosistemde bulunan DKFS'ye kronik maruziyet, hedef olmayan sucul organizmaların metabolizması üzerine toksik etkilere neden olabilmektedir. Çünkü bu ilaçların, birçok hayvan türünde bulunan ve prostanoidlerin sentezinden sorumlu enzim olan siklooksijenaz aktivitesini ve DNA sentezini inhibe ettiği rapor edilmiştir (Felice ve ark., 2012). Ayrıca, anti-inflamatuar ilaç diklofenak Avrupa Komisyonu, 20 Mart 2015 tarih ve 2015/495 sayılı Uygulama Kararı, 2008/105/EC ve 2000/60/EC Su Çerçeve Direktifi kapsamında, öncelikli maddelerin "izleme listesine" dahil edilmiştir (Koba ve ark. 2018).

Zebra balığı, çeşitli kimyasalların ve kirleticilerin gelişimsel toksisite değerlendirmesinde ve teratojenite taramasında yaygın olarak kullanılan model organizmalardan biridir. Geleneksel hayvan modelleriyle karşılaştırıldığında, şeffaflık, küçük boyut, az miktarda test bileşiği gerekliliği, düşük maliyet, kolay bakım ve insan organ sistemlerine büyük benzerlik gibi birçok avantaja sahiptir (Jia ve ark., 2020; Nguyen ve ark., 2021).

Bu çalışmada, DKFS'nin zebra balıklarında kullanılan maruz kalma konsantrasyonları, yüzey sularında ölçülen değerlere nispeten yakın veya daha yüksek seviyelerdedir. Bunun nedeni, hem DKFS'nin sucul ortamda letal konsantrasyonlarını ve subletal etkilerini belirlemek hem de DKFS'nin çevresel sularda konsantrasyonunun artması durumda ne tür etkilere neden olabileceğini göstermektir. Bu amaçla, balık embriyo testi kullanarak DKFS'nin zebra balıkları juvenillerinde hayatta kalma, büyüme ve gelişme, kalp atım oranı, kuluçkadan çıkma oranı ve terotojenite durumu gibi farklı biyolojik yanıtlar belirlenmiştir. Böylece, DKFS'nin zebra balıkları embriyolarında neden olduğu letalite düzeylerinin yanı sıra teratojenitesi ile ilgili sınırlı veriye katkı sunulmuştur.

MATERYAL ve METOD

Test organizması

Çalışmada kullanılan embriyolar, İnönü Üniversitesi Fen Edebiyat Fakültesi, Sucul Omurgalı Deney Hayvanları Birimi, Zebra Balığı Ünitesinde bulunan zebra balığı üretim sisteminde (ZebTec Active Blue, Tecniplast, İtalya) yetiştirilen erişkin zebra balıklarından üretildi. Sürekli su sirkülasyonu olan zebra balığı sisteminde, pH 7.30, iletkenlik 720 µS/cm, sıcaklık 28.2 ℃ ve fotoperiyot 14 saat aydınlık 10 saat karanlık olacak şekilde ayarlandı. Zebra balığı embriyoları, ana sistem ile aynı sucul özelliklere ve doğrudan ana sistem tarafından beslenen su sirkülasyonuna bağlı filtreli bir yetiştirme sistemi (iSpawn, Tecniplast, İtalya) ile elde edildi. Döllenen yumurtalar 3 saat içinde toplandı ve 28.5 ºC sıcaklıkta standart embriyo suyu içerisinde, etüvde muhafaza edildi.

D. rerio yumurta ve larvaları, İnönü Üniversitesi Araştırma Kurulu (Araştırma Protokolü No. 2021/4-2) tarafından onaylanan hayvan protokollerine uygun olarak elde edildi.

Kimyasallar ve maruziyet

Diklofenak sodyum (98% saflıkta, CAS NO: 15307-79-6) ACROS Organics™'den temin edildi. Toksisite testlerinde her bir konsantrasyon için tercih edilen embriyo sayısı ve uygulama şekli OECD 236 nolu Balık Embriyo Akut Toksisite (Fish Embryo Acute Toxicity) testine göre belirlendi (OECD, 2013). Öncelikle, diklofenak sodyumun zebra balıkları embiryolarında öldürücü konsantrasyonunu (LC_{50}) belirlemek için ön toksisite testleri yapıldı. Bu çalışmalardan elde edilen verilerden hareketle LC_{50} belirleme testtinde kullanılacak konsantrasyonlar belirlendi. Balık embriyo-toksisite testi için döllenme sonrası 6-8 saatlik zebra balığı embriyoları 96 saat boyunca 9 farklı konsantrasyonda (5.33-0.21 mg L^{-1}) DKFS'ye 96 kuyucuklu mikroplakalarda maruz bırakıldı. Her bir kuyucuğa, 250 µl olacak şekilde farklı konsantrasyonlarda hazırlanan DKFS çözeltisi ve bir zebra balığı embriyosu eklendi. Her bir konsantrasyon için toplam 24 embriyo kullanıldı. 96 saatlik süre boyunca her 24 saatte bir stereo mikroskop ile incelenen bireylerin ölüm oranları kaydedildi. 48. saatte embriyoların dakikadaki kalp atım sayıları belirlendi. 96. saatin sonunda ise hayatta kalan bireylerin malformasyon oranları ve malformasyon tipleri stereo mikroskop ile belirlenirken boy uzunlukları Euromex Image Focus 4.0 yazılımı kullanılarak ölçüldü.

İstatistiksel analiz

Toplanan verilerin istatistiksel analizi GraphPad Prism 5 (SPSS Inc., ABD) ile yapıldı. Embriyolar için 96 saatlik ortalama öldürücü konsantrasyon (LC_{50}) değerini ve efektif konsantrasyonu (EC_{50}) belirlemek için probit regresyon analizi kullanıldı (EPA, ver. 1.5).

Bütün ölçüm parametreleri normal dağılım göstermediğinden, parametrik olmayan bu veriler Kruskal Wallis ve Dunns Testleri ile istatistiksel olarak karşılaştırıldı. DKFS'nin teratojenik indeks (TI) değeri ise 96h LC_{50} değerinin 96h EC_{50} değerine oranı olarak hesaplandı.

BULGULAR ve TARTIŞMA

Farmasötik bileşikler insan ve hayvan sağlığı için elzemdir; bununla birlikte, dünya genelinde insan nüfusu ve hayvan popülasyonlarında meydana gelen yoğun artış, farmasötik bileşiklere olan talebi de arttırarak çevrede bu bileşiklerin birikimine neden olmaktadır. Bu nedenle, son yıllarda farmasötik atıklar için biyolojik verilerin kullanılması giderek artan bir oranda ilgi görmektedir. Farmasötiklerin ekotoksisitesi ile ilgili temel endişe, esas olarak tatlı ve tuzlu sulardaki su kütlelerinde kronik birikimlerinde yatmaktadır (Khan ve ark., 2019). Farmasotik bileşiklerden biri olan DKFS'nin çevresel konsantrasyonlarda bile birçok sucul organizma için toksik olduğu ve bu organizmaların karaciğer, böbrek, solungaç gibi organlarında olumsuz etkilere neden olduğu bildirilmiştir (Guiloski ve ark., 2015; Fu ve ark., 2020).

Yüksek miktarlarda üretilen ve kullanılan farmasötiklerin etkileri, çevreye girme ve çevrede zamanla birikme olasılığı göz önünde bulundurularak belirlenmelidir. Bu çalışmada 0.21-5.33 mg L^{-1} arasındaki konsantrasyonlarda DKFS'ye maruz bırakılan zebra balığının embriyonik gelişimi üzerindeki olası toksisitesini ve teratojenitesini değerlendirmek için, embriyoların hayatta kalma oranı, kuluçka oranı ve vücut uzunluklarındaki değişimleri analiz edilmiştir. DKFS'ye maruz kalan *D. rerio* embriyolarında 48, 72 ve 96 saatlik LC_{50} değerleri sırasıyla 2.04 (1.76-2.38), 1.74 (1.49-2.04) ve 1.55 (1.32-1.83) mg L^{-1} olarak hesaplanmıştır.

Bu çalışmada, DKFS'nin 5.33 ve 3.56 mg L^{-1} konsantrasyonlarına maruz kalan zebra balıklarında ölümler genellikle 24. saatte gözlenmiştir (Çizelge 1). Johnson ve ark. (2007) balık gelişiminde gastrulasyon ve segmentasyon olaylarının meydana geldiği 5-24 saat aralığının balık gelişiminde kritik dönem olarak adlandırıldığını bildirmiştir. Bu durum verilerimizle uyumludur. Diğer yandan, 0.21 ve 0.31 mg L^{-1} konsantrasyonlarına maruz kalan zebra balıklarında ölüm gözlenmemiştir (Çizelge 1).

Çizelge 1. Farklı konsantrasyonlarda DKFS'ye maruz bırakılan *D. rerio* embriyoları için zamana bağlı ölüm seviyeleri

Table 1. Time-dependent mortality levels for D. rerio embryos exposed to different concentrations of DKFS

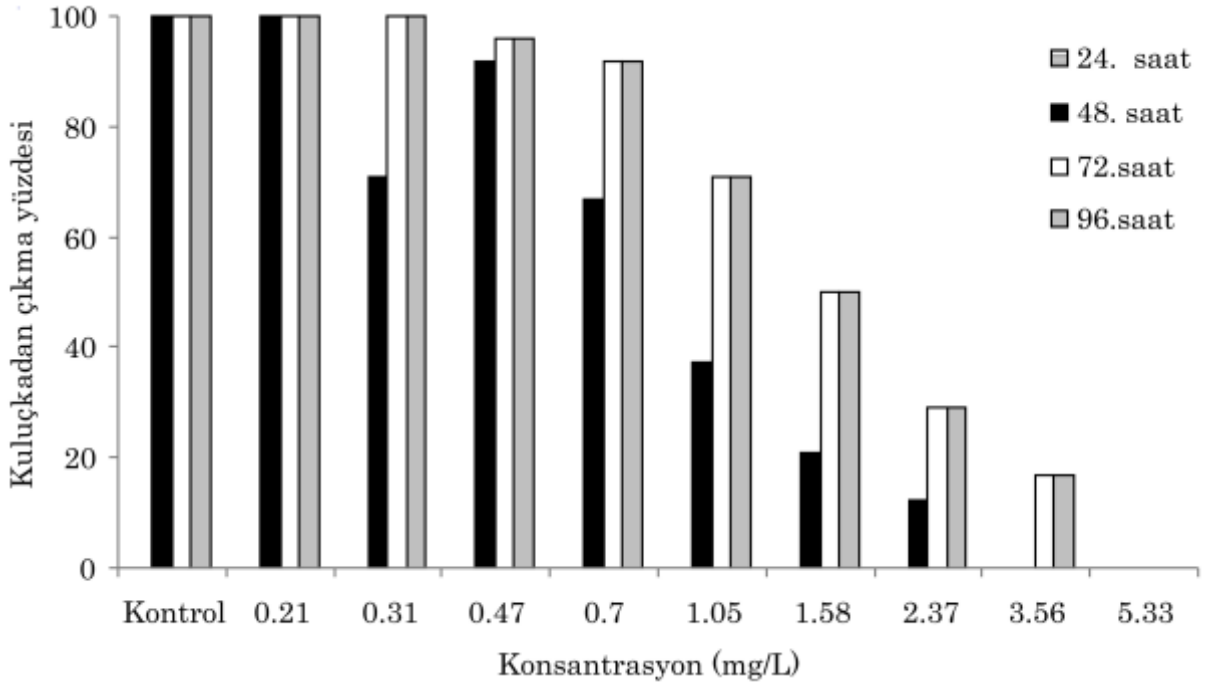
		Σ (Mortalite)			
Konsantrasyon (mg L^{-1})	n	24. saat	48. saat	72. saat	96. saat
Kontrol	24	0	0	0	0
0.21	24	0	0	0	0
0.31	24	0	0	0	0
0.47	24	0	1	1	2
0.70	24	0	1	2	3
1.05	24	2	2	5	7
1.58	24	5	6	9	11
2.37	24	7	14	17	17
3.56	24	18	20	20	21
5.33	24	24	24	24	24

DKFS'ye maruz kalan embriyolarda EC_{50} değeri ise 0.81 (0.69-0.96) mg L^{-1} olarak hesaplanmıştır. Hesaplanan LC_{50} değerinin EC_{50} değerine oranı teratojenite değerini vermektedir. Teratojenite değeri kirleticilerin sucul organizmalar üzerine etkilerinin değerlendirilmesinde kullanılan önemli bir ölçüttür. Teratojenite belirleme çalışmalarında alternatif modellerden biri zebra balığı embriyolarını kullanılarak yapılan testlerdir (Kelly ve ark., 2010; Sipes ve ark., 2011; Tenorio-Chávez ve ark., 2020). Memeli organizmalarda kimyasal maddelerin teratojenik potansiyelini belirlemek için de zebra balığı embriyolarının kullanılmasının uygunluğu bir çok çalışma ile ortaya konmuştur (Teixido ve ark., 2013).

DKFS, Amerika Birleşik Devletleri Gıda ve İlaç Dairesi tarafından gebelik riski sınıfı C ilacı olarak belirlenmiştir. Bu bileşik kadınlarda dismenore ve menorajiyi tedavi etmek için sıkça kullanıldığından, potansiyel teratojenik etkileri değerlendirmek için yeterli ve kontrollü çalışmalar yapılması gerektiği bildirilmiştir. Bu çalışmada, zebra balıkları larvaları

için Terotojenik İndeks (TI) değeri 1.91 olarak hesaplanmıştır. Selderslaghs ve ark. (2009) göre test bileşikleri ile ilgili olarak TI değeri >1 ise bileşik teratojenik olarak kabul edilir. Buna göre bulgularımız DKFS'nin *D. rerio* larvaları için teratojenik olduğunu göstermiştir.

Zebra balıkları embriyolarında kuluçkadan çıkma oranı, çevresel kirleticilerin neden olduğu toksisite değerlendirmesi için kullanılan önemli bir ölçütlerden biridir (Mu ve ark., 2016). 28.2 °C de etüvde kuluçkaya bırakılan zebra balığı embriyolarının 50-60 saat sonunda yumurtadan çıkmaları beklenmektedir (Xia ve ark., 2017). Kontrol grubundaki embriyolar için, kuluçka 48. saatte başlamış ve hayatta kalan embriyoların neredeyse tamamı 72. saatte yumurtadan çıkmıştır. Kontrol grupları ile karşılaştırıldığında, tüm maruziyet gruplarında embriyoların yumurtadan çıkma oranları 72. saatte daha düşüktür, ancak hayatta kalan bireylerin tümü 96. saatte yumurtadan çıkmıştır. Bununla birlikte 3.56 mg L^{-1} konsantrasyonda 72. saatte yumurtadan çıkma oranında önemli düzeyde inhibisyon (%16.7) gözlenmiştir (Şekil 1).

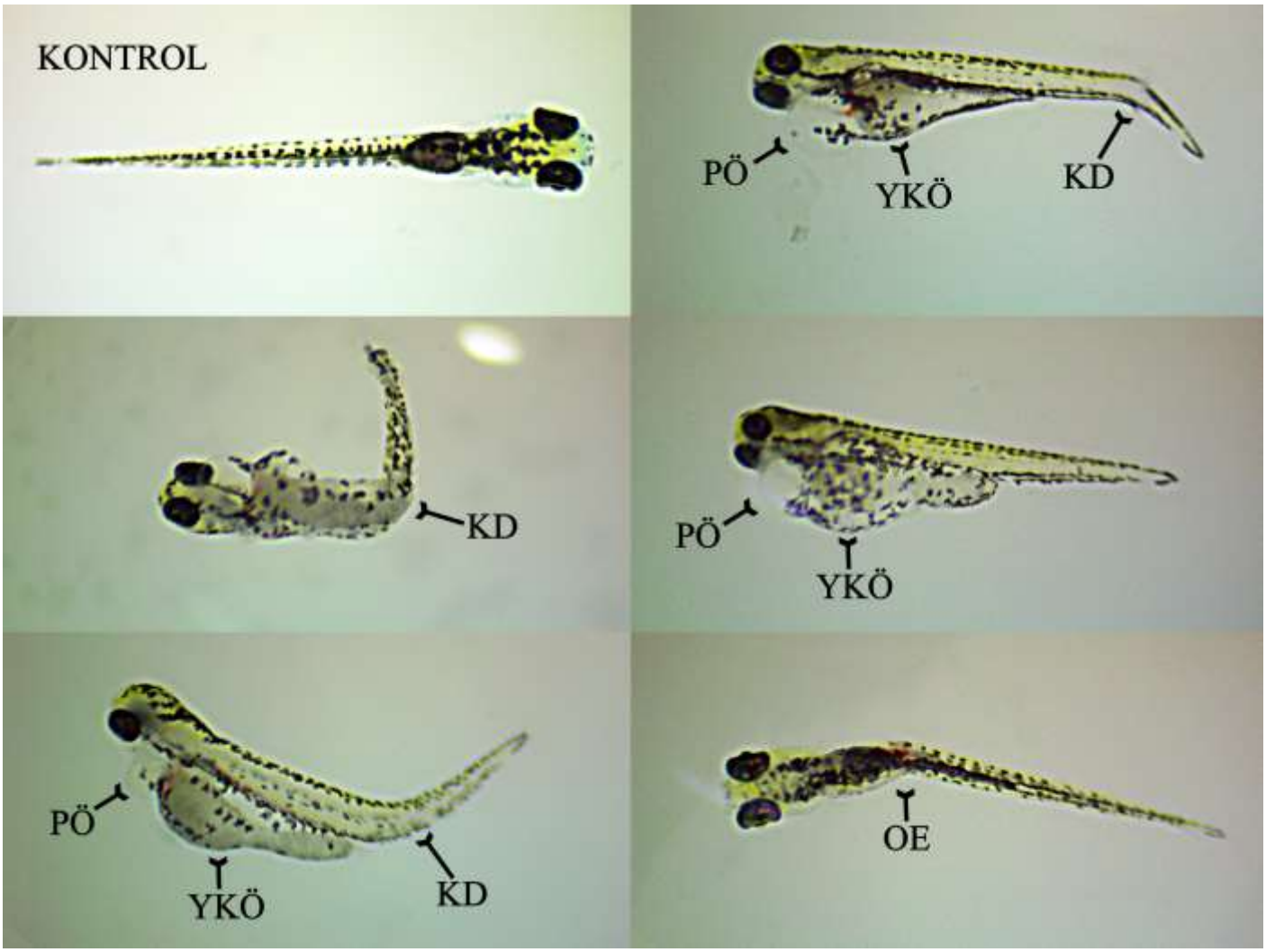


Şekil. 1 Farklı konsantrasyonlarda DKFS'ye maruz kalan *D. rerio* embriyolarında kuluçkadan çıkma yüzdesi
Figure 1. Percent hatching in D. rerio embryos exposed to different concentrations of DKFS

Kuluçka genellikle biyokimyasal, biyofiziksel ve ozmotik mekanizmaların kombinasyonundan oluşmaktadır ve kuluçkada gözlenen inhibisyon, DKFS'nin bu mekanizmaları olumsuz etkilediğini göstermektedir. Ayrıca, kuluçkadan çıkmada meydana gelen gecikme, daha yavaş bir gelişim hızına bağlanabilmektedir (Johnson ve ark., 2007). Aksakal ve Çiltaş (2020), zebra balıklarında kirleticiye maruz kalan embriyoların kuluçka başarısındaki azalmayı kuluçka enziminin inhibisyonuna veya kuluçka bezi hücrelerinin salgılama fonksiyonunun bloke edilmesine bağlanabileceğini bildirmişlerdir. Capriello ve ark. (2021) ise zebra balıkları embriyolarında kuluçkadan çıkma oranındaki inhibisyonun, embriyonun kaslarında meydana gelen etkilere bağlı olarak gerekli hareketlerin engellenmesinden kaynaklanabileceğini ve bu etkilerin kuluçkadan çıktıktan sonra bile yüzme performansında belirgin bir düşüşe neden olduğunu rapor etmişlerdir. Lee ve ark. (2011) tarafından yapılan bir çalışmada 0.001-10 mg L^{-1} konsantrasyonda DKFS'ye maruz bırakılan *Japanese medaka*'nın kuluçkadan çıkma başarısında, konsantrasyona bağlı olarak önemli oranda azalma gözlenmiştir. Ancak Stepanova ve ark. (2013) tarafından yapılan bir çalışmada 3 mg L^{-1} konsantrasyonda DKFS'ye maruz bırakılan sazan balıklarında kuluçkadan çıkma başarısında herhangi bir azalma gözlenmemiştir.

DKFS'ye maruz bırakılan zebra balıklarında perikardiyal ödem (PÖ), yolk kesesi ödemi (YKÖ), kuyruk deformasyonu (KD) ve omurga eğriliği (OE) gibi malformasyonlar belirlenmiştir (Şekil 2).

3.56, 2.37 ve 1.58 mg L^{-1} konsantrasyonlarda hayatta kalan tüm bireylerde malformasyon gözlenmiştir. 1.05, 0.70 ve 0.47 mg L^{-1} konsantrasyonlarda hayatta kalan bireylerde sırasıyla %65, %29 ve %18 oranında malformasyon gözlenmiştir. PÖ ve YKÖ ödemi diğer malformasyon tiplerine göre daha sık gözlenmiştir (Çizelge 2).



Şekil 2. 96 saat boyunca farklı konsantrasyonlarda DKFS'ye maruz bırakılan embriyolarda görülen malformasyon tipleri. YKS: yolk kesesi ödemi; PÖ: perikardiyal ödem; KD: kuyruk deformasyonu; OE: omurga eğriliği

Figure 2. The types of malformations seen in embryos exposed to DKFS for 96 hours in different concentrations. YKO: yolk sac edema, PÖ: pericardial edema; KD: tail deformation; OE: spinal curvature

Bu çalışmanın sonuçlarına benzer şekilde, Escapa ve ark. (2018) tarafından yapılan bir çalışmada DKFS'nin zebra balıklarında PÖ ve YKÖ'ye neden olduğunu göstermiştir. PÖ oluşumu anormal kardiyak gelişimin, ozmotik veya metabolik fonksiyonlarının bozulmasının göstergesi olabileceği rapor edilmiştir. Ayrıca YKÖ'nün kalbe, besin sağlanmasını engelleyebileceğini ve bunun da PÖ ile sonuçlanabileceği belirtilmiştir. Diğer yandan, bu çalışmaya benzer şekilde Pohl ve ark. (2019) 7.5 ve 15 mg L^{-1} konsantrasyonlarda DKFS'nin zebra balıklarında 48. saatte PÖ ve YKÖ'ye neden olduğunu ve 144. saatte ise %100 ölüme neden olduğunu rapor etmişlerdir.

Zebra balıklarında gözlenen omurga eğriliğinin omurga kolonunda düzensiz wnt sinyalinin veya azalmış kollajenin sonucu oluşabileceği rapor edilmiştir. Omurga deformasyonu, zebra balıklarının normal gelişimi için gerekli olan kalsiyum ve fosfor iyonu eksikliğinden kaynaklanabileceği belirtilmiştir (Gonzalez ve ark. 2021).

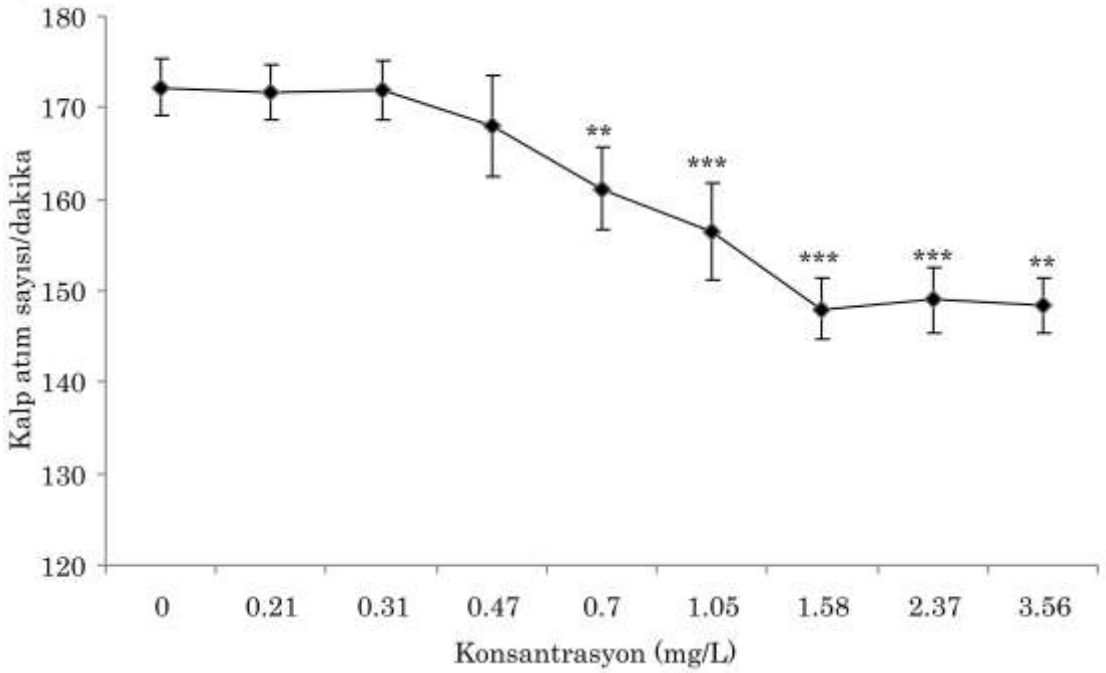
PÖ, DKFS'nin neden olduğu ana malformasyon türü olduğundan, perikardiyal morfolojideki değişikliğin embriyoların kalp fonksiyonunu etkileyip etkilemediğini analiz etmek için kalp atışı hızı belirlenmiştir. Kalp, zebra balıklarında geliştirilen ilk fonksiyonel organdır ve kirleticilerin etkilerinden dolayı kalp atım hızında meydana gelen değişiklik, embriyonik testlerinde kullanılan önemli bir belirteçtir (Zhang ve ark., 2020). Bu çalışmada, DKFS'ye maruz kalan zebra balığı embriyolarının 48. saat kalp atış hızlarında doza bağlı olarak önemli oranda azalmaya neden olduğunu gösterilmiştir (Şekil 3). Literatürde, ilaçların zebra balıkları

embriyolarında kalp atım sayılarında inhibisyona neden olduğunu gösteren çok sayıda çalışma bulunmaktadır (Lin ve ark., 2013; Sun ve ark., 2013; Wang ve ark., 2014; Yin ve ark., 2014; Chen ve ark., 2017; Shen ve ark., 2019). Ayrıca Zhang ve ark., (2020) kirleticilerin öldürücü olmayan dozlarının zebra balıklarında kalp atım hızını etkileyebileceğini rapor etmişlerdir.

Çizelge 2. 96 saat boyunca farklı konsantrasyonlarda DKFS'ye maruz bırakılan *D. rerio* larvalarında malformasyon gözlenen birey ve malformasyon sayıları

Table 2. The number of individuals and malformations observed in D. rerio larvae exposed to different concentrations of DKFS for 96 hours

Konsantrasyon mg L^{-1}	Hayatta kalan birey sayısı	Malformasyon gözlenen birey sayısı	Malformasyon Tipleri			
			PÖ	YKÖ	OE	KY
Kontrol	24	0	-	-	-	-
0.21	24	0	-	-	-	-
0.31	24	0	-	-	-	-
0.47	22	4	3	4	2	2
0.70	21	6	4	6	1	0
1.05	17	11	9	10	4	3
1.58	13	13	11	12	8	5
2.37	7	7	7	7	6	4
3.56	3	3	3	3	1	2
5.33	0	0				



Şekil 3. Farklı konsantrasyonlarda DKFS'ye maruz kalan *D. rerio* embriyolarında 48. saat dakikada kalp atım sayısı

*Figure 3. Heart rate per minute at 48 hours in D. rerio embryos exposed to different concentrations of DKF*S
**Kontrolden önemli ölçüde farklı olan grupları gösterir (p< 0.05)*
*** Kontrolden önemli ölçüde farklı olan grupları gösterir (p< 0.01)*
**** Kontrolden önemli ölçüde farklı olan grupları gösterir (p< 0.001)*

Kirleticilerin balıklar üzerindeki potansiyel etkilerinin değerlendirilmesinde vücut boyu değişiminin belirlenmesi önemli bir parametredir (Yang ve ark., 2018; Seçer ve ark., 2022). Çünkü balıkların boy uzunluğundaki değişim, kirleticilerin etkisine bağlı olarak bireyde oluşabilecek birçok moleküler ve hücresel yanıtları önemli oranda yansıtmaktadır (Cook ve ark. 2005). Bu çalışmada, kontrol grubuna göre DKFS'ye maruz bırakılan embriyoların vücut uzunluklarında önemli oranda bir inhibisyon gözlenmiştir (Çizelge 3). Bu çalışmadan farklı olarak, Horie ve ark. (2018) tarafından yapılan

bir çalışmada 0.4-3.5 mg L^{-1} arası konsantrasyonlarda DKFS'ye maruz bırakılan zebra balıklarının boy uzunluklarında önemli bir fark gözlenmemiştir. Ayrıca Stepanova ve ark. (2013) tarafından yapılan bir çalışmada 3 mg L^{-1} konsantrasyonda DKFS'ye maruz bırakılan sazan balıklarının boy uzunluklarında önemli bir fark belirlenmemiştir.

Çizelge 3. 96 saat boyunca farklı konsantrasyonlarda DKFS'ye maruz bırakılan *D. rerio* larvalarının boy uzunlukları

Table 3. Length of D. rerio larvae exposed to different concentrations of DKFS for 96 hours

Konsantrasyon mg L^{-1}	Hayatta kalan birey sayısı	Boy uzunluğu (mm)[a]	
Kontrol	24	3.47 ± 0.13	
0.21	24	3.41 ± 0.15	
0.31	24	3.40 ± 0.16	
0.47	22	3.32 ± 0.16	
0.70	21	3.27 ± 0.15	*
1.05	17	3.12 ± 0.43	**
1.58	13	3.13 ± 0.24	**
2.37	7	2.68 ± 0.30	***
3.56	3	2.46 ± 0.40	**
5.33	0		

Her konsantrasyon için 24 birey maruz bırakıldı.
[a]Uzunluklar ortalama ± standart hatalar olarak ifade edilir. Bu değerler hayatta kalan bireylerin uzunluklarından elde edilmiştir.
*Kontrolden önemli ölçüde farklı olan grupları gösterir ($p<0.05$)
** Kontrolden önemli ölçüde farklı olan grupları gösterir ($p<0.01$)
*** Kontrolden önemli ölçüde farklı olan grupları gösterir ($p<0.001$)

Bu çalışmada, DKFS'nin 3.56-1.05 mg L^{-1} arası konsantrasyonlarına maruz kalan zebra balıklarında, hem boy uzunluklarında meydana gelen azalma hem de YKÖ'nün yoğun olarak gözlenmesi bu bireylerde gelişim geriliği oluştuğunu düşündürmektedir. Johson ve ark. (2007) yolk kesesi alanının artmasını ve buna eşlik eden boy uzunluğundaki azalmayı, toksisite testlerinde yaygın olarak görüldüğünü ve bu durumu gelişim geriliği ile sonuçlandığını rapor etmişlerdir. DKFS'nin, sucul organizmalarda gelişimsel toksisite, teratojenite ve embriyogenez üzerinde olumsuz etkilere neden olabileceğinden bu etkilerin altında yatan mekanizmaların ayrıntılı olarak değerlendirilmesi gerekmektedir.

SONUÇ ve ÖNERİLER

Bu çalışma DKFS'nin zebra balığı embriyolarında doza bağlı olarak gelişimsel toksisiteye neden olduğunu göstermiştir. Toksisite verileri ve hesaplanan TI değeri DKFS'nin zebra balıkları embriyoları için teratojen olduğunu da göstermiştir. Yolk kesesi ödemi, perikardiyal ödem, kuluçka anormallikleri, kuyruk deformasyonu ve omurga eğriliği gibi yanıtlar toksisite ve teratojenite ile ilgili iddiayı desteklemektedir. DKFS'nin yüzeysel sularında potansiyel birikiminin sucul organizmalarda neden olabileceği etkiler bu kontrollü deneyle çeşitli yönlerden ortaya konmuştur. Ancak yapılacak yeni çalışmalar ile hem DFKS toksisitenin hem de teratojenitesinin olası mekanizmalarının biyokimyasal ve moleküler düzeyde değerlendirilmesi önemli bir gerekliliktir. Diğer yandan, bu tür çevresel kimyasalların sucul organizmalar için toksik konsantrasyonları ve toksisite mekanizmalarının belirlemenin yanı sıra Pohl ve ark. (2019) tarafından yapılan, ozonlayarak DFKS'nin toksik etkileri giderimi şeklindeki çalışmalarla bu tür maddelerin sudan uzaklaştırılması veya en azından toksik etkilerinin giderilmesi ekosistem ve insan sağlığı açısından yerinde olacaktır.

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Divergences of Biochemical Features of Three Reared Trouts; Brook Trout (*Salvelinus fontinalis*, Mitchill 1814), Rainbow Trout (*Oncorhynchus mykiss* Walbaum, 1972), and Black Sea Trout (*Salmo trutta labrax* Pallas 1811)

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ABSTRACT
The objective of this present study is to signify biochemical features of three reared trouts (brook trout, rainbow trout, Black Sea trout) that are economically consequential and reared fish species in the Eastern Black Sea region. The rainbow trout has been reared successfully for a long period of time. However, brook trout and the Black Sea trout have been two new species to be reared in the region with a high potential contribution to the economy. Therefore, there is a strong need to determine and report the differences between the fish (especially the two new species), levels of carbohydrates, energy, carotenes (Vitamin A), fatty acid, and proximate composition (protein, lipid, moisture, and ash). In addition, the lipid indices [Atherogenic Index (AI), Thrombogenic Index (TI), and polyene index (PI)] were also measured and reported for the trouts. Moreover, the color of the fillets of the fish was also measured. The results of this present study show that the average levels of moisture and protein varied while the average levels of lipid and ash were close to each other. The highest levels of carbohydrates, energy, and carotenes were determined in the muscle of brook trout in this study. The highest meat yield was also obtained from brook trout followed by Black Sea trout and rainbow trout. A total of 19 fatty acids were determined for Black Sea trout and brook trout and 17 fatty acids for rainbow trout in the present study. The omega-3 levels of the all trout used for the present study were roughly twice as much as that of the omega-6, except for rainbow trout.

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Yetiştiriciği Yapılan Üç Tür Alabalığın; Kaynak Alabalığı (*Salvelinus fontinalis*, Mitchill 1814), Gökkuşağı Alabalığı (*Oncorhynchus mykiss* Walbaum, 1972) ve Karadeniz Alasının (*Salmo trutta labrax* Pallas 1811) Biyokimyasal Özelliklerinin Farklılıkları

ÖZET
Bu çalışmanın amacı, Doğu Karadeniz Bölgesi'nde ekonomik olarak önemli ve yetiştirilen üç alabalık (kaynak alabalığı, gökkuşağı alabalığı ve Karadeniz alabalığı) türünün bazı biyokimyasal özelliklerini belirlemektir. Gökkuşağı alabalığı uzun süredir başarıyla yetiştirilmektedir. Ancak kaynak alabalığı ve Karadeniz alabalığı bölgede yetiştirilmeye başlanan ve ekonomiye katkısı yüksek iki yeni tür olmuştur. Bu nedenle, balıklar (özellikle iki yeni tür) arasındaki karbonhidrat, enerji, karoten (A Vitamini) ve yağ asidi ve besin bileşenleri (protein, lipit, nem ve kül) seviyelerindeki farklılıkları belirlemeye ve raporlamaya güçlü bir ihtiyaç vardır. Ayrıca, balıkların lipit indeksleri [Aterojenik Indeks (AI), Trombojenik Indeks (TI) ve polien indeksi (PI)] de hesaplanmıştır. Ek olarak, bu çalışmada balıkların fileto renkleri de ölçülmüştür. Balıklarda ortalama nem ve protein seviyeleri farklılık gösterirken, lipit ve kül seviyeleri birbirine yakın bulunmuştur. Bu çalışmada en yüksek karbonhidrat, enerji ve karoten seviyeleri kaynak alabalığında belirlenmiştir. En yüksek et verimi yine kaynak alabalığından elde edilmiştir, bunu Karadeniz alası ve gökkuşağı alabalığı izlemiştir. Çalışma için Karadeniz alası ve

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Anahtar Kelimeler
Besin bileşenleri
Alabalık
Karoten (A vitamini)
Fileto rengi
Yağ asitleri

kaynak alabalığında toplam 19 yağ asidi ve gökkuşağı alabalığı için 17 yağ asidi belirlenmiştir. Çalışmada kullanılan tüm alabalıkların yaklaşık omega-3 seviyelerinin, gökkuşağı alabalığı hariç, omega-6 seviyelerine oranları kabaca iki katı olduğu belirlenmiştir.

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INTRODUCTION

Brook trout, rainbow trout, and Black Sea trout are three reared fish species in the Eastern Black Sea region. These fish have their places on the counters in fish markets, and they compete with each other to get the attention of the customers. The brook trout and Black Sea trout species have challenges in the rearing field comparing the rainbow trout, which is relatively easy to rear. Although rainbow trout have many advantages in rearing conditions, the brook trout and Black Sea trout seem to be one step ahead in marketing prices. Recently, the brook trout, Black Sea trout, and rainbow trout are all subjected to many scientific studies not only in Turkey but also in many other countries such as the USA, Canada, Sweden, Ukraine, and China (Memiş et al., 2020; Çankiriligil & Berik, 2020; Latiu et al., 2020; Martling et al., 2020; Závorka et al., 2020; Zhang et al., 2021; Turan &Aksu, 2021; Barylo et al., 2021; Bayar et al., 2021; İspir et al., 2021; Baesu et al., 2022).

Fish and fish-related dishes nearly come first as a preferred food in diets for a variety of reasons. The taste and lipid content of the fish that make it a healthy food are among the many reasons. Consumption of fish provides some health benefits because fish contains bioactive constituents that improve nutritional quality (Li et al., 2020; Tacon et al., 2020; Chen et al., 2021). Department of health in many countries encourages fish consumption to develop a healthy population of people.

The main parts in muscle of fish are generally known to provide moisture, protein, lipid, and ash which are known proximate components and may vary from species to species (Öksüz & Özyılmaz, 2010; Kayım et al. 2011; Şahin et al., 2011; Öksüz, 2012; Yeşilayer & Genc, 2013; Ozyilmaz, 2019; Çankiriligil & Berik, 2020; Memiş et al., 2021). Fish consumption may give us an opportunity to get health benefits Individuals should consume fish regularly to get the benefits out of it.

Choosing the right fish for individual consumption, therefore, could be a crucial decision to obtain all beneficial ingredients available in the fish. The aim of this present study is to investigate the amounts of ingredients such as protein, lipid, ash, moisture, meat yield, carbohydrate, energy, carotene (Vitamin A), and fatty acids, and their lipid indices (Atherogenic Index, Thrombogenic Index, Polyene Index) available in three different salmonids (Brook trout, rainbow trout, Black Sea trout) in order for us to evaluate the similarities and differences. The fillet colors of these three reared trouts were also determined instrumentally to measure the color changes. The present study provides data on the biochemical attributes of these three fish species, specifically on fatty acid, protein, lipid, moisture, and ash of reared fish, providing unique knowledge to decide what type of fish should be included in an individual's diet.

MATERIALS and METHODS

Fish Materials

Brook trout (*Salvelinus fontinalis*, Mitchill 1814), rainbow trout (*Oncorhynchus mykiss*, Walbaum, 1972), and Black Sea trout (*Salmo trutta labrax*, Pallas 1811) have a growing importance in the Eastern Black Sea region. The fish used in the present study were purchased after regular harvesting times whenever they were ready for sale by the owner of the fish rearing unit. We did not interfere with any prior steps of our purchase of the fish samples however we asked the sellers of the fish about their life history of the fish and recorded the information in detail. Because we observed three different trouts that were reared in a commercial fish rearing unit in Trabzon/Turkey in this research, we present the information that we gathered in the following section.

The fish were grown from eggs to harvest in the commencal fish rearing units and fed with commercial feed. More specifically, the fish were fed with a meal called "range, nurse-s fish hatchery diet" until fish was 1 g in weight. Later on, the fish were fed with another type of feed (Sürsan, Aquamax, extruded fish meal, Muğla, Turkey) for the trouts from 1 g to harvesting. The fish meals were ordinary fish meals that were regularly used in rearing units in the region. The fish used in this current study was

an average of 18-20 months old. The spring water was generally used by the units however the river water was also used whenever the spring water was not adequate.

A total of 14, 18, and 22 fish out of approximately 3000 kg capacity units were randomly selected for brook trout, rainbow trout, and Black Sea trout, respectively for the present study. The trouts were harvested late in March, in the middle of April, and early in May, which are the three months when the fish were sold frequently in the markets. Fish in ice boxes were transported to the laboratory. Their measurements (length and weight) were taken before filleting the fish. Only dorsal parts of the fish were used for the chemical analysis. The inside of the fillets was used for color measurements. The skinless fillets were finely chopped, gathered in a container, and mixed for the biochemical analysis.

Methods

The official methods were performed for the proximate compositions (the crude moisture, ash, protein, and lipid) which were given in detail in Öksüz (2012). The calculations used for carbohydrate contents and energy values were provided by Güner et al. (1998).

Determination of Total Levels of Carotene (vitamin A)

The spectrophotometric determination method described in TS, 1987 (Turkish Standard, number: 5036) and Morello et al. (2004) method were used to determine total carotene levels for all three fish species. A total of 7.5 g muscle lipid of Brook trout, rainbow trout, and Black Sea trout was weighed and dissolved with cyclohexane in a 25 mL volumetric flask. The mixture was made up to 25 mL volume with cyclohexane. The absorbance was read at 470 nm with a spectrophotometer (Shimadzu Hitachi U-1900 model). Its absorbance was measured with a spectrophotometer (Morella et al., 2004). The following equation was used for calculations.

Amount of carotenoids (mg carotenoids/kg lipid) = (A470 × 106)/(2000 × 100 × L)

A = Absorbance

L = Light path (cell thickness, mm)

Color measurements

A chroma meter (CR-400, Minolta, Osaka, Japan) was used to measure the color of the fillets of all three fish specimens. Inside of the fillets, places of the anterior, posterior, and caudal locations, were used for colorimetric analysis which was explained in Öksüz (2012).

Fatty Acid Methyl Esters

After obtaining lipids from all three fillets of the trouts, fatty acid methyl esters were carried out by using the lipid. GC-MS (Gas Chromatography-Mass Spectrometry) was used to determine the fatty acids of the lipids for all three fish muscles. Preparation, conversion, and separation of the fatty acid methyl esters were described in Öksüz and Özyılmaz (2010). The instrument and column conditions were also detailed in the same study, except for the column.

Lipid quality indexes

The data obtained from the fatty acid composition were used to calculate the Atherogenicity Index (AI), and Thrombogenicity Index (TI). The following equations reported by Ulbricht & Southgate (1991) were used to calculate Lipid Quality Indexes (AI and TI).

AI=[(4xC14:0)+C12:0+C16:0]/[(ΣPUFA-n6+ΣPUFA-n3)+ΣMUFA]

IT=[(C14:0+C16:0+C18:0)/(0.5xMUFA+0.5xPUFA-n6+3xPUFA-n3+PUFA-n3/PUFA-n6)]

Additionally, the following equation was used the calculate polyene index (PI), which evaluates the PUFAs damage (Lubis & Buckle, 1990).

(PI) = (C20:5 + C22:6)/C16:0

Statistical analysis

Statistical analysis was performed with SPSS (22.0). Significance was established at $P<0.05$. The data obtained from this study regarding three different trout species were subjected to a one-way analysis of variance (ANOVA), and a mean comparison was carried out by using Duncan's Multiple Range test to see if there are any statistically significant differences between groups. The homogeneity of variances was tested before ANOVA analysis was performed.

RESULTS and DISCUSSION

In this study, the length and the total weight of the brook trout, rainbow trout, and Black Sea trout were tabulated in Table 1. The average lengths were measured in the range of 25.82±2.12 -27.93±1.01 cm for the trouts. The highest weight obtained from rainbow trout was followed by brook trout and Black Sea trout. The highest length was also obtained from rainbow trout.

Table 1. Total length and total weight of the brook trout, rainbow trout, and Black Sea trout

Çizelge 1. Kaynak alabalığı, gökkuşağı alabalığı ve Karadeniz alasının boy ve ağırlığı

Fish species	Length (cm)	Total Weight (g)
Brook Trout	25.82±2.12	232.93±43.01
Rainbow Trout	27.93±1.01	272.61±13.86
Black Sea Trout	25.89±1.07	199.77±10.41

n=14 brook trout, n=18 rainbow trout, and n=22 Black Sea trout

The proximate composition, meat yield, carbohydrate, energy, and carotenes levels of the brook trout, rainbow trout, and Black Sea trout were shown in Table 2. The meat yield diverged in trout species. This divergence was measured to be statistically significant (P<0.05). The highest meat yield was obtained from brook trout followed by Black Sea and rainbow trout in this study. Şahin et al. (2011) study reported that the meat yield of brook trout was higher than that of Black Sea trout. The present study obtained similar results regarding meat yield. Additionally, Özyılmaz (2019) reported the meat yield of rainbow trout as 66.23±0.71% which was higher than that of the rainbow trout in the present study.

Table 2. The proximate composition, meat yield, carbohydrate, energy, and carotene (Vitamin A) levels of the brook trout, rainbow trout, and Black Sea trout

Çizelge 2. Kaynak alabalığı, gökkuşağı alabalığı ve Karadeniz alasının besin bileşenleri, et verimi, karbonhidrat, enerji ve karoten (A vitamini) düzeyleri

Fish species Components	Brook Trout	Rainbow Trout	Black Sea Trout
Moisture (%)	73.92±0.68[a]	76.19±0.88[b]	77.630±99[b]
Lipid (%)	1.14±0.08[a]	1.31±0.05[b]	0.81±0.04[c]
Ash (%)	1.15±0.02[a]	1.37±0.05[b]	1.51±0.06[c]
Protein (%)	22.29±0.83[a]	19.92±0.56[b]	18.67 ±0.34[c]
Meat yield (%)	56±0.02[a]	48±0.03[b]	50±0.01[c]
Carbohydrate (%)	1.50±0.54[a]	1.21±1.03[a]	1.38±0.77[a]
Energy	445.12±11.46[a]	406.58±13.93[b]	369.30±14.84[c]
Total carotenes (µg 100 g^{-1})	94.22±7.90[a]	36.00±0.88[b]	79.72±1.34[c]

n=3 for the chemical analysis

[a,b,c] Values within same row with different superscripts diverge significantly at P<0.05

The average moisture levels were calculated in the following order; brook trout<rainbow trout<Black Sea trout (Table 2). Only the amount of moisture in brook trout was statistically different from the two others (P<0.05). The brook trout in this study has got the least moisture levels. Additionally, while the findings relating mean moisture levels of Black Sea trout were found to be higher than that of wild brown trout (74.8±1.1%) and reared rainbow trout (75.6±1.2%) (Yeşilayer & Genc., 2013), they were found to be very closer to the that of wild brown trout (77.80±0.3%) (Kayım et al., 2011).

Ranges of the average lipid levels of the trouts were calculated to be 0.81±0.04-1.31±0.05%. The lipid levels of the trouts varied only in a small amount. Although small changes were observed among the lipid levels in trouts, it was evaluated to be statistically significant (P<0.05). Given that all trout species have less than 2% lipid level, they were all defined as lean fish. The fish were harvested after the spawning period. The reason for having less lipid level could be the result of the period. One other reason could be their diet. Another reason could be the environmental effects. The wild Black Sea trout came from two different places, cultured Black Sea trout came from three different fish farming units, and three filial generations were measured in the range of 6.13±0.18% - 8.11±0.21% in the study of Çankırılıgil & Berik (2020). Lipid levels of the trouts in the present study were found lower than that stated in this previous study (Çankırılıgil & Berik, 2020). On the other hand, Ateş et al. (2013) measured the levels of the lipid in wild brown trout in winter as 1.48%, which was close to the levels of lipids in Black Sea trout in this current study. According to Özyılmaz (2019), the lipid of rainbow trout was 10.61±0.05%, which was higher than that of the rainbow trout, brook trout, and Black Sea trout in the present study. The differences in lipid levels of the trouts in the present study and that in the previous studies can be attributed to the changes in the feed and farm conditions.

The highest ash levels were measured in the Black Sea trout, followed by rainbow trout and brook trout. The ash levels among trouts differed from each other. Differences in ash content were found to be statistically significant (P<0.05). According to the study of Çakmak et al. (2018), the ash level of the fifth generation of Black Sea trout was 1.51±0.12%, the results very similar to the one reported in the present study.

The protein levels of the brook trout were the highest among all three trouts in the present study. Similarly, Barylo et al. (2021) reported the protein levels of three salmonids namely brown trout, rainbow trout, and brook trout. Accordingly, the brook trout was the highest one as to their protein levels. Additionally, the protein levels of the brook trout were measured to be 22.29±0.83% which was higher than that of rainbow trout, and Black Sea trout. Moreover, Çakmak et al. (2018) reported that the protein level of the fifth generation of Black Sea trout as 15.22±0.48% which was lower than that of Black Sea trout as well as all other trouts used in the

present study. The protein levels of wild Black Sea trout from two different rivers (Altındere River and Çağlayan River), three different rearing units, and three different filial generations were found to be in the range of 17.94±0.10-17.52±0.18% (Çankırılıgil & Berik, 2020). These values of protein in wild Black Sea trout, reared Black Sea trout, and filial generation of the Black Sea trout in study of Çankırılıgil & Berik (2020) were lower than those of protein in reared Black Sea trout which was 18.67±0.34% in the present study.

The carbohydrate amounts, energy value, and total carotene levels of the brook trout, rainbow trout, and Black Sea trout were shown in Table 2. The carbohydrate amounts of all trouts used in this current study were in the range of 1.21±1.03-1.50±0.54 %. Çakmak et al. (2018) reported the carbohydrate level of the fifth generation of Black Sea trout as 0.89±0.02%. The findings of the carbohydrate level of Black Sea trout as well as the other two salmonids in this study are higher than those of previously published findings (Çakmak et al., 2018). The brook trout also got the highest percentage of energy value compared to the two other salmonids. Similar energy values were measured for three freshwater fish species while lower carbohydrate levels were stated for those three freshwater fish species (Ozyilmaz et al., 2016). The total carotene levels are statistically significant from each other in the present study ($P<0.05$).

The average levels of the total carotene (vitamin A) in the brook trout were almost three times higher than those of the total carotene in the rainbow trout. The mean amount of the total carotene in the liver oil of guitarfish, string ray, and eagle ray (249.72±69.6 µg $100g^{-1}$, 401.49±4.06 µg 100 g^{-1}, and 104.53±2.10 µg 100 g^{-1}, respectively) were reported to be higher than that of carotene in all trouts under investigation in the present whereas that of carotene levels in bignose shark (29.26±2.83 µg $100g^{-1}$) were lower (Özyılmaz & Öksüz, 2015). The amounts of total carotenes in the livers of the smooth-hound and cownose ray were reported to be 83.78±3.53 µg 100 g^{-1} and 73.22±0.35 µg 100 g^{-1}, respectively. Findings of total carotenes in the livers of these two different cartilaginous fish species were in the range of carotene levels of all trouts in this present study (Özyılmaz & Öksüz, 2015).

The color of the muscle in the fishery sector can be considered a crucial issue to many customers as well as retailers. That is why the color of the fish fillets was measured for the present study. We aimed at figuring out whether the flesh color of these three salmonids can be a distinctive character to distinguish the trouts from each other. Although these three salmonids in this study belonging to the same family, their color of the muscle are different from each other visually and instrumentally. The color measurements of fillets' brook trout, rainbow trout, and Black Sea trout were tabulated in Table 3.

Table 3. Color measurements of fillets' brook trout, rainbow trout, and Black Sea trout
Çizelge 3. Kaynak alabalığı, gökkuşağı alabalığı ve Karadeniz alası filetolarının renk ölçümleri

		Lx (Lightness)	C (Chroma)	H (Hue)
Anterior,	Brook Trout	47.31±1.05	10.85±0.26	50.53±0.56
	Rainbow Trout	56.86±0.35	6.07±0.19	66.42±0.44
	Black Sea Trout	45.41±1.15	3.85±0.18	77.38±1.02
Posterior	Brook Trout	48.59±0.89	10.31±0.33	78.52±0.64
	Rainbow Trout	48.75±0.57	4.57±0.34	71.15±1.01
	Black Sea Trout	39.46±0.65	6.80±0.41	47.69±0.64
Caudal	Brook Trout	61.64±0.49	23.78±0.30	73.46±0.51
	Rainbow Trout	48.53±0.77	7.45±0.46	62.08±1.08
	Black Sea Trout	47.65±0.70	5.55±0.43	66.72±0.63

The average means and standard deviations for measurements of fillets (n=3)

The lightness (L*) values in the trouts generally differed in the anterior, posterior, and caudal parts of the muscle, except for, a few minor similarities. Only the average chroma (C) values in the anterior and posterior parts of the brook trout were similar. Other than that all chroma values divert in fillets of the brook trout, rainbow trout, and Black Sea trout. Like the chroma values, Hue (H) values also varied in different parts (anterior, posterior, and caudal) of the same fillets for each fish species. The highest lightness and chroma levels were measured in caudal parts of the brook trout fillet whereas the highest hue values were determined in the fillets of anterior parts of the Black sea trout and posterior parts of the brook trout. All three salmonid species had different fillet colors in anterior, posterior, and caudal parts which can be evaluated to be some possible identifiers for the processed fish fillets in the present study.

According to Erikson & Misimi (2008), there are different factors (e.g., perimortem handling stress, rigor mortis, ice storage) that affect skin and fillet color changes in Atlantic salmon. while healthy wild and reared Mediterranean amberjacks were measured to be similar with regard to their fillet colors (Öksüz, 2012), three salmonid species in the present study were not similar in their fillet colors.

A total of 19 fatty acids were determined for Black Sea trout and brook trout, and a total of 17 fatty acids were determined for rainbow trout in the present study. The fatty acid components of the brook trout, rainbow trout, and Black Sea trout were tabulated in Table 4. All of the trouts have less than 24% of total saturated fatty acid and have higher than 26% total monounsaturated fatty acids. Their total monounsaturated fatty acids were determined in the range of 48.18-50.84%. The lowest total saturated fatty acids were measured in rainbow trout. Palmitic acid (C16:0) was the highest fatty acid in total saturated fatty acid followed by stearic acid (C18:0) and myristic acid (C14:0) in the present study. Additionally, Pentadecylic acid (C15:0) and Arachidic acid (C20:0) were not found or found in lower amounts than detection limits in the muscle of rainbow trout.

The mean amount of C16:0 in brook trout and Black Sea trout was found to be different however these differences were not statistically significant ($P>0.05$). The amount of C16:0 in the muscle of rainbow trout was found to be significantly lower than that of C16:0 in the muscle of brook trout and Black Sea trout ($P<0.05$). In addition, the average levels of C16:0 in brook trout and Black Sea trout in the present study were found to be higher than those of C16:0 in brook trout and Black Sea trout as reported in the study of Şahin et al. (2011). These differences for the same fish species in two different studies could be attributed to the differences in the environmental conditions of the fish species.

Among monounsaturated fatty acids; palmitoleic acid (C16:1n9), oleic acid (C18:1n9), vaccenic acid (C18:1n7), and eicosenoic acid (C20:1n9) were determined in the muscle of brook trout, rainbow trout, and Black Sea trout in this study. The levels of C18:1n9 in the flesh of all trouts were the highest fatty acid in total monounsaturated fatty acids. The percentages of C18:1n9 out of all monounsaturated fatty acids determined in the present study were calculated to be 70.71%, 77.53%, and 79.20% in the muscle of brook trout, rainbow trout, and Black Sea trout, respectively.

The amount of C18:1n9 in brook trout was found to be similar to that of C18:1n9 in Black Sea trout ($P>0.05$) whereas the mean level of the same fatty acid was higher in rainbow trout ($P<0.05$). The average level of C18:1n9 in rainbow trout was found to be 22.44±0.25% which was higher than that of C18:1n9 in wild caught rainbow (18.83±1.91%) and lower than that of cage reared rainbow (26.56±1.21%) and pond reared rainbow (24.29±2.82%) (Ural et al., 2017). The mean amounts of C18:1n9 in brook trout and Black Sea trout in this study were found to be lower than those of C18:1n9 in male and female brook trout and Black Sea trout and their hybrids (Şahin et al., 2011). The difference can be attributed to diet differences.

We have separated polyunsaturated fatty acids in two groups in Table 4 (omega-6 and omega-3). Linoleic acid (LA, C18:2n6), eicosadienoic acid (C20:2n6), and arachidonic acid (ARA, C20:4n6) have been classified as omega-6 fatty acids. The linolenic acid (ALA, C18:3n3), stearidonic acid (C18:4n3), eicosatrienoic acid (C20:3n3), eicosatetraenoic acid (C20:4n3), eicosapentaenoic acid (EPA, C20:5n3), docosapentaenoic acid (DPA, C22:5n3), and docosahexaenoic acid (DHA, C22:6n3) have been classified as omega-3 fatty acids in all trouts investigated for this study. Therefore, a total of three omega-6 fatty acids and six omega-3 fatty acids were determined in polyunsaturated fatty acids.

The mean levels of DHA in the muscles of brook trout and Black Sea trout were found to be the highest in all polyunsaturated fatty acids. DHA is considered a health promoted fatty acid for consumers (Zhang et al., 2021). On the other hand, the greatest amount of fatty acid for rainbow trout in all polyunsaturated fatty acids were seem to be the LA in the study. The average levels of ALA were higher than that of EPA for all trouts.

The average amounts of LA in the muscle of brook trout and Black Sea trout were found to be the greatest in all omega 6 groups and second greatest in all polyunsaturated fatty acids. On the other hand, rainbow trout had the highest amount of LA followed by DHA and ALA. Studies related to reared fish fatty acid simply showed that LA amounts in reared fish tended to be higher depending on the fish feeding ingredients (Öksüz, 2012; Yeşilayer & Genç, 2013; Yeşilayer et al., 2014; Dernekbaşı et al., 2015; Dernekbaşı et al., 2017; Özyılmaz, 2019; Dernekbaşı & Karatas, 2020; Dernekbaşı & Karayücel, 2021).

Fatty acid related lipid indices namely atherogenic index (AI), thrombogenic index (TI), and polyene index (PI) of the brook trout, rainbow trout, and Black Sea trout showed some differences. Some of these differences were found to be statistically significant ($P<0.05$). The differences between brook trout and Black Sea trout related to the values of AI, TI, and PI were always statically significant ($P<0.05$) whereas that of rainbow trout did not ($P>0.05$). The AI, TI, and PI point out the quality of the lipid for their health benefits. The amounts of AI, TI, and PI in the lipid of the brook trout, rainbow trout, and Black Sea trout investigated in this present study varied from species

to species in the same salmonid family. Küçükgülmez et al. (2018) reported that values of AI, TI, and PI for golden grey mullet and gold band goatfish differed from season to season.

Table 4. The fatty acid components (% of total fatty acid) and related lipid indices [Atherogenic Index (AI), Thrombogenic Index (TI), and Polyene Index (PI)] of the brook trout, rainbow trout, and Black Sea trout
Çizelge 4. Kaynak alabalığı, gökkuşağı alabalığı ve Karadeniz alasının yağ asidi bileşenleri (toplam yağ asidinin yüzdesi) ve lipit indeksleri [aterojenik indeks (AI), trombojenik indeks (TI) ve polien indeksi (PI)]

Fatty Acids	Brook Trout	Rainbow Trout	Black Sea Trout
C14:0	1.83±0.23[a]	1.76±0.01[a]	1.18±0.01[b]
C15:0	0.89±0.27[a]	ND	0.84±0.16[a]
C16:0	17.26±1.36[a]	13.68±0.04[b]	16.22±0.33[a]
C18:0	3.60±0.25[a]	4.11±0.06[b]	3.96±0.08[b]
C20:0	0.32±0.03[a]	ND	1.82±0.51[b]
∑SFA	**23.90[a]**	**19.54[a]**	**24.02[b]**
C16:1n9	2.92±0.43[a]	2.62±0.01[a]	1.76±0.03[b]
C18:1n9	20.45±1.37[a]	22.44±0.25[b]	20.60±0.39[a]
C18:1n7	2.26±0.26[a]	1.90±0.10[b]	2.51±0.12[a]
C20:1n9	3.30±0.65[a]	1.98±0.03[b]	1.14±0.18[b]
∑MUFA	**28.92[a]**	**28.94[a]**	**26.01[a]**
C18:2n6	12.49±1.38[a]	21.78±0.15[b]	13.78±0.37[a]
C20:2n6	0.94±0.13[a]	0.79±0.03[a]	0.91±0.04[a]
C20:4n6	0.85±0.02[a]	0.81±0.03[a]	1.11±0.18[b]
n6	**14.28[a]**	**23.38[b]**	**15.80[a]**
C18:3n3	5.69±0.54[a]	6.80±0.08[b]	5.22±0.07[a]
C18:4n3	1.51±0.16[a]	1.34±0.03[a]	0.81±0.07[b]
C20:3n3	1.03±0.16[a]	0.91±0.03[a]	0.59±0.24[a]
C20:4n3	0.66±0.13[a]	0.52±0.03[a]	1.19±0.27[b]
C20:5n3	4.64±0.96[a]	2.94±0.06[b]	3.13±0.04[b]
C22:5n3	1.44±0.17[a]	1.11±0.04[a]	2.06±0.46[b]
C22:6n3	16.93±1.55[a]	13.83±0.23[b]	21.24±0.67[c]
n3	**31.90[a]**	**27.46[b]**	**34.23[c]**
∑PUFA	**46.18[a]**	**50.84[b]**	**50.03[c]**
AI	0.33±0.02[a]	0.26±0.00[b]	0.28±0.01[b]
TI	0.19±0.01[a]	0.18±0.00[ab]	0.17±0.00[b]
PI	1.25±0.06[a]	1.23±0.02[a]	1.50±0.02[b]
SFA	**23.90[a]**	**19.54[a]**	**24.02[b]**
MUFA	**28.92[a]**	**28.94[a]**	**26.01[a]**
PUFA	**46.18[a]**	**50.84[b]**	**50.03[c]**
n6	**14.28[a]**	**23.38[b]**	**15.80[a]**
n3	**31.90[a]**	**27.46[b]**	**34.23[c]**
n3/n6	**2.23[a]**	**1.17[b]**	**2.17[a]**
n6/n3	**0.45[a]**	**0.85[b]**	**0.46[a]**
EPA	**4.64[a]**	**2.94[b]**	**3.13[b]**
DHA	**16.93[a]**	**13.83[b]**	**21.24[c]**
EPA/DHA	**0.27**	**0.21**	**0.15**
DHA/EPA	**3.65**	**4.70**	**6.79**
PUFA/SFA	**1.93**	**2.60**	**2.08**
EPA+DHA	**21.57**	**16.77**	**24.36**

[a,b,c] Values within same row with different superscripts diverge significantly at P<0.05
AI (Atherogenic Index), TI (Thrombogenic Index), and PI (Polyene Index)]

CONCLUSIONS

The highest average moisture and ash level were found in the flesh of rainbow trout. In addition, all three fish have lower lipid levels and higher amounts of protein and carotene (vitamin A) which are considered very important for a healthy diet. Palmitic acid (C16:0) and oleic acid (C18:1n9) were the highest level of fatty acids in saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA), respectively. Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) in polyunsaturated fatty acids (PUFA) were found to be different from each other ($P<0.05$). Although the levels of EPA and DHA in fish flesh are different from each other, they are good for health. All trouts in the present study seem suitable for consumption, and they should be added to diets in order to take full benefits.

Statement of Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical statement

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

Authorship Contribution Statement

The contribution of the authors is equal

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Use of Multidimensional Scaling Analysis Together With Multivariate Analysis of Variance in Determining Differences Between Groups

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ABSTRACT
Multidimensional Scaling analysis (MDS) and Multivariate analysis of variance (MANOVA) are among the commonly used multivariate statistical methods. While MANOVA is used to evaluate whether there are statistically significant differences between the mean vectors of the experimental groups in terms of more than one independent variable; MDS analysis is used both for dimension reduction and to classify individuals/variables according to their differences. In cases where the relationships between individuals/variables are not known, but the distances between them can be calculated, MDS analysis allows to reveal the relationships between individuals by using these distances, and unlike MANOVA, it does not require any assumptions. In this study, the numerical values produced by the simulation technique were used as the input data, with reference to the real data regarding 5 kinds of pistachios in terms of 13 fatty acids. These data were evaluated with both the MDS analysis and the MANOVA test and the results were interpreted. Considering the convenience in the evaluation of the data, the usability of the MDS analysis as supportive to the MANOVA test and subsequent multiple comparison tests was evaluated.

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Gruplar Arası Farklılıkların Belirlenmesinde Çok Boyutlu Ölçekleme Analizinin Çok Değişkenli Varyans Analizi ile Birlikte Kullanımı

ÖZET
Çok Boyutlu Ölçekleme (ÇBÖ) analizi ve Çok değişkenli varyans analizi (MANOVA) yaygın olarak kullanılan çok değişkenli istatistik yöntemler arasında yer almaktadır. MANOVA, birden fazla bağımsız değişken bakımından deney gruplarının ortalama vektörleri arasında istatistik olarak önemli farklılıklar olup olmadığını değerlendirmede kullanılırken; ÇBÖ analizi, hem boyut indirgeme hem de bireyleri/değişkenleri farklılıklarına göre sınıflandırmak için kullanılır. ÇBÖ analizi, bireyler/değişkenler arasındaki ilişkilerin bilinmediği fakat aralarındaki uzaklıkların hesaplanabildiği durumlarda, bu uzaklıklardan yararlanarak bireyler arasındaki ilişkilerin ortaya koyulmasına olanak tanır ve MANOVA'nın aksine herhangi bir varsayım gerektirmez. Bu çalışmada girdi verisi olarak 13 yağ asidi bakımından 5 çeşit antepfıstığına ilişkin gerçek veriler referans alınarak simülasyon tekniği ile üretilen sayısal değerler kullanılmış ve bu veriler hem ÇBÖ analizi hem de MANOVA testi ile değerlendirilmiştir. Her iki test sonucunda da elde edilen bulguların benzer olduğu ve verilerin analizi, yorumlanması ve sonuçların değerlendirilmesindeki kolaylıklar göz önünde bulundurulduğunda, MANOVA testi ve sonrasında yapılacak olan çoklu karşılaştırma testlerini destekleyici olarak ÇBÖ analizinin kullanılabilirliği değerlendirilmiştir.

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INTRODUCTION

Multivariate analysis methods aim to obtain a general result by considering the relationships between two or more random variables as a whole. However, with the increase in the number of variables studied, the number of dimensions also increases, making it difficult to interpret the results obtained. For this reason, most of the multivariate statistical analysis methods are based on dimension reduction (Jobson, 1992). One of them is Multidimensional Scaling (MDS) analysis.

MDS analysis is a multivariate method used to classify variables/individuals, which allows modelling nonlinear relationships between variables and evaluating all data types. Unlike other multivariate methods, it does not require assumptions like data type, relationships between variables, and multivariate normal distribution (Yiğit & Mendeş, 2016). MDS analysis evaluates the differences and similarities between data, individuals, variables and even events, yielding graphical results that can be easily interpreted by anyone. Due to these advantages, it has found a wide range of use in practice (Jaworska & Anastasova, 2009).

One of the areas where MDS analysis is widely used is agriculture. Many researchers classify the trial material they are working on with MDS analysis in terms of various properties. In the study conducted by Suarez et al. (2016) on 30 sweet potato varieties; the nutritional composition, mineral and trace element amounts of potatoes were determined and significant differences were found between the varieties in terms of these characteristics. Then, sweet potato varieties were classified by MDS analysis. Yamamoto et al. (2015) improved a image analysis system which can simultaneously assess multiple appearance properties of strawberries, in detail. Then, they tried to reveal the efficiency of the system using clustering, MDS and discriminant analysis. Can et al (2021) evaluated the heavy metal accumulation that occurs as a result of intensive production in fruit and vegetables produced today. For this purpose, the most common fruity vegetables in Kyrgyzstan markets (ten different fruity vegetables including tomato (2), pepper (5), eggplant, cucumber and zucchini) were included in the study and their B, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb and Zn contents were measured. Differences between fruity vegetables in terms of measurements were evaluated by Kruskal Wallis test and these fruity vegetables classified by MDS analysis. s a result of the MDS analysis, the fruity vegetables evaluated within the scope of this study were clearly divided into four groups in terms of mineral nutrients and heavy metal contents. In the study by Lopes et al (2017), the results obtained by electrical impedance spectroscopy (EIS) and standard chemical analyses were compared regarding characterization different wine varieties. Hence, impedance parameters and chemical analysis results of 16 Portuguese wines were evaluated using MDS analysis. Consequently, it was observed that the wines could be classified with the impedance data obtained from the EIS, based on the strong correlations found between the electrical measurements of the wine and its chemical properties. This conclusion has been confirmed through MDS-maps.

In this study, numerical values produced by simulation technique, by taking the real values of 13 fatty acids of 5 types of pistachios as reference, were used as input data. These data were evaluated with metric-MDS analysis and the similarity of the results obtained with the MDS method and multivariate analysis of variance (MANOVA) technique was shown.

MATERIAL and METHOD

The data used in this study were produced by simulation technique with the help of Microsoft Power Station Developer Studio and IMSL Library in the FORTRAN PowerStation 4.0 package program, with reference to the mean, standard deviation and correlation structure of the fatty acids (*myristic acid, palmitic acid, palmitoleic acid, margaric acid, margaoleic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, arachidic acid, gadoleic acid, behenic acid and lignoseric acid*) measured in 5 varieties of pistachio (*Siirt, Uzun, Halebi, Kırmızı, Ohadi*) in the study of Çınar (2012). The mean and standard errors for the simulated data were given in Table 1.

As a result of the simulation study, the data produced in terms of 13 fatty acids for a total of 50 pistachios, 10 of each variety, were evaluated using the NCSS 2007 Version 07.1.5.

Multidimensional Scaling Method

In the MDS method, depending on the type of variables, the configuration distances (δ_{ij}) that will represent the original distances (d_{ij}) with the least error are determined and displayed graphically in a less dimensional space. For this, the data coordinates must be converted to graphical representation coordinates with the least error. This criterion, which measures the discrepancy/difference between the original distances and the configuration distances, is called the stress value (Özdamar, 2004).

The MDS method can generally be grouped under two

headings, metric and non-metric. If the data to be analysed is obtained in nominal or ordinal scale, non-metric, if it is obtained in interval or ratio scale, metric MDS analysis is used. In the metric method, the solution is done with an approach similar to the principal component analysis, while in the non-metric method, the analysis is made by using the rank numbers of the distances. While performing MDS analysis, various input (similarity or dissimilarity) matrices can be used according to the data structure and purpose of the researcher (Cox & Cox, 2001).

Table 1. Descriptive statistics of simulated data
Çizelge 1. Üretilen verilere ait tanıtıcı istatistikler

Fatty Acid *Yağ asidi*	Siirt (n=10)	Uzun (n=10)	Halebi (n=10)	Kırmızı (n=10)	Ohadi (n=10)
Myristic	0.07±0.001	0.10±0.003	0.09±0.001	0.10±0.001	0.08±0.004
Palmitic	7.61±0.034	8.67±0.031	8.81±0.023	8.81±0.027	8.52±0.034
Palmitoleic	0.53±0.006	0.69±0.018	0.77±0.006	0.65±0.011	0.65±0.006
Margaric	0.04±0.001	0.04±0.001	0.04±0.002	0.05±0.007	0.06±0.004
Margaoleic	0.08±0.002	0.06±0.002	0.08±0.010	0.08±0.004	0.06±0.001
Stearic	1.96±0.008	1.77±0.002	1.82±0.012	2.11±0.012	1.26±0.009
Oleic	69.78±0.149	64.08±0.077	71.95±0.193	71.05±0.123	58.21±0.095
Linoleic	18.48±0.044	23.07±0.048	14.94±0.088	15.65±0.055	29.98±0.028
Linolenic	0.34±0.006	0.35±0.006	0.34±0.012	0.36±0.019	0.34±0.004
Arachidic	0.17±0.003	0.14±0.003	0.16±0.004	0.20±0.005	0.12±0.011
Gadoleic	0.65±0.003	0.52±0.013	0.43±0.006	0.47±0.006	0.55±0.018
Behenic	0.19±0.015	0.18±0.014	0.19±0.009	0.25±0.017	0.23±0.018
Lignoseric	0.13±0.006	0.16±0.002	0.1±0.006	0.17±0.037	0.22±0.002

In MDS analysis, the distances (d_{ij}) between the i^{th} and j^{th} individuals/variables in the data set are calculated and these distances are represented in a geometric space (Euclidean space, etc.). In the obtained p-dimensional Euclidean space, the relationship between the original distances (d_{ij}) and the configuration distances (δ_{ij}) can be graphically represented by the Shepard diagram. Shepard diagram is a scatterplot with observed distances on the Y-axis and configuration distances on the X-axis. By looking at the Shepard diagram and the pseudo-R^2 statistics, which is an index similar to the determination coefficient in regression analysis, the goodness of fit between the observed distances and the configuration distances can be observed (Shepard, 1962; Özdamar, 2004; Yiğit, 2007; Gündüz, 2011; Mair et al., 2016).

Metric-multidimensional scaling method

The first foundation of this method, known as metric or classical MDS, was laid by Young and Householder (1938) in the 1930s. Later, the Psychometrics group at Princeton University, which included Messick-Albelson (1956) and Torgerson (1952), conducted studies on this subject. Torgerson first demonstrated the applicability of the metric-MDS method for interval and ratio data in a paper he published in 1952 (Young and Hamer, 1987).

Metric-MDS analysis uses nxn dimensional proximity matrix as input data. The objective is to find estimated distances (configuration distances) which are approximately equal to the observed ones, in the distance matrix (D) for k-dimensional space. Computation of the coordinates to represent individuals is possible by finding the eigenvectors of the B-matrix from which the coordinates of the distance matrix will be obtained. Therefore, in the metric-MDS method, the B-matrix must be obtained first. For this purpose, the following steps are followed (Tatlıdil, 2002; Sığırlı et al., 2006; Alpar, 2013).

1. Firstly, the data is standardized using the appropriate method. Later, nxn-dimensional distance matrix (D) is created by using the distance measure (usually Euclidean distance is preferred in practice) that we determine in accordance with the structure of the data.
2. D-matrix is not a positive semi-definite matrix because its diagonal elements are '0'. However, by using this matrix positive definite B-matrix can be obtained. For this purpose, the matrix A must first be obtained primarily. The nxn dimensional A-matrix is obtained by Equation 1.

$$A = (a_{ij}) = \left(-\frac{1}{2} d_{ij}^2 \right) \quad (1)$$

d_{ij}: elements of the D-matrix (observed distances)

3. Using the A-matrix, the B-matrix (Equation 2.), which is a symmetrical matrix that can be divided into diagonal elements and column vectors, is created.

$$B = -\frac{1}{2}\left[I_n - \frac{1}{n} i_n i'_n \right] D^2 \left[I_n - \frac{1}{n} i_n i'_n \right] \quad (2)$$

I_n: Identity matrix with nxn dimensional, i_n: Unit vector with nx1 dimensional, D^2: The matrix obtained by squaring the elements of the matrix D.

4. The eigenvalues and eigenvectors of the B-matrix are calculated. Since the B-matrix is a positive semi-definite matrix, the number of positive eigenvalues is equal to the number of dimensions of the distance matrix (matrix D). The B-matrix is expressed as $B = V\Lambda V'$ (V: matrix of eigenvectors of the B-matrix, Λ: matrix whose diagonal elements are eigenvalues).

5. After finding the eigenvalues and eigenvectors of the B-matrix, the graphical coordinates are found with the help of $\sqrt{\lambda_i} v_i$. (λi: The eigenvalue of the B-matrix calculated for the i^{th} dimension, v_i: Eigenvectors corresponding to the i^{th} dimension of matrix B.

6. It is necessary to decide in how many dimensional space data matrix will be represented. Therefore, MDS solutions are obtained for each dimension. Then, the goodness of fit of each analysis to the real distance matrix, that is, the stress value, is calculated and it is decided which analysis will be applied. In practice, 2 or 3 dimensions are generally preferred for easy interpretation.

Stress Value

The stress value is used to determine whether the number of dimensions obtained as a result of the MDS analysis is appropriate. The stress value is the sum of the deviations of the points from the regression line in the Shepard diagram and is calculated to determine the correspondence between the observed distances and the configuration distances. The stress value is, in a way, a statistic similar to the correlation coefficient. However, it does not measure the goodness of fit, but the badness of fit. The Stress (STandardized REsidual Sum of Squares) value is a measure of the difference between the multidimensional (p-dimensional) real model and the model estimated in reduced (k-dimensional) space. In other words, it measures the discordance between the observed distances and the configuration distances and is calculated as in Equation 3 (Borg and Groenen, 2005).

$$Stress = \sqrt{\frac{\sum_{i=1}^{n-1}\sum_{j=i+1}^{n}(d_{ij} - \delta_{ij})^2}{\sum_{i=1}^{n-1}\sum_{j=i+1}^{n} d_{ij}^{\,2}}} = \sqrt{\frac{\sum (d_{ij} - \delta_{ij})^2}{\sum_{i<j} d_{ij}^{\,2}}} \quad (3)$$

d_{ij}: observed distances between i^{th} and j^{th} points, δ_{ij}: Estimated configuration distances between the i^{th} and j^{th} points as a result of the c^{th} iteration.

According to the magnitude of the stress value obtained as a result of the MDS analysis, the fitness of the configuration distances with the observed distances can be classified as in Table 2. The main purpose in both metric and non-metric approach; is to minimize the stress value, which is an indicator of the discordance between the observed and the configuration distances.

In MDS analysis, it is desired that the stress value be as close to zero as possible. A stress value of exactly zero indicates perfect fit, while a stress value equal to 1 indicates complete incompatibility (Borg and Groenen, 2005; Borg et al., 2013).

Table 2. Classification of stress value
Çizelge 2. Stres-değerinin sınıflandırılması

Stress value *Stres değeri*	Goodness of fit *Uyum iyiliği*
0.20 ≤ stress	Poor
0.10 ≤ stress < 0.20	Fair
0.05≤ stress < 0.10	Good
0.025≤ stress < 0.05	Excellent
stress < 0.025	Perfect

In the MDS analysis, increasing the number of dimensions decreases the stress value. However, it is necessary to establish a balance between the stress value and the number of dimensions in order to interpret the obtained dimensions and express the results easily (Cox and Cox, 2001; Alpar, 2013).

Shepard Diagram and Pseudo-R^2 Statistic

The diagram showing how well the obtained MDS model fits the data and the compatibility of observed distances and configuration distances is called Shepard diagram. With this diagram, the linearity of the fit can be examined. If the fit is good, the points are located on or around the 45^0 line. In the Shepard diagram, the distances observed on the Y-axis and the configuration distances on the X-axis are located (Shepard, 1962).

How well the configuration distances adapt to the observed distances is measured by the degree of linear relationship between the two features in question, that is, the square of the correlation coefficient (R^2). The pseudo-R^2 statistic calculated in the MDS analysis is an index similar to the coefficient of determination in the regression analysis. The pseudo-R^2 statistic gives a measure of how much of the sum of the squares of the mean-corrected dissimilarity values can be explained by using the number of dimensions determined as a result of the MDS analysis. Pseudo-R^2 statistic can be calculated using the Equation 4 (Cox and Cox, 2001; Alpar, 2013).

$$Pseudo\text{-}R^2 = 1 - \frac{\sum_{i=1}^{n}\left(d_{ij} - \delta_{ij}\right)^2}{\sum_{i=1}^{n}\left(d_{ij} - \bar{d}\right)^2} \quad (4)$$

d_{ij} : Observed distance or proximity values, δ_{ij} : Configuration distances, $\bar{d}$: Average of observed distances.

In order for the number of dimensions obtained as a result of the MDS analysis to be sufficient, the pseudo-R^2 statistic must be greater than 0.80 (in some sources, it is 0.60). Thus, it is understood that the obtained configuration distances are in good agreement with the observed distances (Gevrekçi et al., 2011; Alpar, 2013).

Multivariate Analysis of Variance (MANOVA)

In most biological events, the effects of factors on more than one variable is curious. For this purpose, MANOVA test is widely used and applied by following the steps below:

1. The experimental units should be chosen randomly from the population, the observation values should be independent from each other, the data should be continuous and show multivariate normal distribution, the number of experimental units should be more than the number of variables, and the variance-covariance matrix should be homogeneous.

2. After determination the control hypothesis (H_0 : The differences between the mean vectors of the groups in terms of the studied features are not statistically significant) and the alternative hypothesis (H1: The difference between the mean vectors of at least two groups in terms of the studied features is statistically significant), the test statistic is calculated.

3. The most commonly used test statistics for hypothesis control are; Wilks' lambda, Hotelling's trace, Pillai's trace, and Roy's largest roots statistics. The Pillai's Trace test statistic used in this study is obtained as in Equation (5).

$$T = \sum_{i=1}^{p} \frac{\lambda_i}{1+\lambda_i} \qquad (5)$$

In this equation, the λ_i values are the eigenvalues of the BW^{-1} matrix product (B: sum of squares matrix between groups, W: sum of squares matrix within groups). Calculated T-value is then converted to the F_T value, showing the F-distribution with '$s(2m+s+1)$' and '$(s(2+s+1))$' degrees of freedom, using Equation (6).

$$F_T = \frac{2n+p+1}{2m+p+1} \times \frac{T}{p-T} \qquad (6)$$

n: The number of observation in each group, p: the number of variable, $m = \frac{|p-(k-1)|-1}{2}$,

$s = \min(k-1, p)$, k : the number of mean vectors,

$$\tilde{n} = \frac{N-p-k-1}{2}$$

4. If the control hypothesis is rejected as a result of the hypothesis test, it is determined that the differences between the mean vectors of which groups are statistically significant with the appropriate multiple comparison test. For this purpose, commonly used test are: simultaneous confidence interval method, Bonferroni confidence interval method and Mahalanobis distance. However, in terms of convenience, the most common application is to perform ANOVA test for each variable separately, although it ignores the relationships between variables (Al-Abdullatif et al 2019). In addition to these, Discriminant Analysis is also widely used for this purpose (Al-Abdullatif, 2020). The Mahalanobis distance used in this study can be calculated with Equation (7).

$$D_{ij}^2 = (\mu_i - \mu_j)' \; S^{-1} (\mu_i - \mu_j) \qquad (7)$$

μ_i : i. grubun ortalama vektörü, μ_j : j. grubun ortalama vektörü, S^{-1} :ise gruplar içi varyans-kovaryans matrisinin tersi

The calculated D^2 values are then converted to F-values using Equation (8).

$$F = \frac{n_i n_j (n_i + n_j - p - 1)}{p(n_i + n_j)(n_i + n_j - 2)} D^2 \qquad (8)$$

Finally, the F-value obtained by Equation (8) is compared with the F-table value with '(p)' and '(ni+nj-p-1)' degrees of freedom, and it is determined that the differences between the mean vectors of which groups are statistically significant (Jobson, 1992, Alpar 2013).

RESULTS and DISCUSSION

In most of the studies, the assumptions of the MANOVA technique may not be fulfilled. In addition, in many studies, the number of variables may be higher than the number of experimental units. These and similar situations make it impossible to use the MANOVA technique. Even if all the assumptions are fulfilled, the multiple comparison tests done after MANOVA test for factorial designs are quite complex. For this purpose, the usability of MDS analysis which was not need any assumptions was evaluated in this study, and the results of MANOVA and MDS analysis were compared.

The most important issue to be considered while performing MDS analysis is to determine the number of dimensions. Although it is desired that the stress value be as low as possible and the pseudo-R^2 statistic be as high as possible in theory, the determination of more than 3 dimensions, in practice, makes it difficult

to evaluate the results of the study. For this reason, the balance between the number of dimensions and obtaining interpretable results should be well established and the number of dimensions should be determined as 2 or 3 maximum. However, in some studies, it is unavoidable to take more than 3 dimensions. In such cases, since it will not be possible for the researcher to show all dimensions on the same map, she/he can interpret the results obtained by creating different 2-dimensional maps for binary combinations of dimensions (Buja et al., 2008; Dumanoğlu et al., 2018).

The eigenvalues obtained as a result of the metric-MDS analysis for the fatty acids studied in pistachios are given in Table 3.

Table 3. Eigenvalues obtained as a result of metric-MDS analysis
Çizelge 3. Metrik-MDS analizi sonucunda elde edilen özdeğerler

Number of dimension *Boyut sayısı*	Eigenvalue *Özdeğer*	Individual % *Bireysel %*	Cumulative % *Birikimli %*
1	2894.78	99.10	99.10
2	20.66	0.71	99.81
3	3.61	0.12	99.94
...	....	...	...

As seen in Table 3, the eigenvalue of the first dimension is 2894.78, which explains 99.1% of the total variation. As it can be easily estimated when looking at the eigenvalues given in Table 4, as a result of the metric-MDS analysis made for the data it was decided that it would be sufficient to draw a map by considering only the first dimension. This situation can be seen more clearly when looking at the stress values given in Table 4.

Table 4. Stress values and pseudo-R^2 statistics
Çizelge 4. Stres-değeri ve yalancı-R^2 istatistiği

Dimension *Boyut*	Stress value *Stres değeri*	Pseudo-R^2 statistic *Yalancı-R^2 istatistiği*
1	0.023	99.870
2	0.007	99.990
3	0.003	100.000
...	...	...

Considering the stress values given in Table 4, it can be seen that the accordance between the observed distances and the configuration distances is "perfect" even for one dimension. Because the stress value calculated for the 1st dimension is below 2.5% (Table 2). Also, Pseudo-R^2 statistic, calculated as 99.87%, expresses the power of the model created for metric-MDS analysis to explain the data. Both the stress value and the pseudo-R^2 statistics show that only one dimension (the first dimension) is sufficient to classify the pistachios in the study in terms of the fatty acids studied. In other words, it is concluded that 99.87% of the variation in the observed distances can be explained by the configuration distances calculated using the first dimension obtained as a result of the metric-MDS analysis.

The relationship between the observed and the configuration distances calculated for the first dimension can be shown with the Shepard diagram as in Figure 1. When Figure 1 is evaluated, the linear relationship between the observed distances and the configuration distances can easily be seen.

According to the stress value obtained as a result of the metric-MDS analysis, the number of sufficient dimensions was determined as 1. The classification map created for one dimension as a result of metric-MDS analysis using simulated data for various fatty acids for *'Siirt'*, *'Uzun'*, *'Halebi'*, *'Kırmızı'* and *'Ohadi'* cultivars is given in Figure 2.

Especially *Ohadi* (41-50), *Uzun* (11-20) and cultivars differed from the others and from each other in terms of fatty acids studied (Figure 2). *Halebi* (21-30) and *Kırmızı* (31-40) varieties are located close to each other on the map. Although the *Siirt* (1-10) cultivar is located relatively close to the *Kırmızı*, it is clustered separately from all other cultivars on the map. However, upon careful examination, it can be seen that these two cultivars do not mix completely and that the *Kırmızı* variety is positioned slightly lower than the *Halebi*. When the Figure 2 is considered in general, it is seen that pistachios are ranked from the lowest to the highest in terms of the fatty acid contents as; *Ohadi-Uzun-Siirt-Kırmızı-Halebi*. In summary, as a result of the MDS analysis, it can be said that the richest pistachio cultivars in terms of 13 fatty acids studied are Kırmızı and Halabi.

The investigator may wonder whether the observed differences between the mean vectors of cultivars for the 13 fatty acids studied are statistically significant or not. For this purpose, multivariate analysis of variance (MANOVA) technique is used. As a result of the MANOVA test to determine whether there is a statistically significant difference between the mean vectors of pistachio cultivars in terms of fatty acids studied; Pillai's trace test statistic and F-value were respectively calculated as 3.747 and 41.024, and the control hypothesis, which indicate there is not

statistically significant difference between mean vectors, was rejected ($p<0.01$). Afterward, Mahalanobis distance was calculated in order to determine which varieties' mean vector statistically significantly differentiated from the others. The results of multiple comparison were given in Table 5.

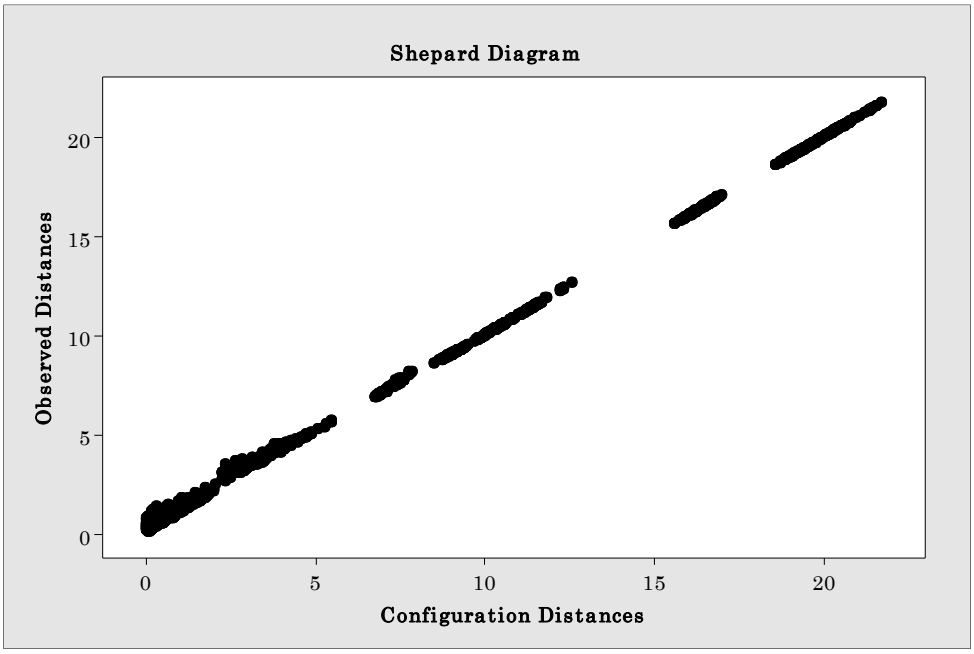

Figure 1. Shepard diagram
Şekil 1. Shepard diyagramı

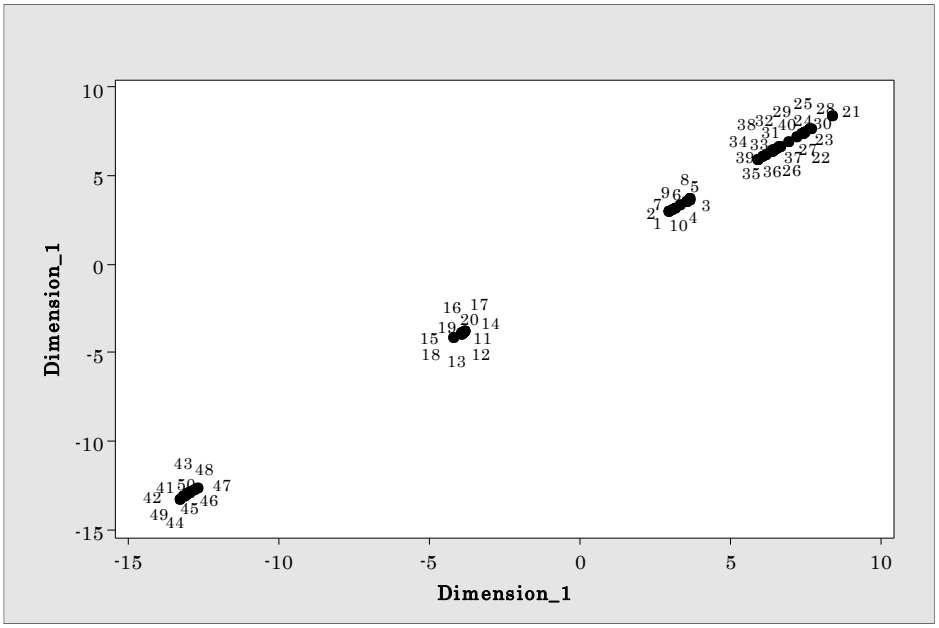

Figure 2. MDS map created to classify pistachios (Codes of pistachios according to varieties: *Siirt*; 1-10 coded, *Uzun*; 11-20 coded, *Halebi*; 21-30 coded, *Kırmızı*; 31-40 coded, *Ohadi*; 41-50 coded)
Şekil 2. Antepfıstıklarının sınıflandırılması amacıyla oluşturulan MDS haritası (Antepfıstığının çeşitlerine göre kodları: Siirt; 1-10, Uzun; 11-20, Halebi; 21-30, Kırmızı; 31-40, Ohadi; 41-50)

When Table 5 is examined, only the differences between the mean vectors of *Halebi –Kırmızı* and *Siirt –Kırmızı* cultivars were not found to be statistically significant. All other differences were statistically significant ($p<0.05$). Therefore, it can be considered that the *Kırmızı* variety of pistachios is a transitional form between *Siirt* and *Halabi* varieties in terms of 13 fatty acids studied. To sum up, it is seen that the pistachio cultivars with statistically higher values than the others in terms of 13 fatty acids studied are Kırmızı and Halabi (Table 5). This inference are also consistent with the MDS analysis.

Table 5. Multiple comparison test results using Mahalanobis distance

Çizelge 5. Mahalanobis uzaklığı kullanılarak yapılan çoklu karşılaştırma testi sonuçları

Varieties *Çeşit*	Results *Sonuçlar*
Siirt (1-10)	B
Uzun (11-20)	C
Halebi (21-30)	A
Kırmızı (31-40)	AB
Ohadi (41-50)	D

* The difference between the mean vectors of cultivars that do not have a common letter is statistically significant ($p<0.05$).

CONCLUSION

The classification results of metric-MDS analysis and the results of MANOVA and its multiple comparison test were evaluated. After the evaluation, although the results of the two tests were not exactly the same, it was observed that they were quite similar. Despite of the MDS analysis has located the Siirt variety slightly apart on the map, it is noteworthy that the results obtained with the Mahalanobis distance almost completely overlap with the MDS analysis. Nevertheless, it is much easier for the researcher to both perform the MDS analysis and interpret the results of the graphical representation compared to the MANOVA analysis. In addition, statistical package programs unfortunately do not include multiple comparison tests to determine which groups' mean vectors are statistically significant if the H0 hypothesis is rejected after the MANOVA test. Determining these by the researcher is quite time consuming and complex. MDS analysis and MANOVA post-hoc test results can be evaluated as supportive and alternative to each other (Kızıl and Aydoğan 2014). Because of these advantages, MDS analysis can be considered as an alternative to the MANOVA test and its multiple comparison tests performed afterwards. In addition, if it was wondered which varieties were different from each other in terms of the data obtained in the nominal or ordinal scale, not the metric measurements, the MANOVA test could not be used because the assumptions were not met. In such a case, varieties can be easily classified by MDS analysis, which does not require any assumptions. For this reason, MDS analysis is an advantageous method in that it provides the researcher with easily interpretable preliminary information about the differences between the means by classifying similar groups (Zech et al 2011).

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Author's Contributions

The contribution of the authors are equal.

Statement of Conflict of Interest

All the authors declare that they have no conflict of interest.

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Feed Value of Emmer Wheat *(Triticum dicoccum)* and By-products for Ruminant Animals

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ABSTRACT

Ancient wheat species attracts more attention recently due to their health benefits and suitability for organic farming. With this attention, the possibilities of using ancient wheat species and especially their by-products in animal nutrition are emerging. Unlike modern wheat varieties, emmer is known as one of the ancient wheat varieties, has hull covering its grains. Approximately 70% of the total plant weight consists of hulls and stalks. Looking at the literature, limited studies has been conducted regarding the evaluation of the hull and stalk parts of emmer wheat as feed. Herein, this study aims to determine the nutritional values of emmer wheat and its by-products. The feed value of the plant was analyzed in five parts (hulled grain, stalk of plant, hull, naked grain and flour). For each part, dry matter (DM), crude protein (CP), ether exract (EE), crude ash (CA), starch, crude fiber (CF), acid detergent fiber (ADF), neutral detergent fiber (NDF), acid detergent insoluble crude protein (ADICP), neutral detergent insoluble crude protein (NDICP), lignin and mineral analyzes were made. In addition, non-fiber carbohydrate (NFC), digestible dry matter (DDM), dry matter intake by animal body weight (DMI_{BW}), total digestible nutriton (TDN_{1X}), net energy lactation (NE_{L3X}), metabolic energy (ME), net energy maintanence (NE_M), net energy gain (NE_G), energy and digestibility calculations were performed. The protein content of emmer grain was observed higher than that of modern wheat. ADF and NDF values of the emmer wheat stalks are lower than modern wheat stalks and therefore have better digestibility values. Additionally, the higher resistance to starch digestion compared to modern wheat varieties may be a reason for preference in ruminant feeding.

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Gernik (Gacer, Kavılca) Buğdayı (*Triticum dicoccum*) ve Yan Ürünlerinin Ruminant Hayvanlar İçin Yem Değeri

ÖZET

Ata buğday türleri, sağlığa faydaları ve organik tarıma uygunlukları nedeniyle son zamanlarda daha fazla ilgi çekmektedir. Bu ilgi ile birlikte ata buğday türlerinin ve özellikle yan ürünlerinin hayvan beslemede kullanım olanakları ortaya çıkmaktadır. Ata buğday çeşitlerinden biri olarak bilinen gernik (gacer, kavılca), tanelerini kaplayan bir kavuza sahiptir. Toplam bitki ağırlığının yaklaşık %70'i kavuz ve saplardan oluşur. Literatür bilgiler incelendiğinde gerniğin kavuz ve sap kısımlarının yem olarak değerlendirilmesine yönelik sınırlı sayıda çalışma yapıldığı görülmektedir. Bu çalışmada ise gernik ve yan ürünlerinin besin değerlerinin belirlenmesi amaçlanmıştır. Bitkinin yem değeri beş kısımda (kavuzlu dane, bitki sapı, kavuz, kavuzsuz dane ve un) analiz edilmiştir. Her kısım için, kuru madde (KM), ham protein (HP), ham yağ (HY), ham kül (HK), nişasta, ham selüloz (HS), asit deterjan fiber (ADF), nötral deterjan fiber (NDF), asit deterjanda çözünmeyen ham protein (ADICP), nötral deterjanda çözünmeyen ham protein (NDICP), lignin ve mineral analizleri yapılmıştır. Bunlara ilave olarak, fiber olmayan karbonhidrat (NFC), sindirilebilir kuru madde (DDM), hayvan vücut ağırlığına göre kuru madde tüketimi ($DMI_{vücut}$

Zootekni

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Anahtar Kelimeler
Ata buğday
Gernik
Gacer
Gernik kavuzu
Triticum dicoccum

ağırlığı), toplam sindirilebilir besin (TDN_{1X}), net enerji laktasyon (NE_{L3X}), metabolik enerji (ME), net enerji yaşama (NE_M), net enerji verim (NE_G) gibi enerji ve sindirilebilirlik hesaplamaları yapılmıştır. Emmer buğday danesinin protein içeriğinin modern buğday çeşitlerine göre daha yüksek olduğu gözlemlenmiştir. Emmer buğdayı sap kısımlarının ADF ve NDF değerleri modern buğday saplarına göre daha düşük belirlenmiştir ve bu nedenle daha iyi sindirilebilirlik değerlerine sahiptir. Ayrıca modern buğday çeşitlerine göre nişasta sindirim direncinin daha yüksek olması ruminant beslemede tercih sebebi olabilir.

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INTRODUCTION

In recent years, it is seen that interest in ancient wheats has increased due to the orientation to healthy and sustainable food resources and genetic erosion of modern species. Einkorn (*T. monococcum*), emmer (*T. dicoccum*) and spelt (*T. Spelta*) are the oldest wheat types and are known as the ancestors of modern wheats. The need for diversification of products, increasing demand for healthy foods, therapeutic properties of foods, increasing obesity and metabolic disorders have increased the interest in the ancient wheat species (Arzani & Ashraf, 2017). Studies have shown that hulled wheats may offer healthier and better nutritional value than modern wheats (Kulathunga et al., 2020). Ancient wheat varieties are sustainable and suitable for organic agriculture (Bencze et al., 2020), and considering that it is advantageous in terms of healthy nutrition, ancient wheat varieties have gained importance. (Longin & Würschum, 2016). Many studies have been conducted on the use of ancient wheat species for food purposes and their benefits to human health, and they are still being grown in different regions of the world. However, the high productivity and other quality related properties of modern wheat varieties resulted in replacement of the ancient wheat varieties.

Emmer (*T. dicoccum*), one of the ancient wheat species, is a hulled wheat. It is called as "gernik, gacer, kavılca" in different regions among the people in Turkey (Bulut, 2022; Atak, 2017). Emmer wheat has durable hull, brittle and thin stalks. After harvest, the hull on the grain should be separated by mechanical operation. By-products such as straw and hull obtained after harvest are used as animal feed. Due to its high adaptability, emmer can be grown in areas such as Kastamonu and Sinop at an altitude of 1000-2000 meters above sea level (Köksel & Çetiner, 2015). It is also grown in rural areas of Çankırı, Kars, Kayseri and used in both human and animal feeding (Gurcan et al., 2017). It is stated in some sources that emmer wheat constitutes 1% of the total wheat production area in the world (Bilalis et al., 2017).

Emmer has gained popularity in recent years in terms of being a healthy food and traditional food products prepared with emmer are preferred in many parts of the world. Although the nutritional content of emmer wheat differs depend upon various environments, it has a rich bioactive and slower digestible starch content. (Dhanavath & Prasada Rao, 2017; Lachman et al., 2012). Emmer wheat is rich in protein (18-23%) minerals and fiber. Studies have shown that as a healthy grain, it is suitable for the diets of people with diseases such as high blood cholesterol, colitis and allergies (Marino et al., 2011). Emmer is resistance to plant diseases, pests, biotic and abiotic stress, and also has high protein quality and rich micronutrient (Zn, Mn, Fe, etc.) concentration of its seed (Zaharieva et al., 2010).

In a study on the use of emmer and spelt wheat as forage, emmer could be recommended for hay production harvested at booting stage given its valuable nutritional characteristics, whereas spelt appears to be more adaptable for silage production when harvested at the early dough stage (Cazzato et al., 2013). In an another research paper, hulled wheat has been evaluated as a new animal feed source with its features such as high organic matter digestion, CP and EE content and low ADF, NDF, methane production (Kaplan et al., 2014).

With the increase in the production amounts of ancient wheats over time, the use of these wheats and their by-products in animal nutrition may become widespread. For emmer wheat, grain constitutes approximately 30% of the total plant weight (Kaplan et al., 2014). The remaining 70% (hull, stalk) is used for animal feeding. Current study, the chemical components, energy and digestibility values of emmer wheat and its by-products in terms of feed value for ruminant animals were determined.

MATERIAL and METHODS

Material

Emmer wheat evaluated in the study was obtained from Develi district of Kayseri and it is called "gacer"

by the local people in this the region. Samples were collected during two harvest years (2019 and 2020). For the emmer plant reviewed in five parts (A-straw of emmer, B-hull of emmer, C-hulled emmer, D-naked emmer, E-flour of emmer), 5 replication analyzes were made for each sample group and each year. Samples were taken from the same field. The production process of the harvested emmer plant is schematized (Figure 1). Chemical analyzes were carried out in the feed analysis laboratory in Kayseri Yem Sanayi A.Ş. Mineral analyzes were performed at Erciyes University, Faculty of Agriculture. Pictures of the parts of the emmer plant whose nutritional values were determined are given in Figure 2.

Chemical Analyses

Dry matter (DM), crude protein (CP), ether exract (EE), crude ash (CA), crude fiber (CF), acid detergent fiber (ADF), neutral detergent fiber (NDF), acid detergent lignin (ADL), acid detergent insoluble protein (ADICP), neutral detergent insoluble protein (NDICP) and starch were analyzed. DM values were determined in an oven (~ 48 hours, 60 °C ±1) and approximately 300 g of sample was used for analysis. The dried samples were milled in a 1 mm particle size in a laboratory mill (IKA MF.10) and transferred to locked plastic bags and stored for analysis. Abbreviations of the terms above-mentioned parameters are also used in Table 1.

CP was determined by the DUMAS method (AOAC, 2006). This method, burning the sample in the furnace in the device (VELP NDA 701), reducing all nitrogen forms in it to elemental nitrogen by converting them to nitrogen oxide gases, and determining the amount of nitrogen by thermal conductivity method, multiplying this amount by the protein factor and determining the crude protein value. Crude fat analysis was performed by extraction method (ANKOM XT15) and petroleum ether was used as solvent (AOCS, 2004). Crude ash analysis was performed by burning the samples in a 550 °C ash furnace (CARBOLITE ELF 11/6) (AOAC, 2005). Crude fiber analysis was carried out based on the detection of the burning part by boiling the defatted samples first in sulfuric acid then sodium hydroxide solution and then burning the remaining mass (ISO 6865, 2000). ADF analysis was performed by boiling the sample in acid detergent solution and NDF analysis in neutral detergent solution by determining the amount of remaining mass. Acid detergent lignin analysis was carried out by determining the amount of the remaining samples after ADF analysis treated with concentrated (72%) sulfuric acid for a certain period of time (3 hours) (AOAC, 2002, 1997). ANKOM^{2000} analyzer was used for CF - ADF - NDF and ADL analyzes. For ADICP and NDICP, CP analysis was performed on the basis of the method given above from the residues resulting from ADF and NDF analysis. Starch analysis was determined by polarimetric method (ISO 10520, 1997).

For mineral content (calcium, potassium, magnesium, phosphorus, sulfur, boron, cadmium, copper, iron, manganese, nickel, lead, zinc), 0.5 g of samples were taken into vessel tubes, 10 ml HNO_3 and 2 ml HCl were added and 200 °C was subjected to 15 minute microwave (ANTON PAAR) thawing at 1600 W. After the process, it was cooled to room temperature and the 0.2 µm syringe tip was filtered, and then the mineral amounts were determined in the ICP-OS (AGILENT 5800) device (AOAC, 2009).

Calculated Parameters

Chemical analysis results were placed in the equations specified in Nutrient Requirements of Dairy Cattle (NRC, 2001), digestibility and energy parameters were calculated. These parameters are non-fiber carbohydrate (NFC), hemicellulose, digestible dry matter (DDM), dry matter intake (DMI_{BW} %, body weight of animal), relative feed value (RFV), total digestible nutrients (TDN_{1X}), metabolic energy (ME), net energy maintanance (NE_M), net energy gain (NE_G), net energy lactation (NE_{L3X}) values. The term abbreviations expressed in this section are also used in the Table 1.

Statistical Analysis

Statistical analyses were performed with Minitab 16.1 software using a completely random one-way analysis of variance (ANOVA) procedure. All data are expressed as mean and standard deviation (mean ±stdsap).

RESULTS and DISCUSSION

Emmer plant are divided into 5 parts within the scope of the study. These parts are expressed as A-straw of emmer wheat, B-hull of emmer wheat, C-hulled emmer wheat, D-naked emmer wheat, E-flour of emmer wheat. The chemical contents of these parts regarding nutrients, minerals, digestibility and energy are given Table 1. The contents of the samples with the same name varied according to the years. It has been evaluated that the results are not similar between years, and may be caused by factors such as climate and fertilization.

Straw of emmer wheat is shown Figure 1-A and analysis results are shown in Table 1. According to the results obtained, nutritional values were different on a yearly basis. Looking at the averages of both years, CP, ADF and NDF were determined as 32.7, 424.4 and 690.4 g kg^{-1}. ME and NE_{L3X} were calculated as 1.79 and 0.94 mkal kg^{-1}, respectively. The average of potassium was found to be 11.7 g kg^{-1}, higher than the other macro mineral values. In terms of trace minerals, copper, iron and zinc were higher than other trace mineral results as 60.5, 53.0, 45.5 ppm, respectively. Similar results have not been reported in previous

research studies. In one study, the ash value of emmer straw was determined as 37.6 g kg^{-1} (Wiwart et al., 2017). In the present study, the crude ash value was determined as 92.0 g kg^{-1}. For straw of modern wheat, CP, ADF, NDF, ME, potassium, copper, iron and zinc values are given as 42.0, 500.0, 775.0 g kg^{-1}, 1.63 mkal kg^{-1}, 11.2 g kg^{-1}, 4.0, 184.0 and 17.0 mg kg^{-1} in an online reference respectively (Feedipedia Animal feed resources information system, 2021). When compared to the straw of modern wheat varieties, emmer wheat straw has lower ADF and NDF values, so it was evaluated that it would be better digested by ruminants. However, more studies are needed on this subject.

Unlike modern wheats, emmer wheat remains hulled after harvest and than hulls are separated from the grain by mechanical treatment . The pictures of these hulls are shown in Figure 2-B and the analysis results are shown in Table 1. In practice, livestock farmers use these hulls in animal feeding, but they do not know their nutritional value. The nutritional value of hulls varied significantly depend on the year of harvest.

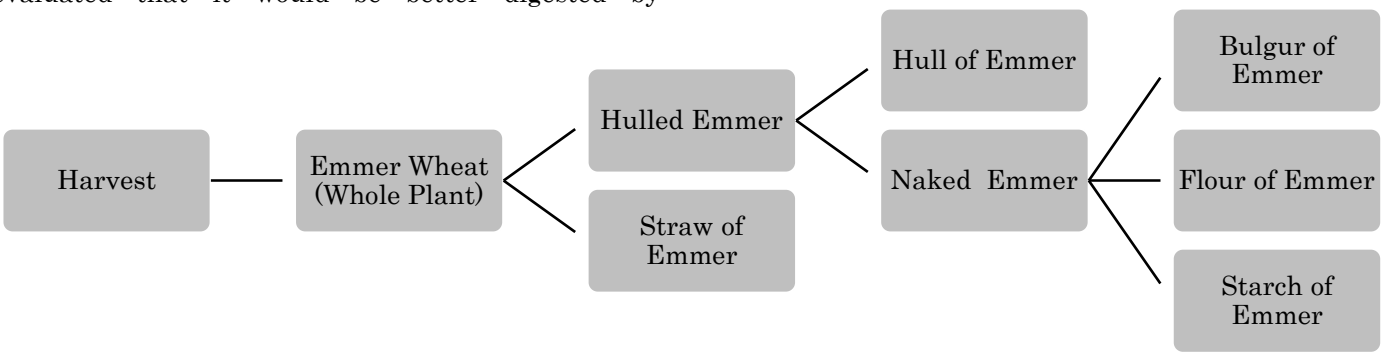

Figure 1. Harvest and process stage process of emmer wheat
Şekil 1. Gernik buğdayının hasat ve işleme süreci

Figure 2. Images of emmer wheat and by-products. A- Straw, B- Hull, C- Hulled emmer, D- Naked emmer, E- Flour of emmer, F- Emmer spike
Şekil 2. Gernik buğdayı ve yan ürünlerinin resimleri. A-Sap, B-Kavuz, C-Kavuzlu gernik, D-Kavuzsuz gernik, E-Gernik unu, F-Gernik başağı

Table 1. Emmer wheat and by-products nutritional value*
*Çizelge 1. Gernik buğdayı ve yan ürünlerinin besinsel değerleri**

	A - Straw	B – Hull	C – Hulled Emmer	D – Naked Emmer	E – Flour of Emmer
Chemical analyzes					
Dry Matter (g kg^{-1})	937.4 ±15.38	947.6 ±5.11	917.7 ±4.36	908.0 ±2.70	910.6 ±5.61
Crude Protein (g kg^{-1})	32.7 ±17.85	40.1 ±14.98	122.8 ±14.80	159.6 ±7.22	159.3 ±1.28
Ether Exract (g kg^{-1})	17.2 ±3.67	17.0 ±1.56	16.0 ±6.92	13.8 ±5.78	20.0 ±4.67
Crude Ash (g kg^{-1})	92.0 ±28.59	123.2 ±19.12	39.3 ±1.07	17.9 ±2.52	19.9 ±1.10
Starch (g kg^{-1})	3.5 ±1.54	53.3 ±41.60	511.2 ±11.52	666.1 ±10.76	663.6 ±9.69
ADF (g kg^{-1})	424.4 ±37.90	434.8 ±18.19	116.5 ±3.76	25.9 ±1.56	19.7 ±3.76
NDF (g kg-1)	690.4 ±79.11	707.5 ±58.88	218.9 ±2.87	62.5 ±4.92	56.5 ±2.20
ADICP (g kg-1)	4.6 ±2.66	4.8 ±1.74	7.9 ±1.11	8.3 ±1.97	8.9 ±1.47
NDICP (g kg-1)	11.0 ±1.96	12.7 ±3.51	15.9 ±3.35	17.9 ±1.69	18.0 ±1.47
Crude Fiber (g kg-1)	363.4 ±52.88	326.4 ±12.36	94.4 ±3.54	21.3 ±6.77	16.4 ±2.45
Lignin (g kg-1)	61.4 ±5.83	80.9 ±4.02	17.1 ±2.93	5.8 ±1.07	4.8 ±1.09
Mineral values					
Calcium (g kg^{-1})	2.6 ±0.10	2.5 ±1.08	1.4 ±0.04	1.51 ±0.04	1.5 ±0.76
Potassium (g kg^{-1})	11.7 ±0.13	7.3 ±0.34	5.3 ±0.74	5.65 ±1.94	4.8 ±0.54
Magnesium (g kg^{-1})	0.9 ±0.21	0.9 ±0.09	1.0 ±0.16	1.36 ±0.46	1.1 ±0.24
Phosphorus (g kg^{-1})	0.4 ±0.20	0.5 ±0.11	1.1 ±0.11	1.89 ±0.81	1.7 ±0.33
Sulfur (g kg^{-1})	1.0 ±0.25	0.8 ±0.08	1.0 ±0.11	1.22 ±0.13	1.0 ±0.22
Boron (mg kg^{-1})	6.5 ±1.72	5.0 ±2.21	6.5 ±6.92	12.5 ±5.89	3.5 ±3.72
Cadmium (mg kg^{-1})	0.0 ±0.00	0.0 ±0.00	0.0 ±0.00	0.0 ±0.00	0.0 ±0.00
Chromium (mg kg^{-1})	1.5 ±0.71	2.0 ±0.47	0.5 ±0.71	0.5 ±0.71	0.0 ±0.00
Copper (mg kg^{-1})	4.5 ±0.85	4.0 ±0.67	6.0 ±1.25	7.5 ±1.90	6.5 ±1.72
Iron (mg kg^{-1})	60.5 ±15.62	115.0 ±67.58	46.5 ±20.76	56.0 ±7.04	55.5 ±23.07
Manganese (mg kg^{-1})	53.0 ±24.49	45.5 ±7.69	33.0 ±5.01	37.5 ±14.55	32.0 ±6.24
Nickel (mg kg^{-1})	1.5 ±0.53	2.0 ±0.67	2.0 ±0.67	1.5 ±0.85	1.5 ±0.85
Lead (mg kg^{-1})	1.0 ±0.00	0.5 ±0.53	0.0 ±0.00	2.5 ±1.65	2.0 ±2.16
Zinc (mg kg^{-1})	45.5 ±3.54	62.0 ±7.76	78.0 ±4.16	109.0 ±17.68	103.0 ±58.20
Calculated parameters					
NFC (g kg-1)	167.8 ±36.37	112.3 ±61.93	603.2 ±9.74	746.3 ±15.38	744.4 ±3.84
Hemicellulose (g kg^{-1})	266.0 ±42.07	272.7 ±41.20	102.4 ±5.57	36.7 ±5.39	36.8 ±5.37
DDM (g kg^{-1})	558.4 ±29.52	550.3 ±14.17	798.2 ±2.93	868.9 ±1.22	873.7 ±2.93
DMI_{BW} %	1.76 ±0.20	1.71 ±0.14	5.5 ±0.07	19.31 ±1.55	21.27 ±0.81
RFV (score)	76.5 ±12.72	72.9 ±7.88	339.3 ±4.10	1300.6 ±104	1440.2 ±52
TDN_{1X} (g kg^{-1})	520.0 ±20.86	453.7 ±42.53	783.5 ±11.75	867.3 ±6.58	874.3 ±7.57
ME (Mkal kg^{-1})	1.79 ±0.07	1.52 ±0.20	3.04 ±0.03	3.44 ±0.04	3.47 ±0.03
NE_{L3X} (Mkal kg^{-1})	0.94 ±0.04	0.77 ±0.13	1.75 ±0.02	2.01 ±0.02	2.03 ±0.02
NE_M (Mkal kg^{-1})	0.95 ±0.07	0.67 ±0.20	2.06 ±0.02	2.39 ±0.03	2.41 ±0.02
NE_G (Mkal kg^{-1})	0.40 ±0.06	0.14 ±0.19	1.40 ±0.02	1.67 ±0.02	1.69 ±0.02

*Results are given on dry matter basis, for each group n=10

When the average of both years is evaluated, CP, ADF, NDF, ME, NE_{L3X} values were determined as 40.1, 434.8, 707.5 g kg^{-1}, 1.52, 0.77 mkal kg^{-1}, respectively. It has been observed that the levels of iron, manganese and zinc (115.0, 45.5 and 62.0 mg kg^{-1}) in terms of mineral are higher than the other minerals. No information has been found in the literature regarding the nutritional values of emmer wheat hulls. As a result, hulls can be used as forage feed.

Hulled emmer wheat appears as a different product when evaluated in terms of animal nutrition. The combination of both structural and non-structural carbohydrates for hulled emmer wheat may be of interest to animal nutritionists. Hulled emmer wheat is shown in Figure 2-C and its nutritional values are given in Table 1. CP, starch, ADF, NDF, TDN_{1X}, ME, NE_{L3X} were determined as 122.8, 511.2, 116.5, 218.9 and 783.5 g kg^{-1}, 3.04 and 1.75 mkal kg^{-1}, respectively. In terms of mineral matter, the levels of iron, manganese and zinc (46.5, 33.0 and 78.0 mg kg^{-1}) were higher than the other trace mineral values. In a study

for hulled emmer wheat, CP, NDF, EE, CA were found as 104.4, 273.2, 18.1 and 4.64 g kg^{-1} in the given order (Zaharieva et al., 2010). Although the hulled emmer is similar in terms of protein content compared to modern wheat but it has less starch content and higher fiber content. In terms of nutritional value of hulled emmer wheat is more similar to barley and it can be used as a combined feed as a source of both fiber and starch. It this sense, further studies should be done on hulled emmer wheat.

Hulless emmer wheat is referred to as naked emmer wheat and is the final product for food. Naked emmer wheat is shown in Figure 2-D and nutritional results are given in Table 1. Analysis results differed according the years. CP, starch, ADF, NDF, TDN_{1X}, ME, NE_{L3X} levels were determined as 159.6, 666.1, 25.9, 62.5 and 867.3 g kg^{-1}, 3.44 and 2.01 mkal kg^{-1}, respectively. In terms of mineral levels, iron, manganese and zinc (56.0, 37.5 and 109.0 mg kg^{-1}) were found higher than other trace mineral values. Calcium, potassium, magnesium and phosphorus values were determined as 1.51, 5.65, 1.36 and 1.89 g kg^{-1}, respectively. In open feed library sources (Dairy One, 2021), the average CP, starch, ADF, NDF, TDN_{1X} values of modern wheat varieties are reported as 135.3, 617.4, 46.5, 129.9 and 838.8 g kg-1, ME and NE_{L3X} values are 3.28 and 1.91 mkal kg^{-1}, respectively. In the same the reference, calcium, potassium, magnesium, phosphorus, sulfur values are expressed as 0.91, 4.44, 1.32, 3.69, 1.48 g kg^{-1}, respectively. In terms of trace mineral, copper, iron, manganese, nickel and zinc were reported as 4.99, 87.58, 41.51 and 30.99 mg kg^{-1}, respectively. In an another reference (Feedipedia, 2021), modern wheat varieties' CP, EE, CA, starch ADF, NDF, CF, lignin are reported 126.0, 17.0, 18.0, 691.0, 36.0, 139.0, 26.0 and 11.0 g kg^{-1} respectively. ME value is 3.13 Mkal kg^{-1}. Calcium, potassium, magnesium, phosphorus 0.7, 4.6, 1.2, 3.6 g kg^{-1} and copper, iron, manganese, zinc are reported as 6.0, 78.0, 40.0 and 31.0 mg kg^{-1}, respectively. When compared with modern wheat grain, protein value of naked emmer wheat was higher, ADF and NDF values were lower, starch value was similar, and ME value was higher. Compared to modern wheat varieties, the mineral content of naked emmer wheat in general is higher, and this is more evident especially in terms of calcium and zinc. In some studies, it has been stated that emmer wheat has high nutritional value (Dhanavath & Prasada Rao, 2017). It has been determined that emmer wheats contain more selenium, iron and zinc compared to other types (Suchowilska et al., 2012). The high value of zinc was similar to the current study.

In one study (Dhanavath & Prasada Rao, 2017), it was determined that the starch of emmer wheat changed between 485.0 and 653.0 g kg^{-1}. In the present study the starch was found to be slightly higher (666.1 g kg^{-1}) than the above mentioned study. In an another study, the starch of emmer wheat was found to be 659.0 g kg^{-1} and it was stated that this value was higher than other ancient wheats (einkorn, spelt) and modern wheats (Kulathunga et al., 2021). These results are similar with our study. In a study examining starch structure in terms of amylose and amylopectin (Dhanavath & Prasada Rao, 2017), amylose content varies between 19.4% and 26.3%. This content is important in terms of starch digestion and the high rate of amylopectin increases the digestive resistance of starch (Singh et al., 2010; Suchowilska et al., 2012). Starch digestion of emmer wheat in *in vitro* studies has been reported to be 40.39 - 47.07 mg glucose/100 g. This results are lower than other cereals (Bhuvaneswari et al., 2004). The slow rate of starch breakdown can be considered as an advantage for ruminants.

In an article in which different studies are compiled, it was stated that the protein ratio in emmer wheat changed between 112.0 and 227.0 g kg^{-1} (Dhanavath & Prasada Rao, 2017). In another study emmer wheat protein was found as 182.0 and 184.0 g kg^{-1} (Gurcan et al., 2017). The study of Biel et al., (2021) CP, EE, CA, CF value of emmer wheat were determined as 154.0, 21.7, 21.6, 50.3 g kg^{-1} respectively. In a paper on the comparison of ancient and modern wheat CP, CA and EE values of emmer wheat were found to be 145.0 - 22.0 - 21.0 g kg^{-1} respectively (Kulathunga et al., 2021). In our study, CP (159.6 g kg^{-1}) is similar with the results of other studies and EE, CA, CF values were determined as 13.8, 17.9, 21.3 g kg^{-1} respectively.

Within the scope of the study, the analyses of emmer wheat flour were also made. In the process of making flour from naked emmer wheat, bran and similar by-products are not separated. When flour is made from modern wheat, by-products such as bran and bonkalit are obtained, whereas in the production of flour from naked emmer wheat, no by-products are formed. In brief, emmer wheat flour is the milled form of naked emmer grains. For this reason, the flour analyzes are similar to the naked emmer wheat analysis results. Analysis results of emmer wheat flour Table 1, its picture is shown in Figure 2-E.

It has been seen in most studies that there is no clear explanation about whether emmer wheat is evaluated as hulled or unhulled. In our present study, the nutritional values of emmer wheat with and without hull were tried to be expressed clearly.

CONCLUSION

The nutritional values of emmer wheat and its by-products vary depending on different factors as in other feeds. The hull of emmer wheat is a different product for feeding animals. Emmer's stalk is thinner and brittle, and its ADF and NDF values are lower than other feeds in the similar category, which can be

a positive point in terms of forage quality. The protein content of emmer wheat has been found to be higher than modern wheat. It is thought that further studies should be performed especially on the resistant starch content and this is an advantageous situation in ruminant feeding. Hulled emmer wheat, the combination of both structural and non-structural carbohydrate content in ruminant feeding (both forage and concentrate) may be of interest to animal nutritionists. Nutrient changes in feeds within their own species are an expected situation. In this study, according to the years variability was observed in the nutritional content of the same type of feed. This change may have been caused by factors such as soil and climatic conditions, fertilization, planting and harvesting time. It is considered that more studies should be done *in vivo* and *in vitro* on emmer wheat as feed. With the increasing interest in ancient wheat species in future, amount of production will increase and the use of these wheats and especially by-products as animal nutrition will become widespread.

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Statement Contribution of the Author

All of this work was done by the author.

Conflict of Interest

The author declared that there is no conflict of interest.

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Karasal İklim Şartlarına Adaptasyon Sürecindeki Bafra Kuzularda Büyüme ve Yaşama Gücü

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ÖZET
Bu çalışma, karasal iklim koşullarında Bafra kuzularda yaşama gücü ve büyüme özelliklerinin incelenmesi amacıyla yapılmıştır. Araştırmada 179 baş Bafra kuzunun büyümenin çeşitli dönemlerinde yaşama gücü, büyüme özellikleri ve bazı vücut ölçüleri incelenmiştir. Büyüme ve vücut ölçülerinin analizinde En Küçük Kareler, yaşama gücünün analizinde Ki-Kare yöntemlerinden yararlanılmıştır. Kuzuların 30. ve 90. gün yaşama gücü sırasıyla %96.65 ve 90.50 olmuştur. Yaşama gücüne doğum tipi ve cinsiyetin etkisinin önemsiz (P>0.05) olduğu belirlenmiştir. Kuzuların doğum, 30., 60. ve 90. gün canlı ağırlıkları yönünden en küçük kareler ortalamaları sırasıyla 3.58±0.541, 8.10±0.127, 12.71±0.223 ve 18.08±0.326 kg olarak bulunmuştur. Kuzu canlı ağırlıklarına incelenen çevre faktörlerinden cinsiyetin etkisin önemsiz (P>0.05), doğum tipinin etkisinin ise 60. güne kadar yüksek düzeyde önemli olduğu (P= 0.000), 60. günde ve sütten kesim döneminde (90. gün) ise tek doğanlarda canlı ağırlıklar rakamsal olarak yüksek olsa da doğum tipinin canlı ağırlığa etkisinin önemsiz (P>0.05) olduğu hesaplanmıştır. Kuzularda sütten kesim öncesi (ortalama 71. gün) cidago yüksekliği, göğüs derinliği, vücut uzunluğu, göğüs çevresi, incik çevresi ve kuyruk çevresi ölçüleri sırasıyla 47.86±0.615, 18.52±0.246, 45.68±0.840, 56.47±0.881, 6.50±0.075, ve 12.01±0.502 cm olmuştur. İncelenen bu vücut ölçülerine doğum tipi ve cinsiyetin etkisi önemli (P>0.05) olmamıştır. Sonuç olarak karasal iklim koşullarında yetiştirilen Bafra kuzuların büyüme ve yasama gücü özelliklerinin iyi düzeyde olduğu tespit edilmiştir.

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Anahtar Kelimeler
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Uyum Kabiliyeti
Büyüme
Yaşama Gücü
Step İklim Koşulları

The Growth and Survival of Bafra Lambs During Adaptation to Steppe Climate Conditions

ABSTRACT
This study investigates the survival and growth characteristics of Bafra lambs born and reared under steppe climate conditions. The survival, growth and certain body measurements of 179 head of Bafra lambs were observed. Least Squares Method was applied for the analysis of growth and body measurements, and Chi square test was used for the analysis of survival rates. The survival rates of the lambs on days 30 and 90 were 96.65% and 90.50%, respectively. Birth type and sex had no significant effect on survival rates (P>0.05). The live weights of the lambs on days birth, 30, 60 and 90th were 3.58±0.541, 8.10±0.127, 12.71±0.223 and 18.08±0.326 kg, respectively. Among the environmental factors affecting the live weights of the lambs, the effect of sex was not significant (P>0.05), while the effect of birth type was highly significant (P=0.000) up until day 60, but decreased thereafter. Furthermore, the effect of birth type on live weight was not significant (P>0,05) in single lambs on day 60 and 90th, even though the live weights were numerically high. The pre-weaning (average 71 day) withers height, chest depth, body length, chest girth, cannon bone circumference and tail circumference of the lambs were 47.86±0.615, 18.52±0.246, 45.68±0.840; 56.47±0.881, 6.50±0.075, and 12.01±0.502 cm,

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respectively. The effect of birth type and sex on these body measurements were not significant (P>0.05). It was concluded that the growth and survival characteristics of Bafra lambs reared under the steppe conditions were at a satisfactory level.

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INTRODUCTION

There are sheep breeding in various parts of the world in terms of importance and the type of production. It is worthy of note, however, that the common purpose is meat production, and there have been many studies investigating approaches to increase fertility characteristics, given the importance for meat production. As a result of these improvement studies, the growth, development and fertility characteristics of sheep breeds have been improved in developed countries (Akçapınar, 2000; Ünal, 2002).

In Turkey, sheep breeding is a form of livestock husbandry that is generally practiced extensively with large flocks. Most of the sheep breeds that are reared are dual-purpose and their fertility characteristics and milk production are low (Akçapınar, 2000). As a result, many foreign breeds have been introduced to the country to improve the production characteristics of native breeds, and crossbreeding has been carried out to this end, although the popularity of the obtained genotypes, excluding the Turkish Merino, Anatolian Merino, Tahirova and Ramlıç breeds, has remained low among local breeders. According to 2019 data, Merino sheep and their crossbreeds account for only 8.2% of the total sheep population in Turkey (TURKSTAT, 2020). This has been attributed to the extensive nature of sheep breeding, in other words, their pasture-dependency – local breeds being well adapted to the conditions in the region in which they are reared – and the preferences of breeders. For this reason, it is necessary to determine the production characteristics of Bafra sheep in different parts of Turkey, being a crossbreed genotype (75% Chios and 25% Karayaka) that is gaining popularity among local breeders as a breed with high fertility and meat quality as well as high milk production.

The crossbreeding studies of the Bafra breed, conducted by the Ankara University Faculty of Veterinary Medicine Department of Animal Breeding and Husbandry in cooperation with the Ministry of Agriculture and Forestry, began in 1982 at the Samsun Karaköy SF (State Farm). These studies investigated the crossbreeding of Chios sheep, which are not suited to steppe conditions, with Karayaka sheep, and the resulting Bafra breed was registered on behalf of the General Directorate of Agricultural Enterprises in 2010 (Akçapınar et al., 2002; Akçapınar & Ünal, 2011).

The vitality trait can be assessed in two periods, namely gestation and birth. The gestation period, as well as the period between birth and weaning, are important times when deaths affect the lambing and weaning rate, as the most important fertility criterion in breeding. The survival rate is the ratio of living lambs at a certain period to the number of lambs born alive (Akçapınar & Özbeyaz, 1999; Akçapınar, 2000).

Growth refers to the increase in the number of tissue cells and is investigated in two main periods – namely before and after birth. The process depends on genotype and continues until 2–3 years of age (Akçapınar, 1978; Akçapınar and Özbeyaz, 1999). Birth weight is an outcome of many factors during the gestation period, such as maternal age, maternal nutrition, genotype, birth type and sex, while growth after birth is greatly influenced by maternal milk production as well as the caring and feeding conditions (Akçapınar, 1978; Akçapınar & Özbeyaz, 1999; Akçapınar, 2000).

The Bafra genotype, known for its high milk production and fertility, was obtained under sea climate conditions. Studies of the breeding performance of Bafra ewes have been conducted in different regions, such as the Gökhöyük SF in the Amasya province (Ünal et al., 2003; Akçapınar et al., 2005), the Lalahan Livestock Research Centre (Ankara province) (Akçapınar et al., 2002), the Çubuk district of Ankara province by local farmers (Güngör & Akçapınar, 2013), the Kazım Karabekir SF in Iğdir province (Işık & Aksoy, 2015a; Işık & Aksoy, 2015b) and the village of Elmalı in Niğde province by local farmers (Yerlikaya & Ulutaş, 2019). Given the preference for the Bafra breed among breeders in different regions, it is necessary to investigate the performance of the breed in different regions. From this point of view, aim of this study was to determine the survival and growth characteristics of Bafra lambs reared under steppe climate conditions.

MATERIALS and METHOD

The farm located in 38° 29' N and 32° 27' E, 1020 m of altitude Konya province in the central Anatolia region

of Turkey and have steppe climate conditions.

Animals

The study was conducted under State Farm conditions in the Konya province with 500 Bafra sheep brought from the Central Black Sea region. Totally 380 ewes were exposed to rams, and 287 ewes have given birth in this season in this study. The survival and growth characteristics of 179 head of Bafra lambs born from 97 ewes (higher than 3 years old) in the second birth season on the state farm were examined. Body measurements were determined from 42 lambs.

Method

For per ewe, 1.5 kg roughage and 0.8 kg concentrate feed (16% CP and 2600 kcal kg^{-1} ME) was given in the last 1.5 month of gestation. However, the ewes had multiple birth had an additional 0.4 kg concentrate feed after delivery. Birth weights were measured 10–18 hours after birth, and live weights controls have started around 15 days after the births started. Live weight gain and lamb mortalities were monitored at 30-day intervals, from the birth season until 180 days. Birth weights were determined using a 1 g precision scale. Other live weights were made at 30-days intervals, using a 50 g precision scale. The wither height, chest depth, body length, chest girth, cannon bone circumference, and tail's widest circumference of the lambs were measured at an average of 71 and 206 days old. The survival rate was the ratio of the number of living lambs on days 30 and 90 (weaning) to the number of lambs born alive. The live weights of the lambs on days 30, 60, 90 and 180 were determined using an interpolation method (Akçapınar et al., 2005).

First of all, it should be noted that sufficient care and feed were provided for the sheep giving birth to multiple lambs and their lambs during the birth season. For this reason, the number of people working on the farm has increased in this period. The lambs were kept with their mothers in individual pens for 1–2 days after birth and then they were included in the groups occurred newborn lambs and their dams. Multiple born lambs were provided bottle milk obtained from sheep high milk yield. In addition, some of these lambs were suckled from mothers giving birth to a dead lamb or mothers with high milk production. Lambs and their dams are kept in this group for 10–15 days were then taken out of these groups and included in larger groups. In this period, groups of lambs were joined with the groups of mothers every morning and evening, and the lambs were allowed to suckle their mothers twice a day. In these groups, the lambs started to consume high quality alfalfa hay and lamb starter feed (18% CP and 2800 kcal kg^{-1} ME) ad libitum. The milking of the sheep began on around day 45, and the lambs started being fed an average total of 500 g concentrated feed (16% CP 2500 and kcal kg^{-1} ME) every morning and evening. The lambs began to be introduced to the pasture with their mothers after the start of milking, and the lambs were weaned on around day 90.

Statistical Analyses

The Least Squares Method was used for the comparison of the live weights and body measurements of the lambs and the effect of birth type and sex. A Chi square test was used to compare the survival rates of lambs. A Tukey's test was applied for the comparison of significance within more than two groups for the effect of birth type, which was investigated in four groups. SPSS software was used for the statistical analyses (SPSS Software 2005).

RESULTS and DISCUSSION

Bafra sheep were a crossbreed 75% Chios and 25% Karayaka native sheep breeds, and known for their impressive high fertility, meat quality and milk production characteristics. The growing preference for the breed among breeders means it is highly important to determine the adaptation and production characteristics of the breed under Turkey's different climate and farm conditions.

Konya region, with its steppe climate, its Turkey's largest agricultural area, and is home to the largest proportion of livestock in the country. Accordingly, there is a need to assess the adaptation of the Bafra breed to steppe conditions as part of a larger study of its potential.

Lamb Survival Rates

Lamb survival was determined during the suckling period, and the rates based on birth type and sex are presented in Table 1. The effects of birth type and sex on survival in lambs were not significant, although the survival rate of female lambs was higher than for male lambs.

A comparison of the survival rates recorded in the present study with those of previous studies of Bafra lambs is presented in Figure 1.

In this study, the survival rate on day 30 (96.65%) was slightly lower than the rate found in 2005 at Gökhöyük SF (Akçapınar et al., 2005) and higher than the rates found in other studies involving the Bafra genotype (Figure 1). The average survival rate of lambs on day 90 was 90.50% in this study. This rate was slightly lower than the rates found during the same period in the two studies conducted at Gökhöyük SF (95.1% and 91.9%) (Ünal et al., 2003; Akçapınar et al., 2005) and the rate found on 140

days in Niğde province by local farmers (90.7%) (Yerlikaya & Ulutaş, 2019). It was, however, much

higher than the survival rates on day 90 found in the studies conducted in the Çubuk district of Ankara province, Kazım Karabekir SF and the Lalahan Livestock Research Institute in Ankara province (Akçapınar et al., 2002; Güngör & Akçapınar, 2013; Işık & Aksoy, 2015b).

Table 1. Survival rates of the lambs
Çizelge 1. Kuzularda yaşama gücü

Item	Number of live lambs			Survival rate (%)	
	Birth	30th day	90th day	30th day	90th day
Birth type					
Single	29	28	25	96.55	86.21
Twin	108	105	99	97.22	91.67
Triplet	32	30	29	93.75	90.63
Quad	10	10	9	100.00	90.00
P-Value				0.732	0.850
Sex					
Female	77	76	72	98.70	93.51
Male	102	97	90	95.10	88.24
P-Value				0.18	0.234
Means	179	173	162	96.65	90.50

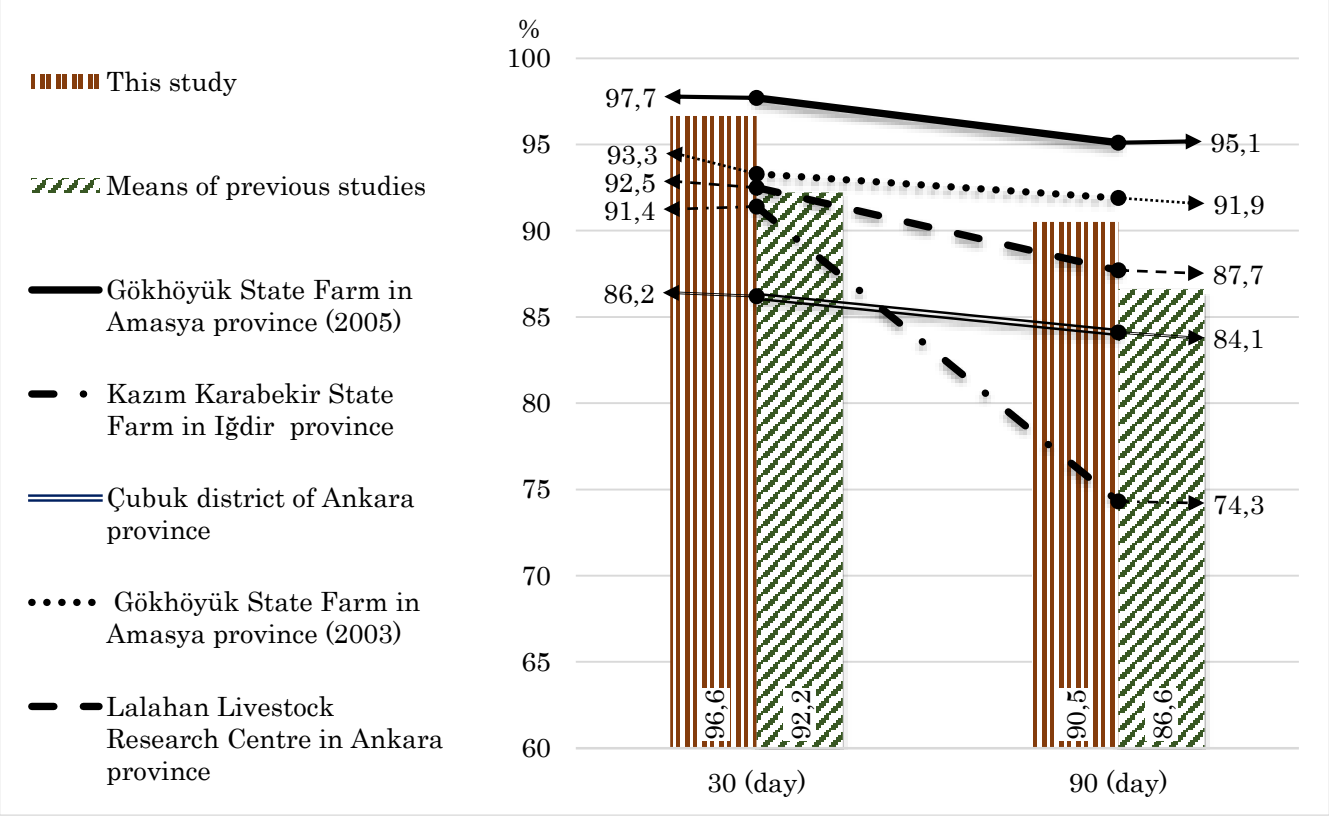

Figure1. Comparison of the survival rates recorded in the present study with those of previous studies (Akçapınar et al., 2002; Ünal et al., 2003; Akçapınar et al., 2005; Güngör & Akçapınar, 2013; Işık & Aksoy, 2015b)

Şekil 1. Bu çalışmada elde edilen yaşama gücü oranlarının daha önce yapılmış çalışmalarda elde edilen sonuçlarla karşılaştırılması (Akçapınar ve ark., 2002; Ünal ve ark., 2003; Akçapınar ve ark., 2005; Güngör & Akçapınar, 2013; Işık & Aksoy, 2015b)

The effect of birth type on survival rate was found to be insignificant. This result was in line with the results of studies conducted at Kazım Karabekir SF (Işık & Aksoy, 2015b) and the Lalahan Livestock Research Institute in Ankara province (Akçapınar et al., 2002) but different from the results of other studies concerning the Bafra genotype. The fact that the effect of birth type on survival is insignificant can

be attributed to caring and feeding opportunities, such as being able to bottle feed twin lambs with milk obtained from sheep with high milk production or allowing twin lambs to suckle from mothers that have given birth to a dead lamb or mothers with high milk production. This is because the number of shepherds that work at the enterprise increases during the birth season. Usually, single-born lambs are expected to have a higher chance of survival. In this study, the survival rate of twin-born lambs on day 90 was not statistically significant, but higher than single-born lambs. This result was also noted in the study conducted in Niğde province with the Bafra lambs (Yerlikaya & Ulutaş, 2019). The reason for this is that the milking process begins at around day 45 in sheep (this will cause the milk intake of lambs to be lower) and it could be said that this is caused by the stress that occurs due to weaning in lambs (day 90). It can also be said that single-born lambs are more dependent on their mothers compared to twin-born lambs and are more affected by these situations.

The effect of sex on survival was found to be insignificant in this study. The survival rate on day 30 was found to be different in male lambs in one study and different in female lambs in the other, between two studies conducted at Gökhöyük SF (Ünal et al., 2003; Akçapınar et al., 2005), where both differences were statistically significant. Other than these two studies, in other studies involving the Bafra lambs it was found that sex did not have a significant effect on survival in male lambs or female lambs. However, there are reports that the survival rate is higher for female lambs in general. The survival rate of female lambs was also high in this study but the difference between male lambs and female lambs in terms of survival was not significant.

Growth Traits of Lambs

Mean values regarding the birth and growth characteristics found in the study are given in Table 2. Effect of birth type on birth weight and live weights of lambs on day 30 was found to be highly significant (P= 0.000). However, this level of significance decreased on day 60 (P= 0.059) and differences between live weights in terms of birth type was found insignificant on day 90 (P= 0.309). The effect of sex on live weights is not significant. Even though male lambs have higher birth weights than female lambs, this difference is not considered statistically significant. The male lambs and female lambs had similar mean live weights on 30, 60 and 90 days.

A comparison of growth characteristic data recorded in the present study with those of studies of Bafra lambs conducted in different parts of Turkey is presented in Figure 2.

Table 2. Least Squares means (± SE) of the lamb live weights at different ages (kg)
Çizelge 2. Kuzu canlı ağırlıklarının farklı yaşlardaki en küçük kareler ortalamaları (± SE), (kg)

	Birth weight		30th day		60th day		90th day		180th day	
Item	n	$\overline{X}\pm S_{\overline{X}}$	n	$\overline{X}\pm S_{\overline{X}}$	n	$\overline{X}\pm S_{\overline{X}}$	n	$\overline{X}\pm S_{\overline{X}}$	n	$\overline{X}\pm S_{\overline{X}}$
Birth type										
Single	29	4.00±0.119^{b}	28	9.03±0.299^{c}	28	13.66±0.536^{b}	25	18.71±0.812	17	23.20±0.972
Twin	108	3.65±0.062^{b}	105	8.08±0.156^{bc}	104	12.60±0.282^{ab}	99	17.78±0.410	52	21.78±0.531
Triplet	32	3.09±0.115^{a}	30	7.54±0.291^{ab}	30	12.38±0.523^{ab}	29	18.44±0.760	13	21.45±1.065
Quad	10	2.86±0.206^{a}	10	6.87±0.511^{a}	9	10.81±0.952^{a}	9	15.96±1.352	6	21.97±1.563
P-Value		0.000		0.000		0.059		0.309		0.581
Sex										
Female	77	3.29±0.104	76	7.83±0.257	75	12.40±0.460	72	17.50±0.660	46	21.23±0.760
Male	102	3.51±0.088	97	7.94±0.222	96	12.33±0.418	90	17.95±0.611	42	22.87±0.790
P-Value		0.100		0.737		0.912		0.616		0.196
Means	179	3.58±0.541	173	8.10±0.127	171	12.71±0.223	162	18.08±0.326	88	22.07±0.548

The average birth weight (3.58 kg) of the lambs in the present study was slightly lower than birth weight reported in the two studies conducted at Gökhöyük SF (3.9 and 3.7 kg) (Ünal et al., 2003; Akçapınar et al., 2005), and slightly higher than those reported in the studies at Kazım Karabekir SF (3.2 kg) (Işık & Aksoy, 2015a), the Lalahan Livestock Research Institute in Ankara province (3.3 kg) (Akçapınar et al., 2002), the Çubuk district of Ankara province (3.2 kg) (Güngör & Akçapınar, 2013) and the village of Elmalı in Niğde province (3.2 kg) (Yerlikaya & Ulutaş, 2019).

The effect of birth type on the birth weight of the lambs was found to be highly significant (P= 0.000), while the effect of sex on birth weight was found to be statistically insignificant, although male lambs had higher birth weights than female lambs. This was an expected result and concurred with the results of other studies of the Bafra lambs.

The effect of birth weight on live weight until day 60 was highly significant (P= 0.000), while the level of significance decreased on day 60 (P= 0.059) and the effect of birth type on live weight became insignificant on day 90. Milking began in the enterprise

approximately 45 days after birth, and at this time the lambs were introduced to the pasture with their mothers after milking. It can be said that this has a greater impact on the single-born lambs, in that a higher mortality was recorded between days 30 and 90 in single-born lambs when the survival rates on days 30 and 90 were assessed according to birth type.

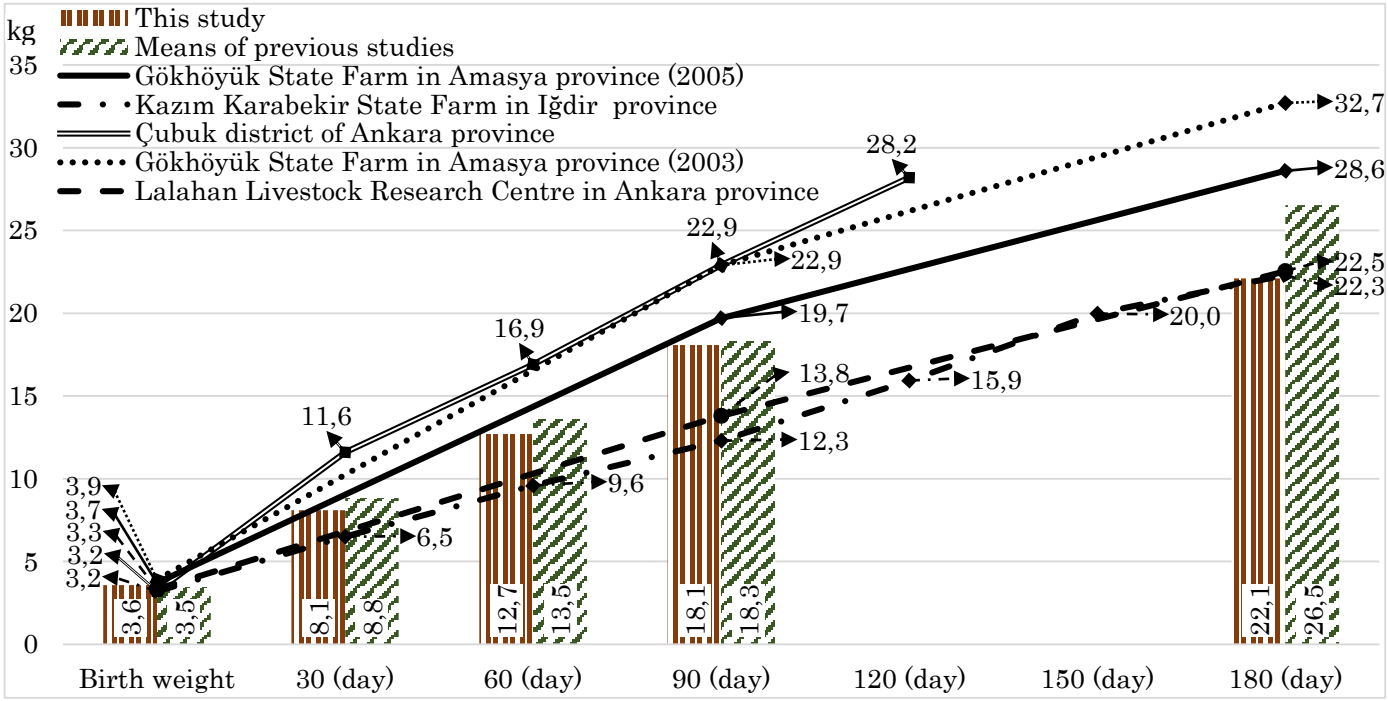

Figure 2. Comparison of the growth traits found in this study with previous studies (Akçapınar et al., 2002; Ünal et al., 2003; Akçapınar et al., 2005; Güngör & Akçapınar, 2013; Işık & Aksoy, 2015a)

Şekil 2. Bu çalışmada elde edilen büyüme özelliklilerinin daha önce yapılmış çalışmalarda elde edilen sonuçlarla karşılaştırılması (Akçapınar ve ark., 2002; Ünal ve ark., 2003; Akçapınar ve ark., 2005; Güngör & Akçapınar, 2013; Işık & Aksoy, 2015a).

The average live weights (18.71 kg) on the weaning (day 90) of the lambs in the present study was slightly lower than those reported in the two studies conducted at Gökhöyük SF, and in the study in the Çubuk district of Ankara province (22.9, 19.7 and 22.9 kg, respectively) (Akçapınar et al., 2002; Ünal et al., 2003; Güngör & Akçapınar, 2013), and higher than the results recorded in the studies at the Kazım Karabekir SF (12.3 kg) and the Lalahan Livestock Research Institute (13.8 kg) (Akçapınar et al., 2002; Işık & Aksoy, 2015a).

When the average live weights of the lambs was assessed on day 180, 22.07 kg recorded in the present study was lower than that found during the same period in the two studies conducted at Gökhöyük SF (32.7 and 28.6 kg) (Ünal et al., 2003; Akçapınar et al., 2005) and the value recorded on day 150 of the study in the Çubuk district of Ankara (28.2) (Güngör & Akçapınar, 2013), but similar to the values recorded in the studies at Kazım Karabekir SF (22.3 kg) (Isik, 2015) and the Lalahan Livestock Research Institute in Ankara province (22.5 kg) (Akçapınar et al., 2002). Lamb growth up to day 90 was slightly lower than, or similar to the results of the two studies at Gökhöyük SF (Ünal et al., 2003; Akçapınar et al., 2005), but lower on day 180, which could be attributed to the fact that the pastures of the farm were not as high quality as those of the Gökhöyük SF.

Body Measurements of Lambs

The mean values of various body measurements determined in different growth periods according to birth type and sex are presented in Table 3. The effects of birth type and sex on body measurements were not found to be significant.

On around day 71, the wither heights and body lengths of the lambs were 47.86 and 45.68 cm. The Bafra lambs' values on 90 days in the studies of the Lalahan Livestock Central Research Institute in Ankara province (48.90 and 49.52 cm) (Akçapınar et al., 2002) and in the Kazım Karabekir SF (46.61 and 48.44 cm) (Işık & Aksoy 2015a) were similar to those in the present study, but lower than the values found in the study conducted in the Çubuk district of Ankara province (55.08 and 56.68 cm) (Güngör & Akçapınar, 2013). This was anticipated taking into consideration the age when the body lengths were determined and the live weights reported in the studies, as the results in the present study were recorded on around 71days ages.

Table 3. The least square means (±SE) of the lamb body measurements at different ages (cm)
Çizelge 3. Kuzu vücut ölçülerinin farklı yaşlardaki en küçük kareler ortalamaları (± SE), (cm)

Age (day)		Mean: 71.24±1.117		Minimum: 56	Maximum: 82		Range: 26
Item	n	Wither height $\bar{X}\pm S_{\bar{X}}$	Chest depth $\bar{X}\pm S_{\bar{X}}$	Body length $\bar{X}\pm S_{\bar{X}}$	Chest girth $\bar{X}\pm S_{\bar{X}}$	Cannon bone circumference $\bar{X}\pm S_{\bar{X}}$	The widest circumference of tail $\bar{X}\pm S_{\bar{X}}$
Birth type							
Single	11	48.63±1.248	18.00±0.499	46.00±1.706	55.46±1.787	6.46±0.152	12.18±1.020
Twin	17	47.59±0.936	18.77±0.374	45.18±1.279	56.21±1.340	6.56±0.114	11.59±0.764
Triplet+	14	46.43±0.986	18.36±0.394	43.64±1.347	55.50±1.411	6.43±0.120	11.36±0.805
P-Value		0.222	0.711	0.163	0.626	0.768	0.461
Sex							
Female	21	47.05±0.926	18.62±0.370	45.14±1.265	56.98±1.325	6.62±0.113	12.00±0.756
Male	21	47.91±0.810	18.24±0.324	44.62±1.107	54.57±1.160	6.36±0.099	11.33±0.662
P-Value		0.861	0.385	0.281	0.057	0.079	0.215
Means	42	47.86±0.615	18.52±0.246	45.68±0.840	56.47±0.881	6.50±0.075	12.01±0.502

Age (day)		Mean: 206.45±1.737		Minimum: 177	Maximum:219		Range: 42
Item	n	Wither height $\bar{X}\pm S_{\bar{X}}$	Chest depth $\bar{X}\pm S_{\bar{X}}$	Body length $\bar{X}\pm S_{\bar{X}}$	Chest girth $\bar{X}\pm S_{\bar{X}}$	Cannon bone circumference $\bar{X}\pm S_{\bar{X}}$	The widest circumference of tail $\bar{X}\pm S_{\bar{X}}$
Birth type							
Single	9	52.89±1.124	23.22±0.473	56.00±1.457	68.56±1.665	6.61±0.144	13.06±0.942
Twin	11	51.73±1.309	22.18±0.551	53.27±1.697	68.64±1.941	6.73±0.168	12.82±1.098
Triplet+	9	51.56±1.124	22.89±0.473	54.67±1.457	68.22±1.665	6.44±0.144	12.33±0.942
P-Value		0.701	0.424	0.723	0.900	0.265	0.599
Sex							
Female	18	51.83±0.836	22.72±0.352	54.17±1.084	69.00±1.240	6.58±0.107	12.86±0.701
Male	11	52.36±1.088	22.73±0.458	55.18±1.411	67.64±1.613	6.64±0.139	12.55±0.913
P-Value		0.763	0.644	0.733	0.580	0.447	0.930
Means	29	52.24±0.686	22.82±0.289	55.06±0.890	68.80±1.017	6.64±0.088	13.00±0.575

The mean wither height and body length of the lambs at around 206 days were 52.24 and 55.06 cm, respectively, which are lower than those of found at the Lalahan Livestock Central Research Institute in Ankara province (Akçapınar et al., 2002) (56.21 and 58.06 cm) and the Kazım Karabekir SF (Işık & Aksoy, 2015a) (54.04 cm and 57.89 cm) on day 180. The chest depth (18.52 cm and 22.82 cm) and chest girth (56.47 cm and 68.80 cm) measurements recorded on around days 71 and 206 of the study were in line with the chest depths (19.11 cm and 23.45 cm) and chest girths (55.28 cm and 68.75 cm) recorded on days 90 and 180 of the study conducted at the Kazım Karabekir SF (Işık & Aksoy, 2015a).

CONCLUSION

It can be concluded that the survival characteristics of Bafra lambs residing in steppe conditions are at an acceptable level, while their growth characteristics are at a satisfactory level. However, growth and development characteristics of lambs of Bafra crossbred genotype should be investigated under different farm conditions and with different breeds.

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Author's contributions

The contributions of the authors are equal.

Conflict of interest

The authors declare no conflict of interest.

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